

**lek. Maksymilian Marek Onyszkiewicz**

**tytuł rozprawy**

**Wpływ kwasu masłowego oraz kwasu walerianowego na regulację  
ciśnienia tętniczego.**

The effect of butyric acid and valeric acid on the regulation of arterial blood  
pressure.

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu  
w dyscyplinie nauki medyczne**

Promotor: Prof. dr hab. n. med. Marcin Ufnal

Zakład Fizjologii i Patofizjologii Eksperymentalnej

Wydział Lekarsko-Stomatologiczny

Warszawski Uniwersytet Medyczny



Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych  
Warszawskiego Uniwersytetu Medycznego

Warszawa 2020

## **Słowa kluczowe**

Metabolity bakterii jelitowych, kwas masłowy, ciśnienie tętnicze krwi, choroby układu krążenia, trimetyloamina, tlenek trimetyloaminy, kwas walerianowy, krótkołańcuchowe kwasy tłuszczowe

## **Key words**

Bacterial metabolites, butyric acid, blood pressure, cardiovascular diseases, trimethylamine, trimethylamine oxide, valeric acid, short chain fatty acids



## **Dedykacje**

*Szczególne wyrazy wdzięczności składam  
Panu Profesorowi Marcinowi Ufnalowi za wielokrotnie okazane  
wsparcie, serdeczność, motywację oraz ogromne zaangażowanie i pomoc w badaniach  
naukowych, których efektem jest niniejsza praca.*

*Współpracownikom z Zakładu Fizjologii i Patofizjologii Eksperymentalnej  
składam podziękowania za przyjazną atmosferę i życzliwość  
oraz nieocenioną pomoc w prowadzeniu badań doświadczalnych.*

*Dziękuję również swoim Bliskim, szczególnie Narzeczonej i Mamie za Ich rady,  
wyrozumiałość, cierpliwość oraz wsparcie.*

## Wykaz publikacji stanowiących pracę dokorską

1. **Onyszkiewicz, M.**, Jaworska, K., Ufnal, M. Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system. *Experimental Biology and Medicine* 2020, volume: 245, pages: 166-175.  
DOI: 10.1177/1535370219900898  
**IF: 3.005, Punkty MNiSW: 70**
2. **Onyszkiewicz, M.**, Gawrys-Kopczynska, M., Konopelski, P., Aleksandrowicz, M., Sawicka, A., Kozniowska, E., Samborowska, E., Ufnal, M. Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors. *Pflugers Archiv - European Journal of Physiology* 2019, volume: 471, pages: 1441-1453.  
DOI: 10.1007/s00424-019-02322-y  
**IF: 3.377, Punkty MNiSW: 100**
3. **Onyszkiewicz M.**, Gawrys-Kopczynska M, Sałagaj M, Aleksandrowicz M, Sawicka A, Koźniowska E, Samborowska E, Ufnal M. Valeric acid lowers arterial blood pressure in rats. *European Journal of Pharmacology* 2020, volume: 877.  
DOI: 10.1016/j.ejphar.2020.173086  
**IF: 3.170, Punkty MNiSW: 100**

Wszystkie prace zostały opublikowane i są zamieszczone w rozprawie doktorskiej w wersji oryginalnej (format PDF)

## Spis treści

|                                                                         |    |
|-------------------------------------------------------------------------|----|
| Słowa kluczowe .....                                                    | 2  |
| Key words .....                                                         | 2  |
| Dedykacje .....                                                         | 3  |
| Wykaz publikacji stanowiących pracę doktorską .....                     | 4  |
| Wykaz stosowanych skrótów .....                                         | 7  |
| Streszczenie w języku polskim .....                                     | 9  |
| Streszczenie w języku angielskim .....                                  | 12 |
| Wstęp uzasadniający połączenie wskazanych publikacji w jeden cykl ..... | 14 |
| Założenia i cel pracy .....                                             | 15 |
| Publikacja nr 1 .....                                                   | 16 |

**Onyszkiewicz, M.,** Jaworska, K., Ufnal, M.

Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system. *Experimental Biology and Medicine* 2020, volume: 245, pages: 166-175. DOI: 10.1177/1535370219900898

|                       |    |
|-----------------------|----|
| Publikacja nr 2 ..... | 27 |
|-----------------------|----|

**Onyszkiewicz, M.,** Gawrys-Kopczynska, M., Konopelski, P., Aleksandrowicz, M., Sawicka, A., Kozniewska, E., Samborowska, E., Ufnal, M.

Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors. *Pflügers Archiv - European Journal of Physiology* 2019, volume: 471, pages: 1441-1453. DOI: 10.1007/s00424-019-02322-y

|                       |    |
|-----------------------|----|
| Publikacja nr 3 ..... | 41 |
|-----------------------|----|

**Onyszkiewicz M**, Gawrys-Kopczynska M, Sałagaj M, Aleksandrowicz M, Sawicka A,  
Koźniewska E, Samborowska E, Ufnal M.

Valeric acid lowers arterial blood pressure in rats. European Journal of Pharmacology 2020,  
volume: 877. DOI: 10.1016/j.ejphar.2020.173086

|                              |    |
|------------------------------|----|
| Podsumowanie i wnioski ..... | 51 |
|------------------------------|----|

|                              |    |
|------------------------------|----|
| Opinia Komisji Etycznej..... | 53 |
|------------------------------|----|

|                                                      |    |
|------------------------------------------------------|----|
| Oświadczenia wszystkich współautorów publikacji..... | 57 |
|------------------------------------------------------|----|

## Wykaz stosowanych skrótów

**(MRM)**-Monitorowanie reakcji następczych

**3-NPH**-3-nitrofenylohydrazyna

**AA**-Kwas octowy

**ACH**-Acetylocholina

**ANT (3-hydroxybutyrate)**-3-hydroksymaślan

**BA**-Kwas masłowy

**BP**-Ciśnienie tętnicze krwi

**BSA**-Surowicza albumina wołowa

**Ca<sup>2+</sup>-free PSS (PSS containing 3 mM EGTA)**-polistyrenosulfonian wolny od jonów wapnia (PSS) zawierający 3 mM kwas etylenoglikol-*O-O'*-bis(2-aminoetyl)-N,N,N',N' tetraoctowy (EGTA)

**DMSO**-Dimetylosulfotlenek

**ECG**-Elektrokardiogram

**EDC·HCl-N (3-dimethylaminopropyl)-N'-ethylcarbodiimide**-chlorowodorek *N*-(3-dimetyloaminopropyl)-*N'*-etylokarbodiimidu

**EDTA**-Kwas wersenowy

**Foley catheter (10F)**-Cewnik Foleya o średnicy 10F

**GMA**-Tętnica wyizolowana z mięśnia smukłego

**HCl**-Chlorowodór

**HPLC**-Wysokosprawna chromatografia cieczowa

**HR**-Częstość rytmu serca

**IBA**-Kwas izomasłowy

**IC**-Podanie dookrężnicze

**IVA**-Kwas izowalerianowy

**LC/MS**-Chromatografia cieczowa sprzężona ze spektrometrią mas

**LQQ**-Granica oznaczalności ilościowej

**MA**-Tętnica krezkowa

**MOPS-PSS**-polistyrenosulfonian (PSS) w roztworach buforowych kwasu 3-(*N*-morfolino) propanosulfonowego (MOPS)

**PA**-Kwas propionowy

**PE**-Fenylefryna

**RR interval**-Odstęp pomiędzy dwoma kolejnymi załamkami R w zapisie EKG

**SCFA**-Krótkołańcuchowe kwasy tłuszczowe

**SEM**- Błąd standardowy

**VA**-Kwas walerianowy

**VA-D9**-Znakowany izotop D9 kwasu walerianowego

## **Streszczenie w języku polskim**

### **Wstęp**

Rosnąca liczba badań wskazuje, że związki wytwarzane przez bakterie jelitowe mogą wpływać na funkcjonowanie układu krążenia w zdrowiu i chorobie. Krótkołańcuchowe kwasy tłuszczowe, takie jak kwas masłowy i kwas walerianowy, powstają w jelicie grubym podczas bakteryjnej fermentacji błonnika pokarmowego. Związki te mogą wywierać zarówno działanie lokalne w jelicie, jak również działanie ogólnoustrojowe, po przekroczeniu bariery jelito-krew. Istnieją pojedyncze prace świadczące o wpływie kwasu masłowego na układ krążenia, jednak mechanizmy działania tego związku nie są poznane. Brak jest badań dotyczących wpływu kwasu walerianowego pochodzącego od bakterii jelitowych na układ krążenia. Celem pracy doktorskiej była ocena wpływu kwasu masłowego i kwasu walerianowego na regulację ciśnienia tętniczego oraz zbadanie mechanizmów związanych z oddziaływaniem tych związków na układ krążenia.

### **Publikacja 1**

W publikacji został przedstawiony przegląd piśmiennictwa dotyczący wpływu krótkołańcuchowych kwasów tłuszczowych i metyloamin, wytwarzanych przez bakterie jelitowe, na układ krążenia. Krótkołańcuchowe kwasy tłuszczowe, takie jak kwas octowy, propionowy oraz masłowy są wytwarzane podczas bakteryjnej fermentacji błonnika pokarmowego. Rosnąca liczba badań wskazuje, że krótkołańcuchowe kwasy tłuszczowe mogą rozszerzać naczynia krwionośne i obniżać ciśnienie tętnicze. Zmiany w stężeniu związków produkowanych przez bakterie jelitowe obserwowane we krwi, wynikające z różnic w ich wytwarzaniu oraz zwiększonej przepuszczalności bariery jelito-krew, mogą istotnie wpływać na funkcjonowanie układu krążenia. W związku z tym produkty metabolizmu bakterii jelitowych mogą być traktowane zarówno jako mediatory jak i markery w chorobach układu krążenia.

### **Publikacja 2**

Kwas masłowy jest jednym z krótkołańcuchowym kwasów tłuszczowych wytwarzanych przez bakterie jelitowe. Przeprowadzone przeze mnie badania wykazały, że stężenie kwasu masłowego jest o trzy rzędy wielkości większe w jelicie niż we krwi obwodowej. Zwiększenie stężenia kwasu masłowego w jelicie grubym, uzyskane poprzez podanie egzogennej roztworu kwasu masłowego, prowadziło do istotnego spadku ciśnienia

tętniczego i zmniejszenia częstości skurczów serca. W badaniu wykazałem również, że efekt hipotensyjny był hamowany przez wykonanie podprzeponowej wagotomii, dożylnie podanie heksametonium, dookreżnicze podanie 3-hydroksymaślanu, ale nie przez dożylnie podanie atropiny. W doświadczeniach ex vivo kwas masłowy wykazał działanie wazodylatacyjne zarówno w stosunku do tętnicy krezkowej, jak i do tętnicy wyizolowanej z mięśnia smukłego. W kolejnych doświadczeniach, w których kwas masłowy był podawany dożylnie, obserwowałem efekt hipotensyjny, który utrzymywał się krócej niż po podaniu dookreżniczym i nie wpływał na częstość skurczów serca. Opierając się na powyższych obserwacjach wydaje się, że jelito grube jest najważniejszym miejscem oddziaływania kwasu masłowego. 2-3 krotne zwiększenie stężenia kwasu masłowego w jelicie grubym znacząco obniża ciśnienie tętnicze za pośrednictwem receptorów GPR41/43 oraz aferentnej impulsacji z jelita za pośrednictwem nerwu błędnego.

### **Publikacja 3**

Kwas walerianowy jest krótkołańcuchowym kwasem tłuszczowym produkowanym przez bakterie jelitowe podczas fermentacji błonnika pokarmowego. Można go również znaleźć w ziołach, takich jak kozłek lekarski (*Valeriana officinalis*). Ponadto, dzięki endogennym esterazom, jest uwalniany z niektórych leków, takich jak walerian estradiolu. Przeprowadzone przeze mnie badania wykazały, że stężenie kwasu walerianowego jest o 4 rzędy wielkości większe w jelicie grubym, niż we krwi obwodowej. Po 5 minutach od podania dookreżniczego egzogenne, znakowanego izotopowo (VA-D9) kwasu walerianowego stwierdzono obecność kwasu w tkankach takich jak mózg, wątroba, nerki oraz serce. Zwiększenie stężenia kwasu walerianowego w jelicie grubym, uzyskane poprzez podanie egzogenne roztworu kwasu walerianowego, prowadziło do krótkotrwałego, istotnego spadku ciśnienia tętniczego, połączonego ze spadkiem częstości skurczów serca. Efekt ten był hamowany przez dookreżnicze podanie 3-hydroksymaślanu, ale nie przez dożylnie podanie atropiny oraz wykonanie podprzeponowej wagotomii. Efekt hipotensyjny uzyskany przez dookreżnicze podanie roztworu kwasu walerianowego został znacząco wydłużony dzięki dożylnemu podaniu heksametonium. W doświadczeniach ex vivo kwas walerianowy wykazał działanie naczyniorozszerzające w stosunku do tętnicy krezkowej, jak również tętnicy wyizolowanej z mięśnia smukłego. Dożylnie podanie kwasu walerianowego u szczurów prowadziło do krótkotrwałego działania hipotensyjnego, jednocześnie nie wywierając istotnego wpływu na częstość skurczów serca. Efekt hipotensyjny uzyskany przy podaniu tego rodzaju został znacząco wydłużony poprzez zastosowanie dożylnego wlewu kwasu



walerianowego w infuzji ciągłej. Niniejsze badanie po raz pierwszy wykazało, że kwas walerianowy szybko przenika przez barierę jelito-krew i docierając do organów zaangażowanych w regulację ciśnienia tętniczego wywołuje istotny efekt hipotensyjny.

### **Podsumowanie**

Wyniki przeprowadzonych prac wskazują, że warunkach fizjologicznych stężenie kwasu masłowego i kwasu walerianowego jest najwyższe w jelicie grubym, które jest głównym habitatem mikrobioty. Kwas masłowy i kwas walerianowy przedostają się z jelita grubego do układu krążenia powodując istotny efekt hipotensyjny, oddziałując zarówno poprzez układ nerwowy, jak również bezpośrednio na naczynia.

## Streszczenie w języku angielskim

### Background

Increasing evidence suggests that molecules produced by gut bacteria affect the health of the host. Short chain fatty acids (SCFAs) such as butyric acid (BA) and valeric acid (VA) are produced by bacterial fermentation of dietary fiber in colon. SCFAs exert local effects on intestines as well as systemic effects after crossing the gut-blood barrier. Several studies suggest that BA affects the circulatory system, however, the mechanisms are not clear. To the best of my knowledge, hemodynamic effects of VA, have not yet been reported. The aim of the study was to assess the effect of two metabolites of gut bacteria i.e. butyric acid and valeric acid on the circulatory system.

### Publication No. 1

This paper reviews research on the cardiovascular effects of microbiota-produced short chain fatty acids and methylamines. Short chain fatty acids such as acetic, propionic, butyric and valeric acids are mainly produced during bacterial fermentation of dietary fiber. SCFAs have been found to dilate blood vessels and lower blood pressure. Changes in plasma levels of gut microbiota-derived products may result from alterations in bacterial metabolism and changes in gut-to-blood penetration of the molecules. Gut microbiota-derived products may serve as mediators and markers in cardiovascular diseases.

### Publication No. 2

BA is one of several short chain fatty acids produced by gut microbiota. The study showed that the concentration of BA in the colon was approximately three orders of magnitude higher than the concentration of BA in the systemic blood. BA administered into the colon (IC) produced a prolonged decrease in arterial blood pressure (BP) and heart rate (HR). Subphrenic vagotomy and IC pretreatment with the antagonist of GPR41/43 receptors (ANT) significantly reduced the hypotensive effect of IC BA. The hypotensive effect was also reduced by IV administered hexamethonium but not by atropine. In *ex vivo* conditions, BA dilated mesenteric (MA) and gracilis muscle (GMA) arteries. Furthermore, I performed analogical series of experiments using intravenous administration. BA administered IV produced shorter decrease in BP than BA administered IC and did not affect HR. In conclusion, the colon appears the most likely site of BA action at physiological

concentrations. A 2-3-fold increase in the concentration of colonic BA lowers BP which seems to be mediated by GPR41/43 and afferent vagal signaling.

### **Publication No. 3**

Valeric acid (VA) is a short chain fatty acid which is produced by gut bacteria during fermentation of dietary fiber. The acid also can be found in herbs such as *Valeriana officinalis*. Furthermore, VA is released from medicines such as estradiol valerate by esterases. The present study showed that the concentration of VA in the colon was approximately four orders of magnitude higher than the concentration of VA in the systemic blood. VA-D9 was detected in the brain, the liver, the kidneys and the heart, 5 min after its administration into the colon (IC). VA administered IC produced a decrease in arterial blood pressure (BP) which was accompanied by a decrease in heart rate (HR). The hypotensive effect of VA was inhibited by the antagonist of GPR41/43 receptors (ANT) but not by the subphrenic vagotomy. Hexamethonium prolonged the hypotensive effect of VA while atropine did not influence the hypotensive effect. In *ex vivo* conditions VA dilated GMA and MA. Furthermore, I performed analogical series of experiments using intravenous administrations of VA. Intravenous bolus of VA produced a short-lasting hypotensive effect with no significant change in HR. The hypotensive effect was significantly prolonged if VA was administered in the continuous intravenous infusion. In conclusion, the study for the first time shows that VA rapidly penetrates from the colon to tissues involved in the control of BP and produces a significant hypotensive effect.

### **Conclusions**

Results of my studies show that the highest concentrations of VA and BA are present in the colon, a major habitat of gut bacteria. BA and VA penetrate from the colon to the systemic circulation and exert a hypotensive effect. The hypotensive effect is mediated via the nervous system and via a direct action of the acids on blood vessels.

## **Wstęp uzasadniający połączenie wskazanych publikacji w jeden cykl**

W ostatnim czasie pojawiły się publikacje wskazujące, że metabolity bakterii jelitowych mogą oddziaływać na układ krążenia. W grupie związków wytwarzanych przez bakterie jelitowe znajdują się krótkołańcuchowe kwasy tłuszczowe, które są wytwarzane podczas bakteryjnej fermentacji błonnika pokarmowego. Nieliczne prace wskazują, że krótkołańcuchowe kwasy tłuszczowe, takie jak kwas masłowy, mogą rozszerzać naczynia krwionośne i obniżać ciśnienie tętnicze.

Dotychczas nie badano wpływu kwasu walerianowego na układ krążenia. Pojedyncze prace opisują działanie hipotensyjne kwasu masłowego, jednak mechanizmy tego działania, w szczególności w odniesieniu do kwasu wytwarzanego w jelicie, nie były badane.

Celem podjętych badań eksperymentalnych była ocena wpływu metabolitów bakterii jelitowych: kwasu masłowego i kwasu walerianowego na ciśnienie tętnicze oraz poznanie mechanizmów regulujących układ krążenia u szczurów.

Badania przeprowadzone w ramach pracy doktorskiej dostarczyły nowych danych dotyczących oddziaływania kwasu masłowego oraz kwasu walerianowego na układ krążenia, wykazując hipotensyjne działanie obu kwasów oraz mechanizmy związane z tym działaniem.

Niniejsza rozprawa doktorska pt. „Wpływ kwasu masłowego oraz kwasu walerianowego na regulację ciśnienia tętniczego.” (*The effect of butyric acid and valeric acid on the regulation of arterial blood pressure.*) tworzy monotematyczny cykl dwóch prac oryginalnych i jednej pracy przeglądowej, opublikowanych w czasopiśmie anglojęzycznym o zasięgu międzynarodowym. Doktorant jest pierwszym autorem we wszystkich publikacjach cyklu. Suma punktów „Impact Factor” przedstawionego cyklu prac wynosi 9.552, natomiast suma punktów MNiSW to 270.

## **Założenia i cel pracy**

Hipoteza badawcza zakładała, że kwas masłowy oraz kwas walerianowy, które są wytwarzane przez bakterie jelitowe, wywierają istotny wpływ na ciśnienie tętnicze.

Główne cele pracy:

1. Zbadanie wpływu kwasu masłowego na ciśnienie tętnicze u szczurów po podaniu dookrężniczym i dożylnym.
2. Zbadanie wpływu kwasu walerianowego na ciśnienie tętnicze u szczurów po podaniu dookrężniczym i dożylnym.

Cele szczegółowe:

1. Zbadanie mechanizmów działania hipotensyjnego kwasu masłowego i walerianowego.
2. Zbadanie fizjologicznych stężeń kwasu masłowego i walerianowego w tkankach oraz ocena stopnia przenikania przez barierę jelito-krew badanych związków.
3. Ocena kardi toksyczności kwasu masłowego i walerianowego.

# PUBLIKACJA nr 1

*Short chain fatty acids and methylamines produced  
by gut microbiota as mediators and markers in the  
circulatory system*

Maksymilian Onyszkiewicz

Kinga Jaworska

Marcin Ufnal

## Minireview

# Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system

Maksymilian Onyszkiewicz, Kinga Jaworska and Marcin Ufnal 

Department of Experimental Physiology and Pathophysiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw 02-097, Poland

Corresponding author: Marcin Ufnal. Email: mufnal@wum.edu.pl

### Impact statement

Despite a progress in the diagnosis and treatment of cardiovascular diseases, there are still significant gaps in understanding complex mechanisms underlying cardiovascular pathology. Increasing evidence suggests that gut microbiota products such as short chain fatty acids or methylamines may affect the circulatory system in health and disease. Hence, the microbiota-derived molecules are potential diagnostic and therapeutic targets in cardiovascular diseases. Therapeutic options may include administration of selected bacterial strains (probiotics) producing desired metabolites or administration of direct gut microbiota products.

### Abstract

Ample evidence suggests that gut microbiota-derived products affect the circulatory system functions. For instance, short chain fatty acids, that are the products of dietary fiber bacterial fermentation, have been found to dilate blood vessels and lower blood pressure. Trimethylamine, a gut bacteria metabolite of carnitine and choline, has recently emerged as a potentially toxic molecule for the circulatory system. To enter the bloodstream, microbiota products cross the gut–blood barrier, a multilayer system of the intestinal wall. Notably, experimental and clinical studies show that cardiovascular diseases may compromise function of the gut–blood barrier and increase gut-to-blood penetration of microbiota-derived molecules. Hence, the bacteria products and the gut–blood barrier may be potential diagnostic and therapeutic targets in cardiovascular diseases. In this paper, we review research on the cardiovascular effects of microbiota-produced short chain fatty acids and methylamines.

**Keywords:** SCFA, TMA, TMAO, bacterial metabolites, blood pressure, cardiovascular diseases

*Experimental Biology and Medicine* 2020; 245: 166–175. DOI: 10.1177/1535370219900898

## Gut bacteria in human homeostasis

The mammalian gut and skin is colonized early after birth by bacteria and fungi.<sup>1,2</sup> The number of gut bacteria is at least equal to the number of human body cells and, at the same time, the composition of human microbiota is very unique for each person. The composition of the gut microbiota depends on numerous factors including geography, age, diet, the mode of delivery, and postnatal feeding.<sup>3–7</sup> For example, it has been shown that vaginal birth and breastfeeding are associated with more diverse microbiota than cesarean birth and formula-feeding.<sup>8–10</sup> The most common bacterial phyla in the human gut include Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria.<sup>11,12</sup>

Increasing research provides evidence that human homeostasis depends on reciprocal interaction with gut

microbiota. On the one hand, gut microbiota produces vital compounds for human homeostasis such as vitamin K or B group (cobalamin, biotin, pyridoxine, thiamine, folates, riboflavin and nicotinic acid), and contributes to the metabolism of bile acids, steroids and xenobiotics.<sup>13–16</sup> On the other hand, gut bacteria produce toxic substances such as ammonia or trimethylamine and may reduce the availability of essential nutrients for the host.<sup>17–19</sup>

Finally, recent research shows that microbiota produced molecules such as hydrogen sulfide (H<sub>2</sub>S), indoles, short chain fatty acids and trimethylamines affect the circulatory system homeostasis, acting on its humoral and nervous control.<sup>20–22</sup> In particular, short chain fatty acids and trimethylamines have attracted a lot of attention, which resulted in the explosion of experimental and clinical papers on the circulatory effects of the two groups of microbiota



products.<sup>23–25</sup> It is also worth noting that some research reported changes in gut bacteria composition also referred to as dysbiosis in cardiovascular diseases (CVDs).<sup>26–28</sup>

### Mechanisms of a crosstalk between gut microbiota and the circulatory system

The mechanisms underlying the interaction between gut microbiota and the circulatory system are far from clear. However, increasing experimental data show that at least two potential pathways are likely (Figure 1). Firstly, gut microbiota-derived products may stimulate afferent (sensory) nerves of the enteric nervous system which may modulate the cardiovascular centers in the brain. The latter affects the activity of the autonomic nervous system that controls the function of the heart, vasculature and glands releasing cardiovascular hormones. Secondly, gut microbiota products may enter the bloodstream and thereby affect the function of organs and tissues that contribute to the circulatory system homeostasis. As most of the gut-derived molecules first pass the liver, the systemic effects of microbiota products may also depend on liver metabolites.<sup>29–31</sup>

It has been well established that biological effects of any molecule depend on its concentration. In this regard, the blood concentration of bacterial metabolites depends on several factors including gut bacteria metabolic activity, the function of the intestinal wall (gut-to-blood

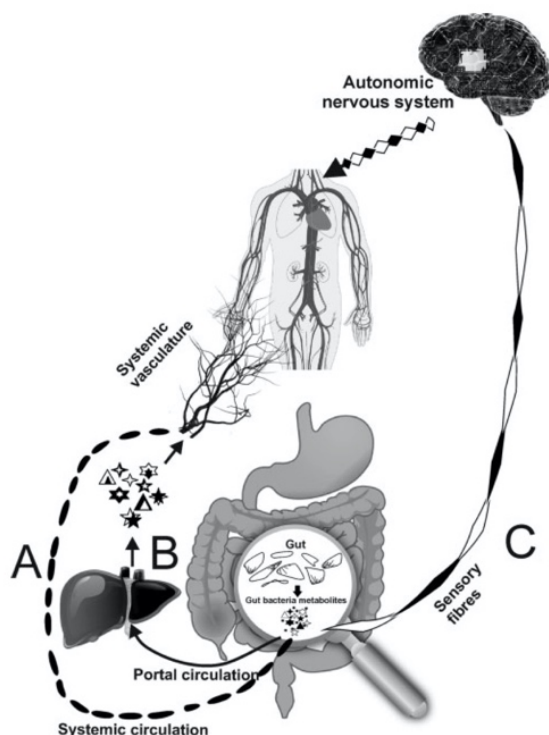
permeability) and the function of the liver and the kidneys (metabolism, detoxification and excretion). Deteriorated function of the kidneys and the liver in cardiovascular diseases is well established and has been described in numerous papers.<sup>32–37</sup> Recently, the decline of the intestinal function and a “leaky gut” syndrome in cardiovascular diseases has gained a lot of attention.<sup>38–40</sup>

In short, to enter the circulation, gut bacteria products need to cross the intestinal wall that forms the gut-blood barrier (GBB). The GBB is a complex system of several biological layers, including vascular endothelium, epithelial cell lining and mucus layer. The integrity and proper functioning of the GBB depend on numerous, not fully understood factors. The GBB enables the absorption of nutrients and beneficial bacterial metabolites such as SCFAs from intestinal lumen and, at the same time, reduces the passage of potentially toxic substances.<sup>41</sup> A pivotal factor for the proper functioning of the GBB is adequate blood perfusion. There is some experimental and clinical evidence showing that CVDs compromise the GBB function by decreasing intestinal blood flow and disrupting the GBB structure. A leaky gut has been reported in patients with heart failure<sup>42</sup> and in several experimental models of CVDs.<sup>39,43</sup> For example, we have found that hypertension in rats is accompanied by morphological and hemodynamic alterations in the colon, and increased permeability of the colon to TMA, a toxic gut bacteria metabolite.<sup>43</sup>

### Short-chain fatty acids

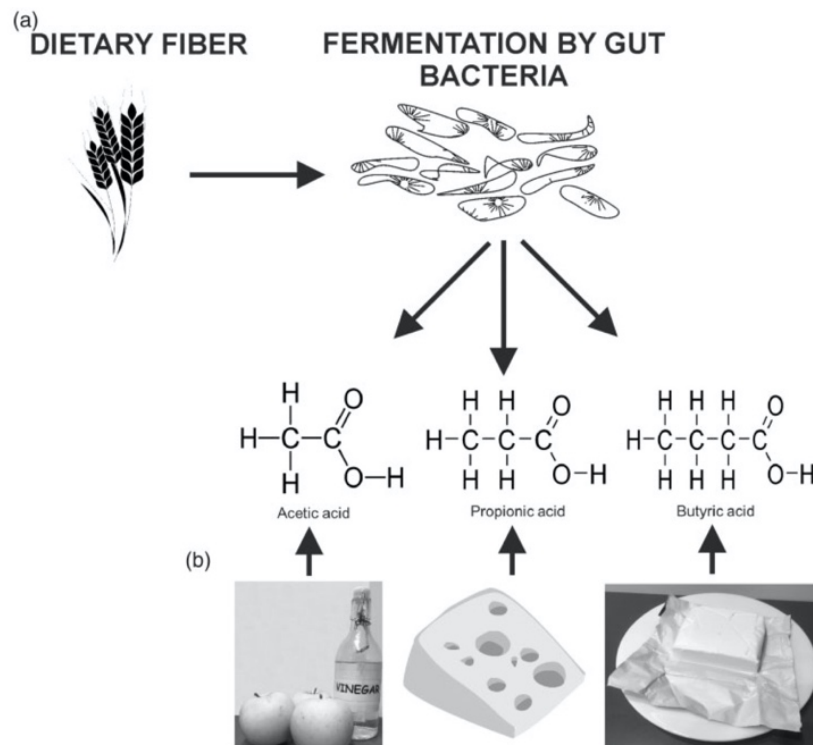
Various biological effect of short-chain fatty acids (SCFAs) have been described in medical literature since ancient times. However, only recently it has been recognized that gut microbiota is an important source of SCFAs in mammalian organisms. SCFAs are produced chiefly by the colon microbiota in fermentation process from dietary fiber. This group of fatty acids consists of aliphatic tail with five or fewer carbons.<sup>44,45</sup> There is evidence that SCFAs are also present in intestinal content of germ-free animals, but at lower concentration than in conventional animals.<sup>46,47</sup> In addition, a group of dietary products constitutes a direct source of short chain fatty acids including acetate, propionate and butyrate (Figure 2).

SCFAs play an important role locally in intestines serving as an energy source for enterocytes and inhibiting the growth of pathogens by reducing pH. In addition, SCFAs may affect the local intestinal microcirculation.<sup>48,49</sup> Furthermore, SCFAs originating from gut microbiota metabolism enter the systemic circulation through the GBB. Biological effects of SCFAs including metabolic, hemodynamic and inflammatory effects are thought to be mediated by GPR41 (also known as Free Fatty Acid Receptor 3, FFAR3), GPR43 (Free Fatty Acid Receptor 2, FFAR2), or Olfr78 receptors which are located in the gastrointestinal tract, adipose tissue, immune cells, and peripheral nerves.<sup>30,50–54</sup> Accumulating evidence suggests that SCFAs may exert blood pressure lowering effect by decreasing vascular tone.<sup>55–59</sup> Furthermore, SCFAs may change blood pressure by mechanisms related to



**Figure 1.** Suggested pathways of cardiovascular actions of gut microbiota products. (A) Gut bacteria products cross the gut-blood barrier, bypass the liver via rectal plexuses and reach cardiovascular tissues. (B) The liver produces derivatives of microbiota metabolites that affect the heart and vasculature. (C) Gut microbiota metabolites stimulate sensory fibers of the enteric nervous system which project to the brain that controls the circulatory system via the autonomic nervous system.





**Figure 2.** Sources of short chain fatty acids (SCFAs) in human organism. (a) SCFAs are produced during fermentation process from dietary fiber; (b) Dietary products constitute a direct source of SCFAs including acetate, propionate and butyrate.

gut-to-brain nervous signaling<sup>60</sup> renal sensory nerves<sup>31,53</sup> and renin-angiotensin-aldosterone pathway.<sup>53,61–63</sup>

### Acetic acid

Acetic acid is a colorless liquid organic compound which consists of methyl group attached to a carboxyl group. It possesses acrid smell and sour taste. Liquid containing 5–20% of acetic acid is called vinegar and was used as a food preservative by Babylonians (c. 5000 BC).<sup>64</sup> Moreover, vinegar was used for dressing wounds by Hippocrates (c. 420 BC) and in American civil war, likely due to its antimicrobial properties.<sup>65</sup> It has also been suggested that acetic acid prevents osteoporosis by increasing calcium intestinal absorption and the concentration of calcium in bones.<sup>66</sup> Moreover, acetic acid was reported to exert antidiabetic effects by improving glucose uptake in skeletal muscles and decreasing fasting and postprandial glucose levels.<sup>67–69</sup> With regard to cardiovascular effects, acetic acid has been found to lower arterial blood pressure in rats.<sup>70,71</sup> The mechanisms behind the hypotensive effect may include the inhibition of ACE activity, decrease in plasma renin activity or increase in tissue level of cAMP.<sup>72–75</sup> In vitro studies report that acetic acid produces vasodilatation in colonic resistance vessels.<sup>76</sup> Additionally, acetate might play a role in flow-mediated vasorelaxation by inducing eNOS phosphorylation in the endothelium.<sup>77</sup> Clinical studies involving patients regularly undergoing hemodialysis with dialysate containing acetate showed a significant decrease in peripheral vascular tone and diminished myocardial contractility.<sup>78–80</sup>

### Propionate

Propionic acid is a colorless oily liquid organic compound, miscible with water, with characteristic unpleasant smell fairly resembling body odor. Antimicrobial effects of propionic acid and its salts have been known for a long time. Propionates are used in the food industry as preservatives in many products such as bread or cheese.<sup>81,82</sup> Propionic acid has been found to exert several potentially health-promoting effects which may lower cardiovascular risk. Firstly, propionate may inhibit lipid synthesis in hepatocytes and thereby decrease cholesterol level.<sup>83,84</sup> Secondly, some evidence suggests that propionic acid has anti-inflammatory effect<sup>85</sup> and improves insulin sensitivity.<sup>86–88</sup> Notably, propionic acid is the strongest ligand for the SCFAs receptors Gpr41 and Gpr43.<sup>89,90</sup>

Finally, some studies show that propionic acid exerts direct cardiovascular effects. A dose-dependent vasodilatory effect have been reported in rat mesenteric arteries and in isolated human colonic resistance arteries.<sup>76,91</sup>

### Butyric acid

Butyrate is a colorless carboxylic acid produced in the mammalian gut by bacterial fermentation of dietary fiber. However, butyric acid is also present in butter and other dairy products. The taste of butyrate is characterized as pungent, with a sweetish aftertaste which can be linked with the taste of ether. The acid is responsible for the obnoxious smell of human vomit.

Butyrate has been found to affect numerous physiological processes including energy homeostasis,



cardiovascular parameters, immune response, and brain functions. The underlying mechanisms may range from metabolic effects of receptor's signaling to enzymatic inhibition.<sup>92,93</sup> Some evidence suggests that butyric acid exerts anticarcinogenic effect that may be explained by the Warburg effect. The opposing effect of butyric acid on normal and cancer cells sometimes is called as butyrate paradox.<sup>94,95</sup>

Numerous studies show that butyrate may affect the regulation of arterial blood pressure. The hypotensive and bradycardic effect of intravenous administration of butyric acid was described already in 1957 by Wretling.<sup>96</sup>

Blood pressure lowering effect of butyrate seems to be dependent on vasodilation. Mortensen *et al.* showed that butyric acid and other SCFAs produce a concentration-dependent dilation of human colonic resistance arteries.<sup>76</sup> Likewise, a weak vasodilatory effect of butyric acid at concentrations above 5 mM was reported in the coronary arteries.<sup>57</sup> The vasodilatory effect of butyric acid was also demonstrated in rat caudal artery.<sup>58</sup> Several mechanisms of the butyric acid-dependent vasodilation have been proposed including stimulation of the cyclic AMP second messenger system<sup>97</sup> and increased synthesis of F2 alpha prostaglandins.<sup>59</sup> It has also been suggested that the hypotensive effect of butyric acid may depend on the inhibition of the intrarenal renin-angiotensin system.<sup>62</sup>

Our recent findings suggest that gut-derived butyric acid affect the circulatory system via the following two mechanisms. Firstly, butyric acid stimulates afferent fibers of the vagus nerve which project from the gut to the brain. This decreases tonic sympathetic activity and thereby lowers arterial blood pressure. Secondly, gut-derived butyric acid crosses the GBB, enters the bloodstream and produces vasodilation acting on GPR41/43 receptors. Interestingly, our study showed that physiological concentration of butyric acid in the colon is three orders of magnitude higher than that in systemic blood, which makes the colon a very likely site of butyric acid action under physiological conditions.<sup>60</sup>

Further indirect evidence for the hypotensive effect of butyric acid produced by gut microbiota is provided by studies showing an inverse correlation between arterial blood pressure and the abundance of bacteria producing butyrate in rats.<sup>98</sup> Similarly, in overweight pregnant women the abundance of butyrate-producing bacteria and butyrate production was associated with lower arterial blood pressure.<sup>99</sup>

Apart from the hypotensive action, butyric acid have been suggested to possess a direct antiatherogenic activity by diminishing vascular smooth muscle cells proliferation.<sup>100</sup>

### Trimethylamines: TMAO vs. TMA

Trimethylamine (TMA) is a simple tertiary aliphatic amine with the formula  $N(CH_3)_3$ . It is synthesized in colon by several bacterial genera including *Clostridium*, *Collinsella*, *Desulfovibrio*, *Lactobacillus*, and *Proteus* from dietary L-carnitine, choline, and their derivatives. After absorption in large intestine TMA passes through the portal vein to the

liver where most of it is oxidized to trimethylamine N-oxide (TMAO) by the flavin-containing monooxygenase (FMO3).<sup>101</sup>

### TMAO

In terms of cardiovascular effects, TMAO has gained much more attention than its precursor TMA as recent studies showed positive correlation between plasma TMAO concentration and cardiovascular risk. However, despite hundreds of studies investigating TMAO, it is not clear whether it is a harmful factor, sign of adaptive response or just a confounder.<sup>23</sup>

It has been shown that plasma TMAO may be used in risk assessment in general population<sup>102</sup> and in patients with heart failure,<sup>103–105</sup> acute myocardial infarction,<sup>106,107</sup> coronary artery disease,<sup>108</sup> peripheral artery disease,<sup>109</sup> stroke,<sup>110</sup> and hypertension.<sup>111,112</sup> In particular, the association between TMAO and atherosclerosis burden has been observed.<sup>113–116</sup> It has also been reported that TMAO contributes to platelet hyperreactivity by increasing intracellular  $Ca^{2+}$  release and enhancing thrombotic potential and it independently predicted incident thrombosis risk.<sup>117</sup> However, there are some issues that call into question the above results. Some research found no evidence linking TMAO to increased cardiovascular risk.<sup>118–121</sup> Other studies suggest that increased TMAO is only a manifestation of decreased kidney function and association with cardiovascular risk become insignificant after adjustment for eGFR.<sup>122–124</sup> It is along with the fact that TMAO is excreted mainly with urine and elevated plasma TMAO level has been widely reported in chronic kidney disease.<sup>114,125,126</sup> It was shown that impaired renal function was the main variable affecting plasma TMAO concentration.<sup>127</sup> It is also worth noting that plasma TMAO concentration may be confounded by the disturbed GBB function<sup>43</sup> or FMO3 activity.<sup>128</sup> FMO3 is mostly active in liver, however, it was detected in lung, kidney and other tissues as well. Besides, its expression is sex- and age-specific,<sup>129,130</sup> which may further interfere with the results. In addition, it has been shown that the association between TMAO and cardiovascular events may vary depending on race<sup>131</sup> or gender.<sup>132</sup> Finally, the connection between TMAO and atherosclerotic progression in patients has been questioned.<sup>133–135</sup>

A lot of experimental studies have been performed to determine a mechanistic insight into how TMAO potentially promotes cardiovascular diseases. Supplementation of TMAO<sup>136,137</sup> or its dietary precursors<sup>116,137–139</sup> augmented atherosclerotic plaque formation in atherosclerosis-prone mice, possibly due to toxic effect on endothelial progenitor cells<sup>140</sup> or acting by CD36/MAPK/JNK pathway.<sup>136</sup> However, some studies do not support these findings<sup>141</sup> and surprisingly, Collins *et al.* suggested even a protective effect of TMAO precursor against atherosclerotic lesion development.<sup>142</sup> Diet enriched by TMAO and its precursors caused a significant increase in heart failure severity in mice<sup>143</sup> and reduction in TMAO concentration resulted in improvement in heart failure symptoms induced by myocardial infarction.<sup>144</sup> Some studies showed that TMAO may induce cardiac fibrosis<sup>145,146</sup> and disturb energy



metabolism in the heart.<sup>147</sup> However, experiments carried out by Querio *et al.*<sup>148</sup> and in our laboratory<sup>149</sup> do not support the damaging effect of TMAO on cardiomyocytes. The connection between TMAO and aging is controversial as well. In mouse model, TMAO treatment for 16 weeks induced vascular aging.<sup>150</sup> On the other hand, TMAO did not increase with age in rat plasma and did not affect smooth muscle cells viability.<sup>151</sup> Indirect evidence of damaging effect of TMAO followed from the fact that its precursors, choline and carnitine, are abundant in red meat which is known to increase cardiovascular risk. However, consumption of fish and other seafood, which are linked to cardio-protective effects, produce substantially greater increase in circulating TMAO than red meat.<sup>152,153</sup> Therefore, the causative role of TMAO in cardiovascular diseases remains under question.

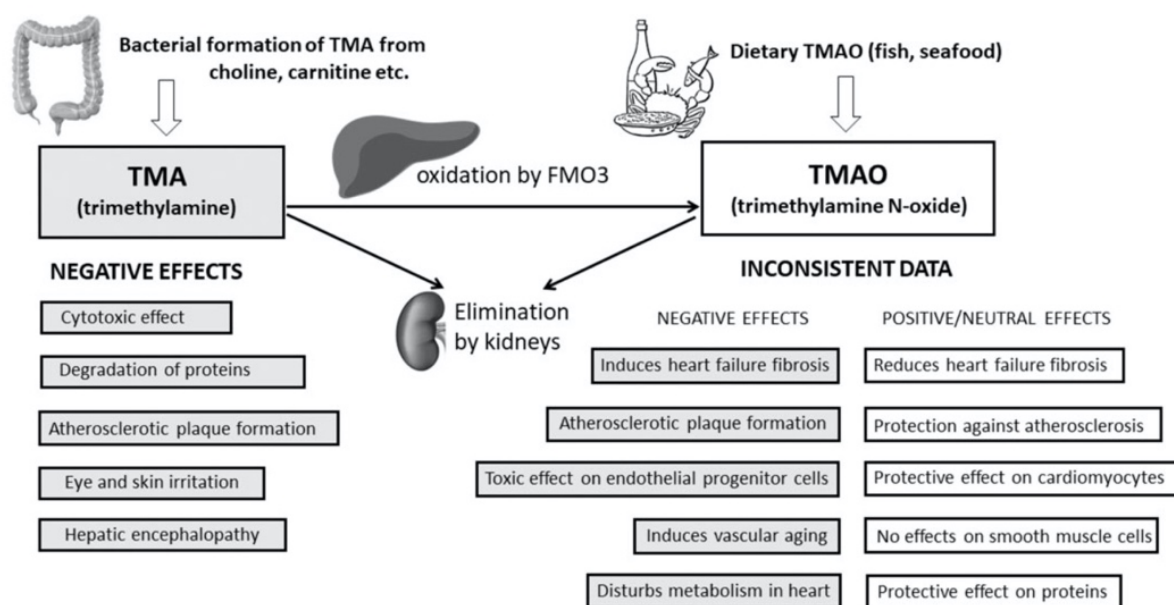
Intriguingly, TMAO has protective functions against osmotic and hydrostatic pressures. Number of biochemical studies show that TMAO stabilizes protein structure and counteracts the effects of urea and other denaturants.<sup>154–156</sup> It may be speculated that TMAO is accumulated as a mechanism of adaptation to hydrostatic pressure or water-electrolyte imbalances which occur in cardiovascular diseases.

### TMA, a toxic precursor of TMAO

On the other hand, cardiovascular effects of TMA, a TMAO precursor, have not been sufficiently studied yet. It is rather surprising because natural occurrence of TMA has been recognized for a long time and its toxic properties have been described in medical literature already in 19th century. Nowadays it is mainly known as a compound responsible for fish odor syndrome i.e. trimethylaminuria. Genetic FMO3 deficiency causes TMA accumulation in the body fluids inducing the characteristic smell of

urine, breath, and sweat in patients suffering from this disease.<sup>157</sup>

TMA is a pollutant widely present in industry, and several guidelines with exposure limit are available, e.g., the Recommendation from the Scientific Committee on Occupational Exposure Limits.<sup>158</sup> Numerous studies show toxic effect of TMA at relatively low concentrations.<sup>159–162</sup> TMA causes eye<sup>160</sup> and skin<sup>163</sup> irritation, developmental toxicity,<sup>161</sup> epilepsy and behavioral disorders<sup>164–166</sup> and promotes hepatic encephalopathy.<sup>167</sup> Strikingly, to the best of our knowledge, there is no research evaluating the association between TMA and cardiovascular risk. Unfortunately, clinical studies focused on TMAO did not assess TMA concentration but it may be speculated that increased TMAO levels were accompanied by increased level of its precursor. Possibly, it is TMA and not TMAO that contributes to increased mortality. There is some evidence to support this view. Increased TMA level has been observed in cardiovascular patients<sup>149,168</sup> and kidney deterioration seems to play a key role in these changes as demonstrated by a significant inverse correlation between level of TMA and eGFR. It must be stressed that TMA has been considered a uremic toxin for almost 40 years<sup>169,170</sup> contributing to neurotoxicity and uremic breath in patients with end-stage renal disease.<sup>171</sup> Moreover, as demonstrated by Srinivasa *et al.*, TMA and not TMAO is associated with atherosclerotic plaque formation in HIV patients.<sup>133</sup> Recently, we have found that TMA and not TMAO elevates blood pressure, exerts cytotoxic effect on cardiomyocytes and vascular smooth muscle cells as well as disturbs LDH and albumin structure.<sup>149,151,172</sup> It is also worth noting that most of experimental studies showing positive effects of reduction in TMAO level were associated with TMA decrease at the same time because they targeted precursors of TMA.<sup>173,174</sup>



**Figure 3.** Dietary sources, metabolism and elimination of trimethylamine (TMA) and trimethylamine N-oxide (TMAO) in humans. Suggested main cardiovascular and other effects of both methylamines based on currently available data (details in the text). FMO3: flavin-containing monooxygenase 3.



Formation and major biological effects of TMA and TMAO are depicted in Figure 3.

## Conclusions

Increasing experimental and clinical evidence suggests that gut microbiota-derived products affect the circulatory system functions. Both beneficial and toxic effects have been described. For instance, short chain fatty acids have been found to lower blood pressure. On the other hand, trimethylamine has been suggested to increase blood pressure and exert negative effect on the circulatory system. Hence, the bacteria products may have a therapeutic potential in cardiovascular diseases. Further studies are needed to establish the role of gut microbiota products in the circulatory system homeostasis.

**Authors' contributions:** All authors have contributed to the authorship of this review article.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

This work was supported by the National Science Centre, Poland grant no. UMO-2016/22/E/NZ5/00647.

## ORCID ID

Marcin Ufnal  <https://orcid.org/0000-0003-0088-8284>

## REFERENCES

- Lai GC, Tan TG, Pavelka N. The mammalian mycobiome: a complex system in a dynamic relationship with the host. *Wiley Interdiscip Rev Syst Biol Med* 2019;11:e1438
- Walker WA. The importance of appropriate initial bacterial colonization of the intestine in newborn, child, and adult health. *Pediatr Res* 2017;82:387–95
- Graf D, Di Cagno R, Fak F, Flint HJ, Nyman M, Saarela M, Watzl B. Contribution of diet to the composition of the human gut microbiota. *Microb Ecol Health Dis* 2015;26:26164
- Ursell LK, Clemente JC, Rideout JR, Gevers D, Caporaso JG, Knight R. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. *J Allergy Clin Immunol* 2012;129:1204–8
- Bibbo S, Ianiro G, Giorgio V, Scadaferri F, Masucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. *Eur Rev Med Pharmacol Sci* 2016;20:4742–9
- Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;14:e1002533
- Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010;90:859–904
- Ho NT, Li F, Lee-Sarwar KA, Tun HM, Brown BP, Pannaraj PS, Bender JM, Azad MB, Thompson AL, Weiss ST, Azcarate-Peril MA, Litonjua AA, Kozyrskyj AL, Jaspan HB, Aldrovandi GM, Kuhn L. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun* 2018;9:4169
- Stinson LF, Payne MS, Keelan JA. A critical review of the bacterial baptism hypothesis and the impact of cesarean delivery on the infant microbiome. *Front Med (Lausanne)* 2018;5:135
- van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V. Shaping the gut microbiota by breastfeeding: the gateway to allergy prevention? *Front Pediatr* 2019;7:47
- Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggianno GAD, Gasbarrini A, Mele MC. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* 2019;7:e14
- Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977;31:107–33
- Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018;57:1–24
- Martin G, Kolida S, Marchesi JR, Want E, Sidaway JE, Swann JR. In vitro modeling of bile acid processing by the human fecal microbiota. *Front Microbiol* 2018;9:1153
- Hill MJ. Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev* 1997;6:S43–5
- Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Front Nutr* 2019;6:48
- Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* 2018;23:716–24
- Vince A, Dawson AM, Park N, O'Grady F. Ammonia production by intestinal bacteria. *Gut* 1973;14:171–7
- Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. *Microbiome* 2017;5:54
- Jaglin M, Rhimi M, Philippe C, Pons N, Bruneau A, Goustard B, Dauge V, Maguin E, Naudon L, Rabot S. Indole, a signaling molecule produced by the gut microbiota, negatively impacts emotional behaviors in rats. *Front Neurosci* 2018;12:216
- Tomasova L, Konopelski P, Ufnal M. Gut bacteria and hydrogen sulfide: the new old players in circulatory system homeostasis. *Molecules* 2016;21:e1558
- Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 2016;165:1332–45
- Nowinski A, Ufnal M. Trimethylamine N-oxide: a harmful, protective or diagnostic marker in lifestyle diseases? *Nutrition* 2018;46:7–12
- Zhu X, Han Y, Du J, Liu R, Jin K, Yi W. Microbiota-gut-brain axis and the central nervous system. *Oncotarget* 2017;8:53829–38
- Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S, Joe B. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol Genomics* 2015;47:187–97
- Ahmadmehrabi S, Tang W. Gut microbiome and its role in cardiovascular diseases. *Curr Opin Cardiol* 2017;32:761–6
- Cui X, Ye L, Li J, Jin L, Wang W, Li S, Bao M, Wu S, Li L, Geng B, Zhou X, Zhang J, Cai J. Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients. *Sci Rep* 2018;8:635
- Kamo T, Akazawa H, Suda W, Saga-Kamo A, Shimizu Y, Yagi H, Liu Q, Nomura S, Naito AT, Takeda N, Harada M, Toko H, Kumagai H, Ikeda Y, Takimoto E, Suzuki JI, Honda K, Morita H, Hattori M, Komuro I. Dysbiosis and compositional alterations with aging in the gut microbiota of patients with heart failure. *PLoS One* 2017;12:e0174099
- Hutsch T, Pham K, Skrzypecki J, Ufnal M. Significance of gut-blood barrier in health and disease. *Eur J Biol Res* 2016;6:193–200
- Jovancevic N, Dendorfer A, Matzkies M, Kovarova M, Heckmann JC, Osterloh M, Boehm M, Weber L, Nguemo F, Semmler J, Hescheler J, Milting H, Schleicher E, Gelis L, Hatt H. Medium-chain fatty acids modulate myocardial function via a cardiac odorant receptor. *Basic Res Cardiol* 2017;112:13
- Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JI, Eichmann A, Peti-Peterdi J, Caplan MJ. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin



- secretion and blood pressure regulation. *Proc Natl Acad Sci USA* 2013;**110**:4410–5
32. Chen YY, Chen DQ, Chen L, Liu JR, Vaziri ND, Guo Y, Zhao YY. Microbiome-metabolome reveals the contribution of gut-kidney axis on kidney disease. *J Transl Med* 2019;**17**:5
  33. Hobby GP, Karaduta O, Dusio GF, Singh M, Zybailev BL, Arthur JM. Chronic kidney disease and the gut microbiome. *Am J Physiol Renal Physiol* 2019;**316**:F1211–17
  34. Ji Y, Yin Y, Li Z, Zhang W. Gut microbiota-derived components and metabolites in the progression of non-alcoholic fatty liver disease (NAFLD). *Nutrients* 2019;**11**:e1712
  35. Konturek PC, Harsch IA, Konturek K, Schink M, Konturek T, Neurath MF, Zopf Y. Gut(-)liver axis: how do gut bacteria influence the liver? *Med Sci (Basel)* 2018;**6**:e79
  36. Safari Z, Gerard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci* 2019;**76**:1541–58
  37. Wang L, Wan Y-JY. The role of gut microbiota in liver disease development and treatment. *Liver Res* 2019;**3**:3–18
  38. Nowinski A, Ufnal M. Gut bacteria-derived molecules as mediators and markers in cardiovascular diseases. The role of the gut-blood barrier. *Kardiologia Pol* 2018;**76**:320–7
  39. Ufnal M, Pham K. The gut-blood barrier permeability – a new marker in cardiovascular and metabolic diseases? *Med Hypotheses* 2017;**98**:35–7
  40. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Lobaton GO, Stewart DC, Rubiano A, Simmons CS, Garcia-Pereira F, Johnson RD, Pepine CJ, Raizada MK. Hypertension-linked pathophysiological alterations in the gut. *Circ Res* 2017;**120**:312–23
  41. Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol* 2017;**11**:821–34
  42. Kuehn BM. Gut microbes role in heart failure explored. *Circulation* 2019;**140**:1217–8
  43. Jaworska K, Huc T, Samborowska E, Dobrowolski L, Bielinska K, Gawlak M, Ufnal M. Hypertension in rats is associated with an increased permeability of the colon to TMA, a gut bacteria metabolite. *PLoS One* 2017;**12**:e0189310
  44. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;**54**:2325–40
  45. Pluznick JL. Microbial short-chain fatty acids and blood pressure regulation. *Curr Hypertens Rep* 2017;**19**:25
  46. Hoverstad T, Midtvedt T. Short-chain fatty acids in germfree mice and rats. *J Nutr* 1986;**116**:1772–6
  47. McNabney SM, Henagan TM. Short chain fatty acids in the colon and peripheral tissues: a focus on butyrate, Colon cancer, obesity and insulin resistance. *Nutrients* 2017;**9**:e1348
  48. Alexander C, Swanson KS, Fahey GC, Garleb KA. Perspective: physiologic importance of short-chain fatty acids from nondigestible carbohydrate fermentation. *Adv Nutr* 2019;**10**:576–89
  49. Scheppach W. Effects of short chain fatty acids on gut morphology and function. *Gut* 1994;**35**:S35–8
  50. Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, Kobayashi M, Hirasawa A, Tsujimoto G. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci USA* 2011;**108**:8030–5
  51. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem* 2003;**278**:25481–9
  52. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemo-attractant receptor GPR43. *Nature* 2009;**461**:1282–6
  53. Pluznick J. A novel SCFA receptor, the microbiota, and blood pressure regulation. *Gut Microbes* 2014;**5**:202–7
  54. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JL. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA* 2008;**105**:16767–72
  55. Bauer W, Richards DW. A vasodilator action of acetates. *J Physiol (Lond)* 1928;**66**:371–8
  56. Daugirdas JT, Nawab ZM. Acetate relaxation of isolated vascular smooth muscle. *Kidney Int* 1987;**32**:39–46
  57. Hulsman WC. Coronary vasodilation by fatty acids. *Basic Res Cardiol* 1976;**71**:179–91
  58. Nutting CW, Islam S, Daugirdas JT. Vasorelaxant effects of short chain fatty acid salts in rat caudal artery. *Am J Physiol* 1991;**261**:H561–7
  59. Kristev A, Mitkov D, Lukanov J. Influence of short-chain fatty acids on vascular tone. *Int J Exp Pathol* 1991;**72**:475–80
  60. Onyszkiewicz M, Gawrys-Kopczynska M, Konopelski P, Aleksandrowicz M, Sawicka A, Kozniwska E, Samborowska E, Ufnal M. Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via Colon-vagus nerve signaling and GPR41/43 receptors. *Pugers Arch* 2019;**471**:1441–53
  61. Natarajan N, Pluznick JL. Olfaction in the kidney: ‘smelling’ gut microbial metabolites. *Exp Physiol* 2016;**101**:478–81
  62. Wang L, Zhu Q, Lu A, Liu X, Zhang L, Xu C, Liu X, Li H, Yang T. Sodium butyrate suppresses angiotensin II-induced hypertension by inhibition of renal (pro)renin receptor and intrarenal renin-angiotensin system. *J Hypertens* 2017;**35**:1899–908
  63. Mosienko V, Chang AJ, Alenina N, Teschemacher AG, Kasparov S. Rodents and humans are able to detect the odour of L-Lactate. *PLoS One* 2017;**12**:e0178478
  64. Johnston CS, Gaas CA. Vinegar: medicinal uses and antiglycemic effect. *Med Gen Med* 2006;**8**:61
  65. Bhattacharya S. Wound healing through the ages. *Indian J Plast Surg* 2012;**45**:177–9
  66. Kishi M, Fukaya M, Tsukamoto Y, Nagasawa T, Takehana K, Nishizawa N. Enhancing effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats. *Biosci Biotechnol Biochem* 1999;**63**:905–10
  67. Mitrou P, Petsiou E, Papakonstantinou E, Maratou E, Lambadiari V, Dimitriadis P, Spanoudi F, Raptis SA, Dimitriadis G. The role of acetic acid on glucose uptake and blood flow rates in the skeletal muscle in humans with impaired glucose tolerance. *Eur J Clin Nutr* 2015;**69**:734–9
  68. Mitrou P, Petsiou E, Papakonstantinou E, Maratou E, Lambadiari V, Dimitriadis P, Spanoudi F, Raptis SA, Dimitriadis G. Vinegar consumption increases insulin-stimulated glucose uptake by the forearm muscle in humans with type 2 diabetes. *J Diabetes Res* 2015;**2015**:175204
  69. White AM, Johnston CS. Vinegar ingestion at bedtime moderates waking glucose concentrations in adults with well-controlled type 2 diabetes. *Diabetes Care* 2007;**30**:2814–5
  70. Kondo S, Tayama K, Tsukamoto Y, Ikeda K, Yamori Y. Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. *Biosci Biotechnol Biochem* 2001;**65**:2690–4
  71. Na L, Chu X, Jiang S, Li C, Li G, He Y, Liu Y, Li Y, Sun C. Vinegar decreases blood pressure by down-regulating AT1R expression via the AMPK/PGC-1 $\alpha$ /PPAR $\gamma$  pathway in spontaneously hypertensive rats. *Eur J Nutr* 2016;**55**:1245–53
  72. Tsuzuki W, Kikuchi Y, Shinohara K, Suzuki T. Fluorometric assay of angiotensin I-converting enzyme inhibitory activity of vinegars. *J Food Sci Technol* 1992;**39**:188–92
  73. Nishikawa Y, Takata Y, Nagai Y, Mori T, Kawada T, Ishihara N. Antihypertensive effect of kurosu extract, a traditional vinegar produced from unpolished rice, in the SHR rats. *J Jap Soc Food Sci Technol* 2001;**48**:73–5
  74. Honsho S, Sugiyama A, Takahara A, Satoh Y, Nakamura Y, Hashimoto K. A red wine vinegar beverage can inhibit the renin-angiotensin system: experimental evidence in vivo. *Biol Pharm Bull* 2005;**28**:1208–10



75. Daugirdas JT, Swanson V, Islam S, Nutting C, Kim DD, Wang XA, Fiscus RR. Acetate causes endothelium-independent increases in cyclic AMP in rat caudal artery. *Am J Physiol* 1988;**255**:H1378–83
76. Mortensen FV, Nielsen H, Mulvany MJ, Hessov I. Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* 1990;**31**:1391–4
77. Sakakibara S, Murakami R, Takahashi M, Fushimi T, Murohara T, Kishi M, Kajimoto Y, Kitakaze M, Kaga T. Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric oxide synthase activity. *Biosci Biotechnol Biochem* 2010;**74**:1055–61
78. Aizawa Y, Ohmori T, Imai K, Nara Y, Matsuoka M, Hirasawa Y. Depressant action of acetate upon the human cardiovascular system. *Clin Nephrol* 1977;**8**:477–80
79. Miller FN, Nolph KD, Joshua IG, Wiegman DL, Harris PD, Andersen DB. Hyperosmolality, acetate, and lactate: dilatory factors during peritoneal dialysis. *Kidney Int* 1981;**20**:397–402
80. Mansell MA, Wing AJ. Acetate or bicarbonate for haemodialysis? *Br Med J (Clin Res Ed)* 1983;**287**:308–9
81. Piwowarek K, Lipinska E, Hac-Szymanczuk E, Kieliszek M, Scibisz I. *Propionibacterium* spp.-source of propionic acid, vitamin B12, and other metabolites important for the industry. *Appl Microbiol Biotechnol* 2018;**102**:515–38
82. Zhang C, Brandt MJ, Schwab C, Ganzle MG. Propionic acid production by cofermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. *Food Microbiol* 2010;**27**:390–5
83. Demigne C, Morand C, Levrat MA, Besson C, Moundras C, Remesy C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br J Nutr* 1995;**74**:209–19
84. Wright RS, Anderson JW, Bridges SR. Propionate inhibits hepatocyte lipid synthesis. *Proc Soc Exp Biol Med* 1990;**195**:26–9
85. Al-Lahham S, Rezaee F. Propionic acid counteracts the inflammation of human subcutaneous adipose tissue: a new avenue for drug development. *Daru* 2019;**27**:645–52
86. Han JH, Kim IS, Jung SH, Lee SG, Son HY, Myung CS. The effects of propionate and valerate on insulin responsiveness for glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes via G protein-coupled receptor 41. *PLoS One* 2014;**9**:e95268
87. Heimann E, Nyman M, Degerman E. Propionic acid and butyric acid inhibit lipolysis and de novo lipogenesis and increase insulin-stimulated glucose uptake in primary rat adipocytes. *Adipocyte* 2015;**4**:81–8
88. Al-Lahham S, Roelofsen H, Rezaee F, Weening D, Hoek A, Vonk R, Venema K. Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest* 2012;**42**:357–64
89. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 2003;**278**:11312–9
90. Liaw CW, Connolly DT. Sequence polymorphisms provide a common consensus sequence for GPR41 and GPR42. *DNA Cell Biol* 2009;**28**:555–60
91. Knock G, Psaroudakis D, Abbot S, Aaronson PI. Propionate-induced relaxation in rat mesenteric arteries: a role for endothelium-derived hyperpolarising factor. *J Physiol (Lond)* 2002;**538**:879–90
92. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, neuroepigenetics and the gut microbiome: can a high fiber diet improve brain health? *Neurosci Lett* 2016;**625**:56–63
93. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J Gastroenterol* 2011;**17**:1519–28
94. Donohoe DR, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol Cell* 2012;**48**:612–26
95. Goncalves P, Martel F. Butyrate and colorectal cancer: the role of butyrate transport. *Curr Drug Metab* 2013;**14**:994–1008
96. Wretling A. Effect of tributyrin on circulation and respiration. *Acta Physiol Scand* 1957;**40**:59–74
97. Aaronson PI, McKinnon W, Poston L. Mechanism of butyrate-induced vasorelaxation of rat mesenteric resistance artery. *Br J Pharmacol* 1996;**117**:365–71
98. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B, Pepine CJ, Raizada MK, Mohamadzadeh M. Gut dysbiosis is linked to hypertension. *Hypertension* 2015;**65**:1331–40
99. Gomez-Arango LF, Barrett HL, McIntyre HD, Callaway LK, Morrison M, Dekker Nitert M, Group ST. Increased systolic and diastolic blood pressure is associated with altered gut microbiota composition and butyrate production in early pregnancy. *Hypertension* 2016;**68**:974–81
100. Mathew OP, Ranganna K, Yatsu FM. Butyrate, an HDAC inhibitor, stimulates interplay between different posttranslational modifications of histone H3 and differently alters G1-specific cell cycle proteins in vascular smooth muscle cells. *Biomed Pharmacother* 2010;**64**:733–40
101. Zeisel SH, Warrier M. Trimethylamine N-oxide, the microbiome, and heart and kidney disease. *Annu Rev Nutr* 2017;**37**:157–81
102. Zheng L, Zheng J, Xie Y, Li Z, Guo X, Sun G, Sun Z, Xing F, Sun Y. Serum gut microbe-dependent trimethylamine N-oxide improves the prediction of future cardiovascular disease in a community-based general population. *Atherosclerosis* 2019;**280**:126–31
103. Salzano A, Israr MZ, Yazaki Y, Heaney LM, Kanagala P, Singh A, Arnold JR, Gulsin GS, Squire IB, McCann GP. Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFPEF study. *Eur J Prevent Cardiol* 2019;**20**:4787319870355
104. Tang WW, Wang Z, Shrestha K, Borowski AG, Wu Y, Troughton RW, Klein AL, Hazen SL. Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. *J Card Fail* 2015;**21**:91–6
105. Troseld M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, Aakhus S, Gude E, Bjorndal B, Halvorsen B, Karlsen TH, Aukrust P, Gullestad L, Berge RK, Yndestad A. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. *J Intern Med* 2015;**277**:717–26
106. Suzuki T, Heaney LM, Jones DJ, Ng LL. Trimethylamine N-oxide and risk stratification after acute myocardial infarction. *Clin Chem* 2017;**63**:420–8
107. Li XS, Obeid S, Klingenberg R, Gencer B, Mach F, Raber L, Windecker S, Rodondi N, Nanchen D, Muller O, Miranda MX, Matter CM, Wu Y, Li L, Wang Z, Alamri HS, Gogonea V, Chung YM, Tang WH, Hazen SL, Luscher TF. Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors. *Eur Heart J* 2017;**38**:814–24
108. Senthong V, Wang Z, Li Xinmin S, Fan Y, Wu Y, Wilson Tang WH, Hazen Stanley L. Intestinal microbiota-generated metabolite trimethylamine-N-oxide and 5-year mortality risk in stable coronary artery disease: the contributory role of intestinal microbiota in a COURAGE-like patient cohort. *J Am Heart Assoc* 2016;**5**:e002816
109. Senthong V, Wang Z, Fan Y, Wu Y, Hazen Stanley L, Tang W. Trimethylamine N-oxide and mortality risk in patients with peripheral artery disease. *J Am Heart Assoc* 2016;**5**:e004237
110. Haghikia A, Li XS, Liman TG, Bledau N, Schmidt D, Zimmermann F, Kränkel N, Widera C, Sonnenschein K, Haghikia A. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol* 2018;**38**:2225–35
111. Nie J, Xie L, Zhao B-X, Li Y, Qiu B, Zhu F, Li G-F, He M, Wang Y, Wang B. Serum trimethylamine N-oxide concentration is positively associated with first stroke in hypertensive patients. *Stroke* 2018;**49**:2021–8
112. Ge X, Zheng L, Zhuang R, Yu P, Xu Z, Liu G, Xi X, Zhou X, Fan H. The gut microbial metabolite trimethylamine N-Oxide and hypertension



- risk: a systematic review and dose-response Meta-analysis. *Adv Nutr* 2019;**pii:nmz064**
113. Sheng Z, Tan Y, Liu C, Zhou P, Li J, Zhou J, Chen R, Chen Y, Song L, Zhao H. Relation of circulating trimethylamine N-oxide with coronary atherosclerotic burden in patients with ST-segment elevation myocardial infarction. *Am J Cardiol* 2019;**123**:894–8
  114. Stubbs JR, House JA, Ocque AJ, Zhang S, Johnson C, Kimber C, Schmidt K, Gupta A, Wetmore JB, Nolin TD, Spertus JA, Yu AS. Serum trimethylamine-N-oxide is elevated in CKD and correlates with coronary atherosclerosis burden. *J Am Soc Nephrol* 2016;**27**:305–13
  115. Senthong V, Li XS, Hudec T, Coughlin J, Wu Y, Levison B, Wang Z, Hazen SL, Tang WH. Plasma trimethylamine N-oxide, a gut microbe-generated phosphatidylcholine metabolite, is associated with atherosclerotic burden. *J Am Coll Cardiol* 2016;**67**:2620–8
  116. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;**19**:576–85
  117. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, Sartor RB, McIntyre TM, Silverstein RL, Tang WH, DiDonato JA, Brown JM, Lusis AJ, Hazen SL. Gut microbial metabolite TMAO enhances platelet hyperactivity and thrombosis risk. *Cell* 2016;**165**:111–24
  118. Haissman JM, Knudsen A, Hoel H, Kjaer A, Kristoffersen US, Berge RK, Katzenstein TL, Svardsdal A, Ueland T, Aukrust P, Lebech AM, Nielsen SD, Troseld M. Microbiota-dependent marker TMAO is elevated in silent ischemia but is not associated with first-time myocardial infarction in HIV infection. *J Acquir Immune Defic Syndr* 2016;**71**:130–6
  119. Kaysen GA, Johansen KL, Chertow GM, Dalrymple LS, Kornak J, Grimes B, Dwyer T, Chassy AW, Fiehn O. Associations of trimethylamine N-oxide with nutritional and inflammatory biomarkers and cardiovascular outcomes in patients new to dialysis. *J Ren Nutr* 2015;**25**:351–6
  120. Stubbs JR, Stedman MR, Liu S, Long J, Franchetti Y, West RE, Prokopenko AJ, Mahnken JD, Chertow GM, Nolin TD. Trimethylamine N-oxide and cardiovascular outcomes in patients with ESKD receiving maintenance hemodialysis. *Clin J Am Soc Nephrol* 2019;**14**:261–7
  121. Yin J, Liao SX, He Y, Wang S, Xia GH, Liu FT, Zhu JJ, You C, Chen Q, Zhou L. Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. *J Am Heart Assoc* 2015;**4**:e002699
  122. Gruppen EG, Garcia E, Connelly MA, Jeyarajah EJ, Otvos JD, Bakker SJ, Dullaart RP. TMAO is associated with mortality: impact of modestly impaired renal function. *Sci Rep* 2017;**7**:13781
  123. Mueller DM, Allenspach M, Othman A, Saelly CH, Muendlein A, Vonbank A, Drexel H, von Eckardstein A. Plasma levels of trimethylamine-N-oxide are confounded by impaired kidney function and poor metabolic control. *Atherosclerosis* 2015;**243**:638–44
  124. Winther SA, Ollgaard JC, Tofte N, Tarnow L, Wang Z, Ahluwalia TS, Jorsal A, Theilade S, Parving HH, Hansen TW, Hazen SL, Pedersen O, Rossing P. Utility of plasma concentration of trimethylamine N-oxide in predicting cardiovascular and renal complications in individuals with type 1 diabetes. *Diabetes Care* 2019;**42**:1512–20
  125. Tang WW, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatista-Boyle B, Li XS, Levison BS, Hazen SL. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* 2015;**116**:448–55
  126. Kim RB, Morse BL, Djurdjev O, Tang M, Muirhead N, Barrett B, Holmes DT, Madore F, Clase CM, Rigatto C, Levin A, Can PI. Advanced chronic kidney disease populations have elevated trimethylamine N-oxide levels associated with increased cardiovascular events. *Kidney Int* 2016;**89**:1144–52
  127. Missailidis C, Hällqvist J, Qureshi AR, Barany P, Heimbürger O, Lindholm B, Stenvinkel P, Bergman P. Serum trimethylamine-N-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. *PLoS One* 2016;**11**:e0141738
  128. Nnane IP, Damani LA. Pharmacokinetics of trimethylamine in rats, including the effects of a synthetic diet. *Xenobiotica* 2001;**31**:749–55
  129. Jannmohamed A, Hernandez D, Phillips IR, Shephard EA. Cell-, tissue-, sex- and developmental stage-specific expression of mouse flavin-containing monooxygenases (FMOs). *Biochem Pharmacol* 2004;**68**:73–83
  130. Lattard V, Lachuer J, Buronfosse T, Garnier F, Benoit E. Physiological factors affecting the expression of FMO1 and FMO3 in the rat liver and kidney. *Biochem Pharmacol* 2002;**63**:1453–64
  131. Shafi T, Powe NR, Meyer TW, Hwang S, Hai X, Melamed ML, Banerjee T, Coresh J, Hostetter TH. Trimethylamine N-oxide and cardiovascular events in hemodialysis patients. *J Am Soc Nephrol* 2017;**28**:321–31
  132. Koupenova M, Mick E, Mikhalev E, Benjamin EJ, Tanriverdi K, Freedman JE. Sex differences in platelet toll-like receptors and their association with cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 2015;**35**:1030–7
  133. Srinivasa S, Fitch KV, Lo J, Kadar H, Knight R, Wong K, Abbara S, Gauguier D, Capeau J, Boccara F, Grinspoon SK. Plaque burden in HIV-infected patients is associated with serum intestinal microbiota-generated trimethylamine. *AIDS* 2015;**29**:443–52
  134. Fukami K, Yamagishi S, Sakai K, Kaida Y, Yokoro M, Ueda S, Wada Y, Takeuchi M, Shimizu M, Yamazaki H, Okuda S. Oral L-carnitine supplementation increases trimethylamine-N-oxide but reduces markers of vascular injury in hemodialysis patients. *J Cardiovasc Pharmacol* 2015;**65**:289–95
  135. Meyer KA, Benton TZ, Bennett BJ, Jacobs DR, Lloyd-Jones DM, Gross MD, Carr JJ, Gordon-Larsen P, Zeisel SH. Microbiota-dependent metabolite trimethylamine N-oxide and coronary artery calcium in the coronary artery risk development in young adults study (CARDIA). *J Am Heart Assoc* 2016;**5**:e003970
  136. Geng J, Yang C, Wang B, Zhang X, Hu T, Gu Y, Li J. Trimethylamine N-oxide promotes atherosclerosis via CD36-dependent MAPK/JNK pathway. *Biomed Pharmacother* 2018;**97**:941–7
  137. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;**472**:57–63
  138. Chen ML, Yi L, Zhang Y, Zhou X, Ran L, Yang J, Zhu JD, Zhang QY, Mi MT. Resveratrol attenuates trimethylamine-N-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. *Mbio* 2016;**7**:e02210–15
  139. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, Org E, Wu Y, Li L, Smith JD, Tang WHW, DiDonato JA, Lusis AJ, Hazen SL. Gamma-butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. *Cell Metab* 2014;**20**:799–812
  140. Chou R-H, Chen C-Y, Chen IC, Huang H-L, Lu Y-W, Kuo C-S, Chang C-C, Huang P-H, Chen J-W, Lin S-J. Trimethylamine N-oxide, circulating endothelial progenitor cells, and endothelial function in patients with stable angina. *Sci Rep* 2019;**9**:4249
  141. Aldana-Hernández P, Leonard K-A, Zhao Y-Y, Curtis JM, Field CJ, Jacobs RL. Dietary choline or trimethylamine N-oxide supplementation does not influence atherosclerosis development in *ldlr*<sup>-/-</sup> and *apoE*<sup>-/-</sup> male mice. *J Nutr* 2019; <https://doi.org/10.1093/jn/nxz214>
  142. Collins HL, Drazul-Schrader D, Sulpizio AC, Koster PD, Williamson Y, Adelman SJ, Owen K, Sanli T, Bellamanna A. L-carnitine intake and high trimethylamine N-oxide plasma levels correlate with low aortic lesions in *ApoE*<sup>-/-</sup> transgenic mice expressing CETP. *Atherosclerosis* 2016;**244**:29–37
  143. Organ CL, Otsuka H, Bhushan S, Wang Z, Bradley J, Trivedi R, Polhemus DJ, Tang WW, Wu Y, Hazen SL. Choline diet and its gut microbe-derived metabolite, trimethylamine N-oxide, exacerbate pressure overload-induced heart failure. *Circ Heart Fail* 2016;**9**:e002314
  144. Li X, Sun Y, Zhang X, Wang J. Reductions in gut microbiota derived metabolite trimethylamine N oxide in the circulation may ameliorate



- myocardial infarction induced heart failure in rats, possibly by inhibiting interleukin 8 secretion. *Mol Med Rep* 2019;**20**:779–86
145. Yang W, Zhang S, Zhu J, Jiang H, Jia D, Ou T, Qi Z, Zou Y, Qian J, Sun A. Gut microbe-derived metabolite trimethylamine N-oxide accelerates fibroblast-myofibroblast differentiation and induces cardiac fibrosis. *J Mol Cell Cardiol* 2019;**134**:119–30
  146. Li Z, Wu Z, Yan J, Liu H, Liu Q, Deng Y, Ou C, Chen M. Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis. *Lab Invest* 2019;**99**:346–57
  147. Makrecka-Kuka M, Volska K, Antone U, Vilskersts R, Grinberga S, Bandere D, Liepinsh E, Dambrova M. Trimethylamine N-oxide impairs pyruvate and fatty acid oxidation in cardiac mitochondria. *Toxicol Lett* 2017;**267**:32–8
  148. Querio G, Antoniotti S, Levi R, Gallo MP. Trimethylamine N-oxide does not impact viability, ROS production, and mitochondrial membrane potential of adult rat cardiomyocytes. *Int J Mol Sci* 2019;**20**:3045
  149. Jaworska K, Hering D, Mosieniak G, Bielak-Zmijewska A, Pilz M, Konwerski M, Gasecka A, Kapon-Cieślicka A, Filipiak K, Sikora E, Hoyst R, Ufnal M. TMA, a forgotten uremic toxin, but not TMAO, is involved in cardiovascular pathology. *Toxins* 2019;**11**:490
  150. Ke Y, Li D, Zhao M, Liu C, Liu J, Zeng A, Shi X, Cheng S, Pan B, Zheng L. Gut flora-dependent metabolite trimethylamine-N-oxide accelerates endothelial cell senescence and vascular aging through oxidative stress. *Free Radic Biol Med* 2018;**116**:88–100
  151. Jaworska K, Konop M, Hutsch T, Perlejewski K, Radkowski M, Grochowska M, Bielak-Zmijewska A, Mosieniak G, Sikora E, Ufnal M. TMA but not TMAO increases with age in rat plasma and affects smooth muscle cells viability. *J Gerontol A Biol Sci Med Sci* 2019;**pii**: glz181
  152. Cho CE, Taesuwan S, Malysheva OV, Bender E, Tulchinsky NF, Yan J, Sutter JL, Caudill MA. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: a randomized controlled trial. *Mol Nutr Food Res* 2017;**61**:e1600324
  153. Krüger R, Merz B, Rist MJ, Ferrario PG, Bub A, Kulling SE, Watzl B. Associations of current diet with plasma and urine TMAO in the KarMeN study: direct and indirect contributions. *Mol Nutr Food Res* 2017;**61**:1700363
  154. Ma J, Pazos IM, Gai F. Microscopic insights into the protein-stabilizing effect of trimethylamine N-oxide (TMAO). *Proc Natl Acad Sci USA* 2014;**111**:8476–81
  155. Venkatesu P, Lee M-J, Lin H-M. Trimethylamine N-oxide counteracts the denaturing effects of urea or GdnHCl on protein denatured state. *Arch Biochem Biophys* 2007;**466**:106–15
  156. Zou Q, Bennion BJ, Daggett V, Murphy KP. The molecular mechanism of stabilization of proteins by TMAO and its ability to counteract the effects of urea. *J Am Chem Soc* 2002;**124**:1192–202
  157. Mitchell S, Smith R. Trimethylaminuria: the fish malodor syndrome. *Drug Metab Dispos* 2001;**29**:517–21
  158. Pospischil E, Johanson G, Nielsen GD, Papametiou D, Klein CL. Recommendation from the Scientific Committee on Occupational Exposure Limits. Luxembourg: Publications Office of the European Union, 2017. DOI: 10.2767/440659
  159. Brieger H, Hodes WA. Toxic effects of exposure to vapors of aliphatic amines. *AMA Arch Ind Hyg Occup Med* 1951;**3**:287–91
  160. Friemann W, Overhoff W, Wolter JR. [Eye diseases in the fishing industry]. *Arch Gewerbepathol Gewerbehyg* 1959;**17**:1–56
  161. Guest I, Varma DR. Selective growth inhibition of the male progeny of mice treated with trimethylamine during pregnancy. *Can J Physiol Pharmacol* 1993;**71**:185–7
  162. Kinney L, Burgess B, Chen H, Kennedy G. Inhalation toxicology of trimethylamine. *Inhal Toxicol* 1990;**2**:41–51
  163. Fluhr JW, Kelterer D, Fuchs S, Kaatz M, Grieshaber R, Kleesz P, Elsner P. Additive impairment of the barrier function and irritation by biogenic amines and sodium lauryl sulphate: a controlled in vivo tandem irritation study. *Skin Pharmacol Physiol* 2005;**18**:88–97
  164. Gajda Z, Gyengesi E, Hermes E, Ali KS, Szente M. Involvement of gap junctions in the manifestation and control of the duration of seizures in rats in vivo. *Epilepsia* 2003;**44**:1596–600
  165. McConnell H, Mitchell S, Smith R, Brewster M. Trimethylaminuria associated with seizures and behavioural disturbance: a case report. *Seizure* 1997;**6**:317–21
  166. Pellicciari A, Posar A, Cremonini MA, Parmeggiani A. Epilepsy and trimethylaminuria: a new case report and literature review. *Brain Dev* 2011;**33**:593–6
  167. Marks R, Dudley F, Wan A. Trimethylamine metabolism in liver disease. *Lancet* 1978;**1**:1106–7
  168. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorn Dahl TC, Bouatra S, Anderson T, Oudit GY, Wishart DS, Dyck JR, Alberta H. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PLoS One* 2015;**10**:e0124844
  169. Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, Argiles A, European Uremic Toxin Work G. Normal and pathologic concentrations of uremic toxins. *J Am Soc Nephrol* 2012;**23**:1258–70
  170. Wills MR, Savory J. Biochemistry of renal failure. *Ann Clin Lab Sci* 1981;**11**:292–9
  171. Simenhoff ML, Ginn HE, Teschan PE. Toxicity of aliphatic amines in uremia. *Trans Am Soc Artif Intern Organs* 1977;**23**:560–5
  172. Jaworska K, Bielinska K, Gawrys-Kopczynska M, Ufnal M. TMA (trimethylamine), but not its oxide TMAO (trimethylamine-oxide), exerts haemodynamic effects: implications for interpretation of cardiovascular actions of gut microbiome. *Cardiovasc Res* 2019;**115**:1948–9
  173. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, Wagner MA, Bennett BJ, Li L, DiDonato JA. Transmission of atherosclerosis susceptibility with gut microbial transplantation. *J Biol Chem* 2015;**290**:5647–60
  174. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 2015;**163**:1585–95



## PUBLIKACJA nr 2

*Butyric acid, a gut bacteria metabolite, lowers  
arterial blood pressure via colon-vagus nerve  
signaling and GPR41/43 receptors*

Maksymilian Onyszkiewicz

Marta Gawryś-Kopczyńska

Piotr Konopelski

Marta Aleksandrowicz

Aneta Sawicka

Ewa Koźniewska

Emilia Samborowska

Marcin Ufnal



# Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors

Maksymilian Onyszkiewicz<sup>1</sup> · Marta Gawrys-Kopczynska<sup>1</sup> · Piotr Konopelski<sup>1</sup> · Marta Aleksandrowicz<sup>2</sup> · Aneta Sawicka<sup>2</sup> · Ewa Koźniewska<sup>2</sup> · Emilia Samborowska<sup>3</sup> · Marcin Ufnal<sup>1</sup>

Received: 2 August 2019 / Revised: 26 September 2019 / Accepted: 7 October 2019 / Published online: 15 November 2019  
© The Author(s) 2019

## Abstract

Butyric acid (BA) is a short-chain fatty acid (SCFA) produced by gut bacteria in the colon. We hypothesized that colon-derived BA may affect hemodynamics. Arterial blood pressure (BP) and heart rate (HR) were recorded in anesthetized, male, 14-week-old Wistar rats. A vehicle, BA, or 3-hydroxybutyrate, an antagonist of SCFA receptors GPR41/43 (ANT) were administered intravenously (IV) or into the colon (IC). Reactivity of mesenteric (MA) and gracilis muscle (GMA) arteries was tested ex vivo. The concentration of BA in stools, urine, portal, and systemic blood was measured with liquid chromatography coupled with mass spectrometry. BA administered IV decreased BP with no significant effect on HR. The ANT reduced, whereas L-NAME, a nitric oxide synthase inhibitor, did not affect the hypotensive effect of BA. In comparison to BA administered intravenously, BA administered into the colon produced a significantly longer decrease in BP and a decrease in HR, which was associated with a 2–3-fold increase in BA colon content. Subphrenic vagotomy and IC pretreatment with the ANT significantly reduced the hypotensive effect. Ex vivo, BA dilated MA and GMA. In conclusion, an increase in the concentration of BA in the colon produces a significant hypotensive effect which depends on the afferent colonic vagus nerve signaling and GPR41/43 receptors. BA seems to be one of mediators between gut microbiota and the circulatory system.

**Keywords** Butyric acid · Blood pressure · SCFA · Bacterial metabolites

## Abbreviations

|     |                                                            |
|-----|------------------------------------------------------------|
| ACH | Acetylcholine                                              |
| ANT | 3-hydroxybutyrate an antagonist of SCFA receptors GPR41/43 |
| BA  | Butyric acid                                               |
| BP  | Arterial blood pressure                                    |

|          |                                                                                    |
|----------|------------------------------------------------------------------------------------|
| BSA      | Dialyzed bovine serum albumin                                                      |
| EDC·HCl  | <i>N</i> -(3-dimethylaminopropyl)- <i>N</i> '-ethylcarbodiimide                    |
| GMA      | Gracilis muscle artery                                                             |
| HR       | Heart rate                                                                         |
| IC       | Into the colon                                                                     |
| LOQ      | Limits of quantification                                                           |
| MA       | Mesenteric artery                                                                  |
| MOPS-PSS | Physiological saline buffered with 3-( <i>N</i> -morpholino) propane sulfonic acid |
| PE       | Phenylephrine                                                                      |
| SCFA     | Short-chain fatty acid                                                             |

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00424-019-02322-y>) contains supplementary material, which is available to authorized users.

✉ Marcin Ufnal  
mufnal@wum.edu.pl

<sup>1</sup> Department of Experimental Physiology and Pathophysiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland

<sup>2</sup> Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

<sup>3</sup> Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

## Introduction

Ample evidence suggests that molecules produced by gut microbiota such as short-chain fatty acids (SCFAs) exert a significant effect on mammalian homeostasis [4, 20]. SCFAs are

used by intestinal cells as an energy source. However, accumulating data imply that these molecules may also produce systemic effects [8, 19, 25, 29] acting via free fatty acid receptors GPR41, GPR43, and olfactory receptors 78 [6, 9, 14, 15, 23].

SCFAs include several carboxylic acids produced by bacterial fermentation of dietary fibers such as butyric acid (BA). There are some studies suggesting that BA affects arterial blood pressure. For example, a biphasic hemodynamic effect of intravenous administration of tributyrin, a BA prodrug, was described in 1957 by Wretling [33]. Recently, a hypotensive effect was reported after administration of BA into the kidney medulla [31] and intraperitoneally [25] in rats. However, those studies did not evaluate BA blood level and it is difficult to speculate whether the reported effects are of physiological, pharmacological, or suprapharmacological importance.

Notably, although BA is produced in the gut, the effects of BA on the gut-circulatory system axis have not been evaluated. The latter is important as ample research shows that gut signaling plays a significant role in interactions between gut microbiota and the host [5, 27].

Finally, the colon, a major site of bacterial metabolism, expresses receptors for SCFA [13, 24, 28].

Therefore, we evaluated whether BA may exert hemodynamic effects via gut signaling. Furthermore, we aimed to establish physiological concentrations of BA in the colon content (stools), portal blood, systemic blood, and urine in rats.

## Methods

### Animals

The experiments were performed according to Directive 2010/63 EU on the protection of animals used for scientific purposes and approved by the I Local Bioethical Committee in Warsaw (permission no 534/2018 and 535/2018). Experiments were performed on 14–16-week-old, male Wistar rats (Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland) fed a standard laboratory diet (Labofeed B standard, Kcynia, Poland), food and water ad libitum. Rats were housed in groups (3–4) in polypropylene cages with environmental enrichment, 12-h light/12-h dark cycle, temperature 22–23 °C, humidity 45–55%.

### Evaluation of BA levels in body fluids

Rats ( $n = 9$ ) were maintained for 2 days in metabolism cages to evaluate 24 h water and food balance and to collect urine for BA analysis. Data from the second day were analyzed. Next, rats were anaesthetized with 15% solution of urethane (i.p. 1.5 g/kg bw, Sigma-Aldrich,

Poznan, Poland) and were implanted with polyurethane catheters inserted into the portal vein and into the inferior vena cava as we previously described [10]. After the blood taking (0.25 ml each sample), rats were killed by decapitation. The evaluation of BA concentration was performed in portal and systemic blood plasma. A 7–8-cm long segment of the colon (a middle part between the cecum and the rectum) was closed with sutures and removed. A sample of 0.5 ml of stools was collected from the removed colon, weighted and homogenized with 1 ml of 0.9% NaCl in a closed 2-ml laboratory tube by vortexing it for 5 min. Afterwards, the sample was centrifuged for 5 min at 5000 rpm, and 1 ml of the obtained supernatant was transferred to a laboratory tube and again centrifuged for 5 min. All procedures were performed at the temperature of 2–5 °C. The supernatant was collected into Eppendorf tubes and frozen at  $-20$  °C. BA concentration in the colon content was calculated as BA concentration in the supernatant multiplied by a factor of 3 (as described above, 1 ml of saline was added to 0.5 ml of colon content to prepare supernatant for analysis).

### Hemodynamic studies

The measurements were performed under general anesthesia with 15% solution of urethane (i.p. 1.5 g/kg b.w, Sigma-Aldrich, Poznan, Poland). Before the measurements, rats were implanted with a polyurethane arterial catheter which was inserted through the femoral artery into the abdominal aorta and connected to the BP recording system, BIOPAC MP160 (Biopac Systems, Goleta, USA). For intravenous treatment, a catheter was implanted into the femoral vein. For electrocardiogram (ECG) recordings, standard needle electrodes were used (Biopac).

The measurements were started 40 min after the induction of anesthesia, and 10 min after connecting the arterial and venous catheters.

Hemodynamic studies comprised the following experimental series performed on separate groups of rats:

#### Intravenous administrations

1. Intravenous administration of a vehicle (saline 0.2 ml/2 min,  $n = 5$ ), saline solution of BA at a dose of 0.14 ( $n = 5$ ), 1.4 ( $n = 5$ ), 2.8 ( $n = 5$ ) and 5.6 mmol/kg ( $n = 5$ ), and ANT at a dose of 5.6 mmol/kg ( $n = 5$ ).
2. Intravenous administration of BA at a dose of 1.4 mmol/kg after the pretreatment with the ANT at a dose of 1.4 mmol/kg ( $n = 5$ ).
3. Intravenous administration of BA at a dose of 1.4 mmol/kg after the pretreatment with L-NAME, a non-



specific nitric oxide synthase inhibitor, at a dose of 0.3 mmol/kg ( $n = 5$ ), and the administration of L-NAME alone ( $n = 5$ ).

### Administrations into the colon

1. Administration of a vehicle (saline, 0.25 ml/30s,  $n = 5$ ), saline solution of BA (Sodium butyrate, Sigma-Aldrich, Poznan, Poland) at a dose of 1.4 ( $n = 5$ ), 2.8 ( $n = 5$ ) and 5.6 mmol/kg ( $n = 5$ ) and ANT at a dose of 5.6 mmol/kg ( $n = 5$ ).
2. Administration of BA at a dose of 5.6 mmol/kg after the pretreatment with the ANT (3-hydroxybutyrate, Sigma-Aldrich, Poznan, Poland) at a dose of 5.6 mmol/kg ( $n = 5$ ).
3. Administration of BA at a dose of 5.6 mmol/kg after the subphrenical vagotomy ( $n = 5$ ), administration of BA at a dose of 5.6 mmol/kg after the sham procedure ( $n = 5$ ) and vagotomy alone ( $n = 5$ ).
4. Administration of BA at a dose of 5.6 mmol/kg during the intravenous treatment with hexamethonium, an autonomic ganglia blocker (Sigma-Aldrich, Poznan, Poland, 15 mg/kg bolus followed by continuous infusion at a rate of 1.5 mg/kg/min,  $n = 5$ ) or after the intravenous treatment with atropine (Atropinum Sulfuricum WZF, Polfa Warszawa, Warsaw, Poland) at a dose of 1 mg/kg ( $n = 5$ ).

After hemodynamic studies, rats were killed by decapitation.

### Vagotomy

The surgical vagotomy, i.e., bilateral abdominal (subdiaphragmatic) truncal vagotomy, was performed as previously described [2] with some modification. In short, after cutting skin and muscles from the xiphoid to the navel, the liver was stabilized with ligatures to visualize the subdiaphragmatic vagus nerves. Saline solution of methylene blue (0.4%) was applied on tissues for better visualization of nerves. The nerves were cut with vascular scissors. After the procedure, the wound was stitched. The sham procedure followed the same steps except that the nerves were not cut.

It needs to be mentioned that in contrast to humans, in rats, all the regions of the colon, except the rectum, are innervated by the branches of the vagus nerve [3].

### Intracolonic administrations

The intracolonic infusions were performed by means of a pediatric Foley catheter (10F) inserted into the colon, 8 cm from the anus as we previously described [10].

### Changes in BA concentration in stools, portal, and systemic blood after the intracolonic (IC) administration of BA

BA concentration in the colon content (stools) was evaluated in control rats ( $n = 5$ ) and rats administered with BA into the colon at a dose of 2.8 mmol/kg ( $n = 6$ ), as we described above. To evaluate portal and systemic blood plasma concentration of BA, blood samples (0.25 ml) from the portal vein and vena cava were collected at baseline and 20 and 60 min after the IC administration of BA at a dose of 2.8 mmol/kg ( $n = 6$ ). The selected time points corresponded to the maximal hypotensive effect and return of BP to baseline according to our findings from the hemodynamic part of the study.

### Ex vivo reactivity studies

#### Isolation of mesenteric (MA) and gracilis muscle arteries (GMA)

Rats ( $n = 11$ ) were anesthetized with an intraperitoneal injection of 15% solution of urethane (1.5 g/kg b.w.). The mesenteric and gracilis muscle arterial bed was dissected and placed in a petri dish filled with cold (4 °C, pH = 7.4) physiological saline buffered with MOPS (3-(*N*-morpholino) propanesulfonic acid) (MOPS-PSS) containing: 3.0 mM MOPS, 144.0 mM NaCl, 3.0 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.5 mM MgSO<sub>4</sub>, 1.21 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.02 mM EDTA, 2.0 mM sodium pyruvate, 5.0 mM glucose, and 1% dialyzed bovine serum albumin (BSA). The branches of the mesenteric artery (MA, 250–370 µm diameter) and gracilis muscle artery (GMA, 225–280 µm diameter) were carefully cleaned of surrounding tissues under a dissecting microscope (SZ51, Olympus, Germany) and transferred to an organ chamber. After cannulation of one end of the vessel, the blood from the lumen was gently removed, and then the other end of the vessel was mounted on the distal pipette, and both ends were secured with a 10–0 nylon suture. The organ chamber was placed on the stage of an inverted microscope (CKX41, Olympus, Germany) equipped with a video camera and a monitor. The transmural pressure was set at 50 mmHg for the MA and 80 mmHg for the GMA. The experiments were performed without intraluminal flow. The extraluminal fluid was switched to MOPS-PSS without BSA, slowly heated to 37 °C and exchanged at a rate of 20 mL/min with the help of a peristaltic pump (Masterflex, Cole-Parmer, USA).

### Experimental protocol

After 60 min equilibration at 37 °C, the arteries were pre-constricted with phenylephrine (PE, 0.5 µM), and after the



contraction reached a steady state, acetylcholine (ACh, 1  $\mu$ M) was added to MOPS-PSS. Arteries which relaxed in response to ACh by more than 90% were considered as endothelium intact vessels.

The responses to increasing concentrations of butyric acid (BA, starting from 5  $\mu$ M up to 1 mM) were studied in pre-constricted GMA and MA branches. The starting point was equal to a physiological blood concentration of BA in the rat.

In separate series of experiments, the ANT was administered in increasing concentrations equimolar to BA (from 5  $\mu$ M to 1 mM) with BA (5  $\mu$ M) in the background.

In conclusion of the experiments with ANT, the response to 1 mM BA was studied to verify whether ANT (1 mM) affects the vasorelaxant effect of BA at this concentration. Only one experimental protocol was carried out on the same vessel.

The effects of each concentration of the tested substances on the inner diameter of MA branches and GMA were assessed 15 min after their administration. At the end of each experiment, the MOPS-PSS bath solution was replaced with  $\text{Ca}^{2+}$ -free PSS (PSS containing 3 mM EGTA) in which the vessels were incubated for 15 min to determine maximal passive diameter.

All values are expressed as means  $\pm$  SE. Vasodilatation, as percent of the maximal diameter, was calculated based on a formula  $(D_{\text{active}} - D_{\text{baseline}})/(D_{\text{passive}} - D_{\text{baseline}}) \times 100\%$ , where  $D_{\text{active}}$  is the measured diameter for a given dose of the tested compound,  $D_{\text{baseline}}$  is the baseline diameter measured before administration of the drug, and  $D_{\text{passive}}$  is the maximal passive diameter.

## BA concentration analysis

BA concentration analysis was performed using Waters Acquity Ultra Performance Liquid Chromatograph

**Table 1** Basic metabolic parameters, concentration of butyric acid (BA) in the colon content, portal blood, systemic blood plasma, urine ( $\mu$ M), and daily urine excretion ( $\mu$ mol/24 h) of BA in Wistar rats ( $n = 9$ ). Presented data are means  $\pm$  SE

|                                         |                   |
|-----------------------------------------|-------------------|
| General metabolic data                  |                   |
| Body weight (g)                         | 314.8 $\pm$ 25.2  |
| Food intake (g)                         | 24.3 $\pm$ 2.6    |
| Water intake (ml)                       | 35.6 $\pm$ 4.3    |
| 24-h urine output (ml)                  | 18.8 $\pm$ 5.0    |
| Stools output (g)                       | 10.3 $\pm$ 2.1    |
| BA concentrations/excretion             |                   |
| Colon content ( $\mu$ M)                | 7,992 $\pm$ 445   |
| Portal blood ( $\mu$ M)                 | 129 $\pm$ 37      |
| Systemic blood ( $\mu$ M)               | 4.25 $\pm$ 1.26   |
| Urine ( $\mu$ M)                        | 15.6 $\pm$ 3.89   |
| Daily urine excretion ( $\mu$ mol/24 h) | 0.294 $\pm$ 0.073 |

**Table 2** Baseline mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats/min) in Wistar rats. Means  $\pm$  SE are presented

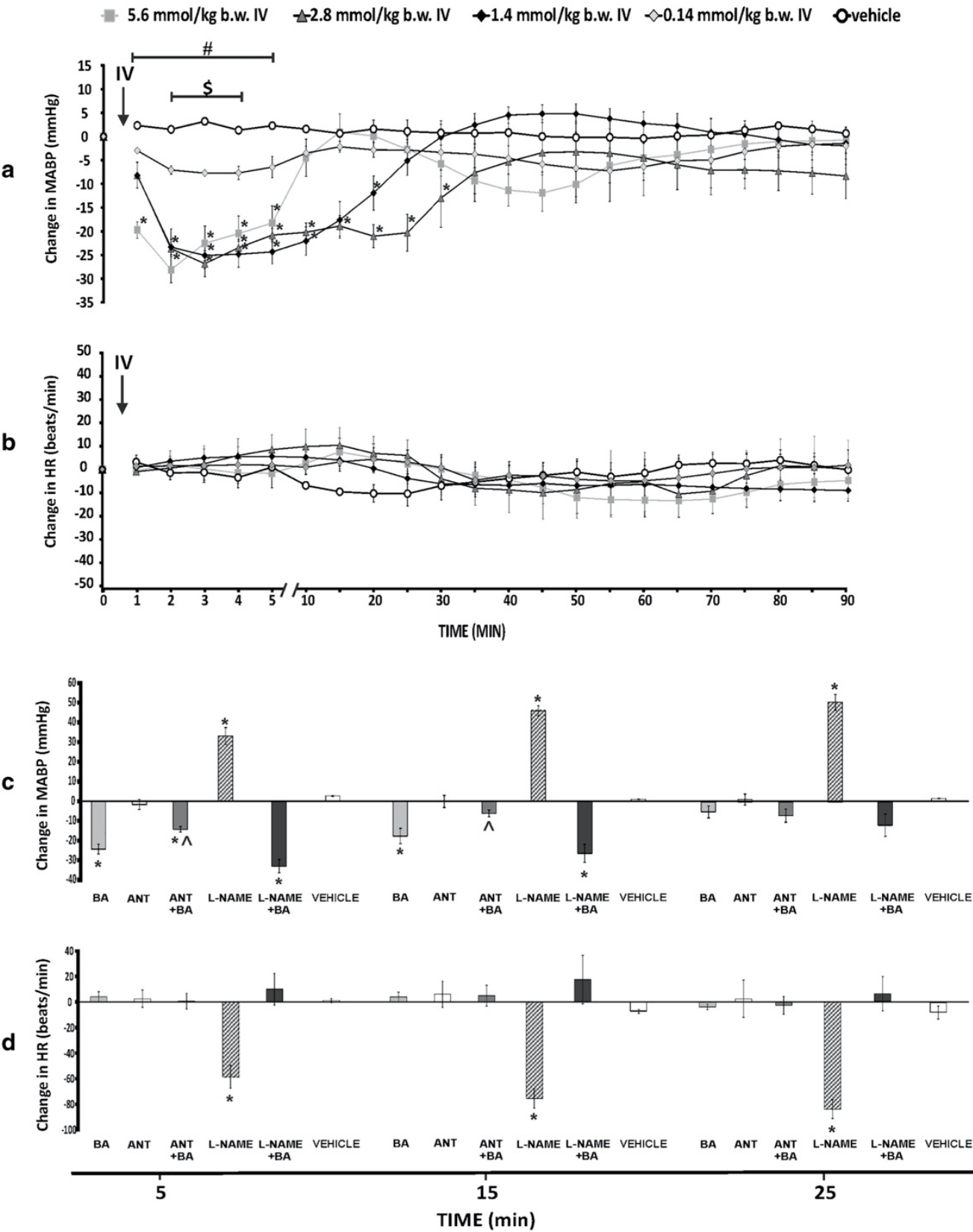
| Series          | MABP            | HR           |
|-----------------|-----------------|--------------|
| Vehicle         | 100.5 $\pm$ 1.3 | 364 $\pm$ 14 |
| ANT             | 101.8 $\pm$ 2.1 | 346 $\pm$ 11 |
| BA 5.6 mmol/kg  | 100.9 $\pm$ 5.2 | 351 $\pm$ 11 |
| BA 2.8 mmol/kg  | 103.2 $\pm$ 0.5 | 331 $\pm$ 16 |
| BA 1.4 mmol/kg  | 95.2 $\pm$ 4.7  | 336 $\pm$ 20 |
| BA 0.14 mmol/kg | 107.2 $\pm$ 3.2 | 385 $\pm$ 12 |
| BA + ANT        | 101.1 $\pm$ 1.3 | 362 $\pm$ 11 |
| BA + L-NAME     | 99.4 $\pm$ 7.3  | 371 $\pm$ 11 |
| L-NAME          | 99.5 $\pm$ 5.6  | 349 $\pm$ 17 |

coupled with Waters TQ-S triple-quadrupole mass spectrometer. For the instrument control and data acquisition, Waters MassLynx software was used. Waters TargetLynx was used to processed data. LC/MS/MS analysis was performed in negative electrospray ionization mode (ESI). The mass spectrometer operated in multiple-reaction monitoring (MRM). The analytes were separated using a Waters BEH C18 column (1.7  $\mu$ m, 2.1 mm  $\times$  50 mm) and Waters BEH C18 guard column (1.7  $\mu$ m, 2.1 mm  $\times$  5 mm). Mobile phase A consisted of 1 mL of formic acid in 1 L water, and mobile phase B consisted of 1 mL of formic acid in acetonitrile. The flow rate of mobile phase was set at 0.6 mL/min.

Sample preparation was as follows: 80  $\mu$ L methanol (containing internal standards) was added to 40  $\mu$ L of sample (plasma, stool extract, urine and calibrators). After vortexing, 20  $\mu$ L of 3NPH solution and 20  $\mu$ L of EDC-pyridine solution were added and the mixture was incubated in room temperature for 30 min. The solution was diluted to 1 mL with 15% aqueous acetonitrile, centrifuged, and aliquot was injected into the apparatus.

To define the relationship between the concentration and detector response for analytes, calibration points were prepared. Calibration curves for BA were generated by

**Fig. 1** **a** Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and **b** heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the intravenous administration (IV) of either a vehicle (0.9% NaCl) or butyric acid (BA) at a dose of 0.14, 1.4, 2.8, and 5.6 mmol/kg; \* $p < 0.05$  vs baseline, # $p < 0.05$ : 1.4, 2.8, and 5.6 mmol/kg BA series vs the vehicle, \$ $p < 0.05$ : 0.14 mmol/kg BA series vs 2.8 and 5.6 mmol/kg BA series. c, d  $\Delta$ MABP and  $\Delta$ HR after the intravenous infusions of BA at a dose of 1.4 mmol/kg, (BA) or 3-hydroxybutyrate, a non-specific antagonist of GPR41/43 receptors at a dose of 1.4 mmol/kg (ANT), or BA after the pretreatment with ANT (ANT + BA) or a non-specific nitric oxide synthase inhibitor at a dose of 0.3 mmol/kg (L-NAME) or the administration of BA after the pretreatment with L-NAME (L-NAME + BA), or the vehicle. \* $p < 0.05$  vs baseline, ^ $p < 0.05$  vs BA series. Means  $\pm$  SE are presented



comparing a ratio of the peak area of the analyzed compound to the peak of the corresponding internal standard against

known analyte concentrations. The limits of quantification (LOQ) were 1  $\mu$ M for BA.



**Table 3** Baseline mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats/min) in Wistar rats. Means  $\pm$  SE are presented

| Series             | MABP                                        | HR                                    |
|--------------------|---------------------------------------------|---------------------------------------|
| Vehicle            | 98.4 $\pm$ 1.8                              | 350 $\pm$ 16                          |
| ANT                | 96.1 $\pm$ 1.9                              | 346 $\pm$ 13                          |
| BA 5.6 mmol/kg     | 102.3 $\pm$ 3.4                             | 352 $\pm$ 13                          |
| BA 2.8 mmol/kg     | 104.1 $\pm$ 1.4                             | 355 $\pm$ 14                          |
| BA 1.4 mmol/kg     | 98.9 $\pm$ 6.3                              | 343 $\pm$ 11                          |
| BA + ANT           | 99.3 $\pm$ 2.4                              | 321 $\pm$ 17                          |
| BA + vagotomy      | 98.6 $\pm$ 2.2                              | 357 $\pm$ 31                          |
| BA + sham vagotomy | 95.5 $\pm$ 4.3                              | 313 $\pm$ 37                          |
| Vagotomy           | 97.9 $\pm$ 5.4                              | 349 $\pm$ 21                          |
| BA + Hexamethonium | 100.3 $\pm$ 4.5/64.8 $\pm$ 2.4 <sup>a</sup> | 365 $\pm$ 16/268 $\pm$ 3 <sup>a</sup> |
| BA + Atropine      | 95.2 $\pm$ 2.6/90.7 $\pm$ 3.4 <sup>b</sup>  | 376 $\pm$ 8/365 $\pm$ 5 <sup>b</sup>  |

<sup>a</sup> After the pretreatment with hexamethonium but before administration of BA

<sup>b</sup> After the pretreatment with atropine but before administration of BA

## Chemicals

Pyridine anhydrous, 3-nitrophenylhydrazine (3NPH·HCl), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC·HCl) were acquired from Sigma-Aldrich (St. Louis, MO, USA). LC-MS grade acetonitrile, HPLC grade acetonitrile, HPLC grade methanol, and formic acid were obtained from J.T. Baker. Ultra-pure water (Mili-Q water) was produced by a water purification system (Mili-Q, Millipore, Milford, MA, USA).

Urethane, DMSO, BA, MOPS-PSS, BSA, ANT-3-hydroxybutyrate, hexamethonium, and all reagents used to study isolated vessels were purchased from Sigma-Aldrich, Poznan, Poland

## Data analysis and statistics

Mean arterial blood pressure (MABP) and heart rate (HR) were calculated on blood pressure tracing by Acq Knowledge software (Biopac Systems, Goleta, USA). To evaluate ECG, lead II was used. The length of QT was manually measured from the onset of QRS complex to the end of T wave. The average of 10 consecutive QT intervals was used for analysis. Corrected QT (QTc) was calculated according to the following formula:  $QTc = QT / (RR/f)^{1/2}$ , where *f* is the normalization factor according to the basal RR interval duration in rats (150 ms) [17]. For the evaluation of changes in hemodynamic parameters and SCFA blood level in response to the treatment, baseline values were compared with values after the treatment by means of one-way analysis of variance (ANOVA) for repeated measures, followed by Tukey's post hoc test. Differences between the groups/series were evaluated by multivariate ANOVA, followed by Tukey's post hoc test or by *t* test, when appropriate. The Kolmogorov-Smirnov test

**Fig. 2** **a** Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and **b** heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the intracolonic administration (IC) of either a vehicle (0.9% NaCl) or butyric acid (BA) at a dose of 1.4, 2.8, and 5.6 mmol/kg. \**p* < 0.05 vs baseline, #*p* < 0.05 - 2.8 and 5.6 mmol/kg BA series vs the vehicle. **c, d**  $\Delta$ MABP and  $\Delta$ HR after the IC administration of BA at a dose of 5.6 mmol/kg (BA), or 3-hydroxybutyrate, a non-specific antagonist of GPR41/43 receptors at a dose of 5.6 mmol/kg (ANT) or BA after the pretreatment with ANT (ANT + BA), or the administration of BA after the sham vagotomy (SHAM + BA) or the administration of BA after the vagotomy (VAG + BA), or the vehicle. \**p* < 0.05 vs baseline, ^*p* < 0.05 vs BA series, \$*p* < 0.05 vs SHAM + BA series. Means  $\pm$  SE are presented

was used to test normality of the distribution. A value of two-sided *p* < 0.05 was considered significant. Analyses were conducted using Dell Statistica, version 13 (Dell Inc, Tulsa, USA).

## Results

### Physiological levels of BA in body fluids in rats

Basic metabolic parameters and concentration of BA in the colon content, portal blood plasma, systemic blood plasma, and urine are presented in Table 1. The concentration of BA was the highest in the colon content  $\approx$  8 mM (Table 1). Portal blood concentration of BA was approximately two orders of magnitude lower whereas systemic blood concentration of BA was approximately three orders of magnitude lower than the concentration of BA in the colon content.

### BA administered intravenously produced a transient decrease in BP

There were no significant differences in hemodynamic parameters between experimental series at baseline (Table 2).

BA at a dose of 1.4, 2.8, and 5.8 mmol/kg produced a significant, transient decrease in MABP (Fig. 1). The hypotensive response was not associated with significant changes in HR (Fig. 1). Administration of the vehicle and ANT did not produce a significant change in MABP and HR (Fig. 1).

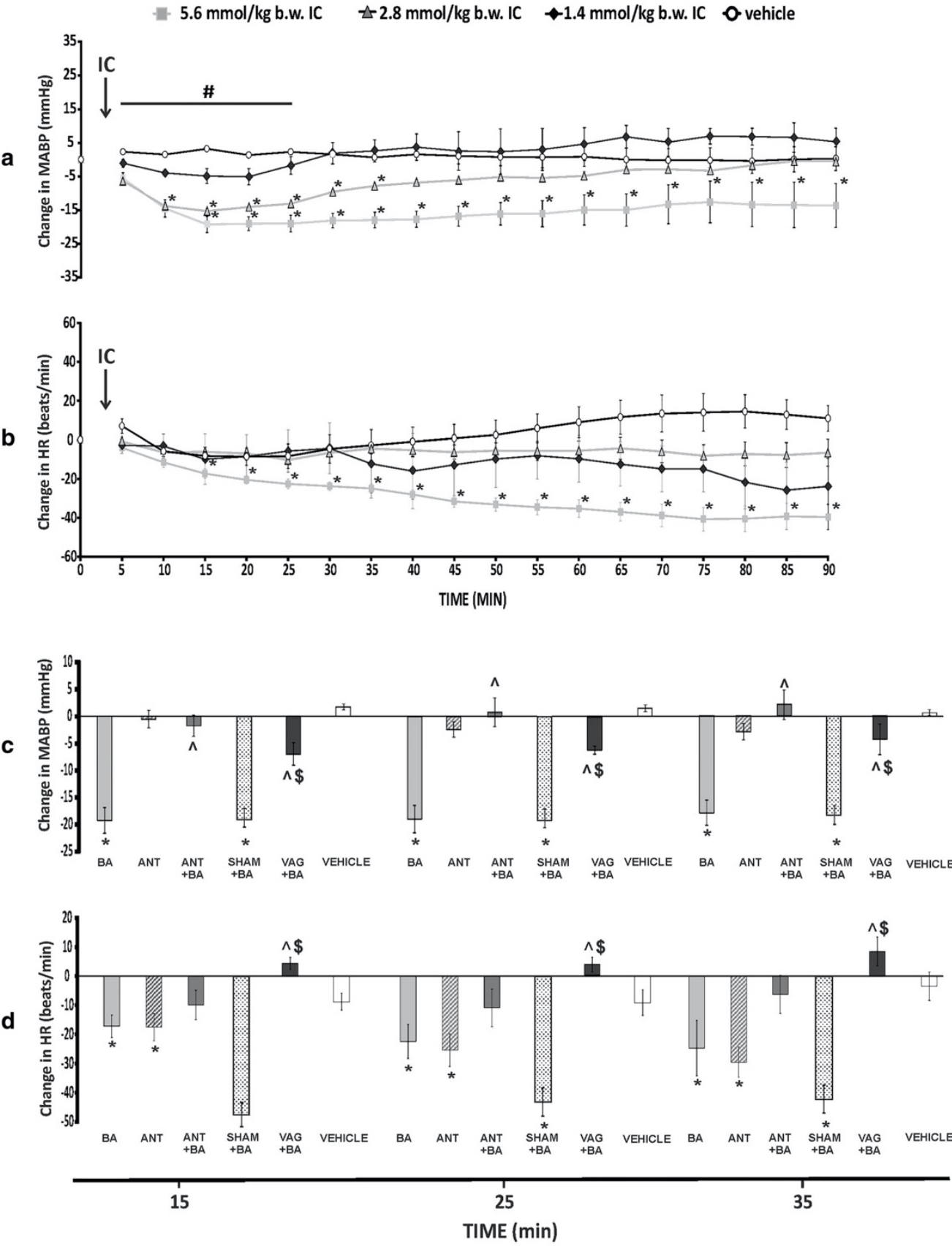
Pretreatment with L-NAME did not affect significantly hypotensive effect of BA, whereas pretreatment with ANT moderately reduced the hypotensive effect of BA (Fig. 1 and Figs. S1–S4).

### BA administered into the colon produced prolonged decrease in BP and HR

There were no significant differences in hemodynamic parameters between experimental series at baseline (Table 3).

BA administered IC produced a dose-dependent decrease in MABP, i.e., 1.4 mmol/kg series showed mild and not significant decrease, whereas 2.8 and 5.6 mmol series showed a significant decrease in MABP which lasted throughout the





**Table 4** Electrocardiographic parameters in rats ( $n = 5$ ) at baseline, 20 and 60 min after the intracolonic administration of BA (IC BA) at a dose of 5.6 mmol/kg. Means  $\pm$  SE are presented

| ECG parameters     | Baseline         | 20 min after IC BA | 60 min after IC BA |
|--------------------|------------------|--------------------|--------------------|
| RR (ms)            | 182.2 $\pm$ 14.6 | 229.3 $\pm$ 20.8*  | 210.5 $\pm$ 16.3*  |
| QT (ms)            | 89.1 $\pm$ 5.4   | 100.6 $\pm$ 3.2    | 102.2 $\pm$ 3.7    |
| QTc (ms)           | 80.9 $\pm$ 2.5   | 82.0 $\pm$ 3.7     | 86.7 $\pm$ 3.1     |
| PR (ms)            | 65.8 $\pm$ 2.2   | 66.2 $\pm$ 1.7     | 70.4 $\pm$ 0.8     |
| QRS width (ms)     | 17.7 $\pm$ 0.6   | 19.1 $\pm$ 0.9     | 18.2 $\pm$ 0.2     |
| QRS amplitude (mV) | 2.9 $\pm$ 0.3    | 2.8 $\pm$ 0.3      | 2.8 $\pm$ 0.3      |

\* $p < 0.05$  vs baseline

experiment (90 min) for 5.6 mmol/kg series (Fig. 2). This was associated with a significant decrease in HR in 5.6 mmol/kg series (Fig. 2), but no significant change in QTc, a marker of drug cardiotoxicity (Table 4).

The IC pretreatment with the ANT significantly reduced the hypotensive effect of BA (Fig. 2 and Fig. S9).

The hypotensive and bradycardic response to BA was significantly reduced by subphrenic vagotomy. Namely, vagotomized rats showed significantly smaller decrease in MABP and HR than sham series after the IC administration of BA (Fig. 2 and Figs. S11 and S12). Likewise, the pretreated with hexamethonium, the autonomic ganglia blocker inhibited the hypotensive response (Fig. S13). In contrast, the pretreatment with atropine, an antagonist of acetylcholine muscarinic receptors, did not affect significantly the hypotensive response to the IC administration of BA (Fig. S10).

The vehicle, the ANT, and vagotomy alone did not affect MABP significantly (Fig. 2 and Figs. S8 and S10). The ANT did not affect significantly MABP, but produced a decrease in HR (Fig. 2 and Fig. S8).

**Table 5** BA concentration in portal blood and systemic blood at baseline, 20 and 60 min after the intracolonic administration (IC) of BA at a dose of 2.8 mmol/kg ( $n = 6$ ). The selected time points correspond to the maximal hypotensive effect and return of BP to baseline according to our findings from the hemodynamic part of the study. Means  $\pm$  SE are presented

| BA ( $\mu$ M) concentration before (baseline) and after BA administration into the colon |                   |
|------------------------------------------------------------------------------------------|-------------------|
| Stools (colon content)                                                                   |                   |
| Colon content                                                                            | 9202 $\pm$ 1723   |
| 20 min after administration                                                              | 24,128 $\pm$ 2158 |
| Portal vein blood                                                                        |                   |
| Baseline                                                                                 | 96.9 $\pm$ 19.4   |
| 20 min after administration                                                              | 528.0 $\pm$ 75.2  |
| 60 min after administration                                                              | 296.9 $\pm$ 40.1  |
| Systemic vein blood                                                                      |                   |
| Baseline                                                                                 | 4.35 $\pm$ 0.42   |
| 20 min after administration                                                              | 107.5 $\pm$ 19.3  |
| 60 min after administration                                                              | 43.9 $\pm$ 6.6    |

## Changes in BA level in portal and systemic blood after administration of BA into the colon

Measurements of the concentration of BA in the colon content and in the portal and systemic blood showed that the lowest effective dose of BA which decreased arterial blood pressure increased colon content of BA by 2–3-fold, portal blood concentration of BA by 5-fold, and systemic blood concentration of BA by 20-fold (Table 5).

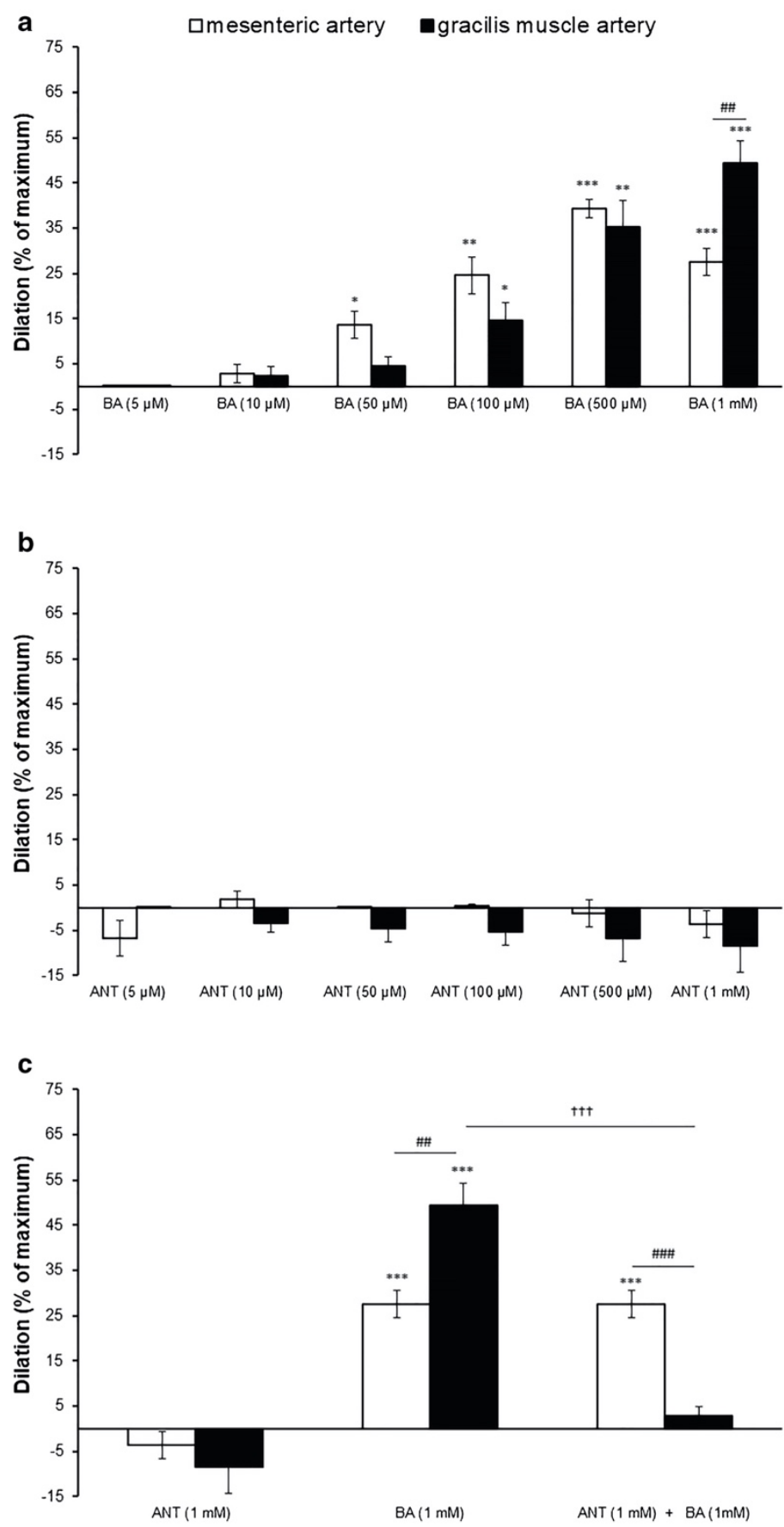
## BA dilated mesenteric and gracilis muscle arteries (ex vivo reactivity studies)

At 50 mmHg, the mean internal diameter of MA branches was 305  $\pm$  12  $\mu$ m ( $n = 10$ ) and at 80 mmHg, the mean internal diameter of GMA was 245  $\pm$  7  $\mu$ m ( $n = 10$ ). All arteries were pre-constricted with phenylephrine (0.5  $\mu$ M), which decreased vessels diameter by around 50%.

## Effect of BA on MA branches and GMA diameter

Administration of BA at a dose equal to the physiological concentration of this acid in the systemic blood (5  $\mu$ M) did not change the diameter of MA or GMA. A significant relaxation of MA by 14  $\pm$  3% was observed at the threshold concentration of 50  $\mu$ M. The response of the GMA to BA was shifted to the right, i.e., threshold concentration which resulted in increase of the GMA diameter by 15  $\pm$  4% amounted to 100

**Fig. 3** Response of the pre-constricted with PE mesenteric artery branches and gracilis muscle arteries to **a** increasing concentration of butyric acid (BA, from 5  $\mu$ M up to 1 mM,  $n = 10$ ); **b** increasing concentration of 3-hydroxybutyrate (ANT, from 5  $\mu$ M to 1 mM,  $n = 10$ ) administered concurrently with BA (5  $\mu$ M), and **c** the effect of 3-hydroxybutyrate (ANT, 1 mM) administered concurrently with BA on vasorelaxant responses of mesenteric artery branches ( $n = 5$ ) and gracilis muscle artery ( $n = 5$ ) to BA (1 mM). Dilation is expressed as a percentage of maximum diameter (0  $\text{Ca}^{2+}$ , EGTA 3 mM). Values are means  $\pm$  SE of  $n$  arteries. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ : a significant vasorelaxation; ### $p < 0.01$ , ### $p < 0.001$ : a significant difference between the MA vs GMA response to the BA or ANT; ††† $p < 0.001$  a significant effect of ANT (1 mM) on the vasodilation evoked by BA (1 mM)





$\mu\text{M}$ . There were no significant differences in the BA response between MA and GMA, except for the last tested dose (1 mM) at which GMA responded more strongly than MA ( $49 \pm 5\%$  vs  $28 \pm 3\%$ , respectively) (Fig. 3a).

### Effect of 3-hydroxybutyrate (ANT) on MA branches and GMA diameter

ANT regardless of the applied concentration did not affect the MA and GMA diameters (Fig. 3b). The ANT did not affect the vasorelaxant effect of BA (1 mM) in MA branches. In contrast, ANT abolished GMA relaxation produced by BA (Fig. 3c).

## Discussion

A new finding of our study is that BA at a dose which increases the concentration of BA in the colon by 2–3-fold exerts a significant hypotensive effect. The hypotensive effect seems to involve nervous control of arterial blood pressure including afferent colonic vagus nerve signaling and SCFA receptors GPR41/43.

Gut bacteria and their metabolites including hydrogen sulfide, indoles, and SCFA may influence the circulatory and the nervous system functions [10, 11, 14]. The mechanisms of such interaction are not clear. Nevertheless, the following two pathways are possible. Firstly, bacterial metabolites may stimulate sensory fibers of the enteric nervous system which communicate with the central nervous system [5]. Secondly, gut bacteria-derived molecules after entering the systemic circulation may reach virtually all organs involved in arterial blood pressure control.

Our study shows that physiological concentration of BA in the colon is three orders of magnitude higher than that in systemic blood, which makes the colon a very likely site of BA action. Furthermore, BA administered into the colon produced a significant hypotensive effect which was diminished by the subphrenic vagotomy and intracolonic pretreatment with a non-specific antagonist of GPR41/43. These findings suggest that the afferent arm of the hypotensive response involves colonic afferent vagus nerve signaling and GPR41/43 receptors. In this regard, the afferent fibers of the vagus nerve are known to modulate the brain centers involved in the control on the autonomic nervous system activity and blood pressure [7]. Notably, the hypotensive effect of BA administered into the colon was associated with only 2–3-fold increase in the colon concentration of BA, suggesting that such effect may be of physiological importance.

We would speculate that the hypotensive response to BA may be mediated by the inhibitory effect of BA on tonic sympathetic activity, as hexamethonium, an autonomic ganglia blocker, but not atropine, an antagonist of the acetylcholine receptors reduced the hypotensive effect of BA. The

hypotensive response may also depend on a direct vasodilatory action of BA. Namely, in ex vivo studies, we found that BA produced a significant, dose-dependent vasodilation in MA and GMA.

Interestingly, in GMA, the vasodilatory effect of BA was reduced by the ANT whereas the ANT did not affect significantly the BA-induced vasodilation in MA, suggesting that the mechanisms of relaxation to BA in these two vessels differ. Although both MA and GMA are resistance vessels, they differ in the structure of the internal elastic lamina [16] and in the response to TRP agonists [30]. Our findings suggest that BA-induced vasorelaxation is dependent on GPR41/43 receptors in GMA but not in MA. It seems that the vasorelaxant effect of BA in the latter may be mediated by other than GPR41/43 receptors or may be dependent on a direct relaxation of vascular smooth muscles as postulated by Aronson and collaborators [1]. Although the relaxation of various resistance blood vessels in response to other SCFA is well documented, the underlying mechanisms are complex and not fully elucidated. Previously, Mortensen et al. showed that acetic, propionic, and BA produce a concentration-dependent (0.1–30 mM) vasorelaxant effects in human colonic resistance arteries [20]. Also, a weak vasodilatory effect of BA at concentrations above 5 mM was shown in the coronary arteries [12]. The vasodilatory effect of SCFA was also demonstrated in rat caudal artery at 0.8 mM and 1.9 mM mean effective concentrations for butyrate and propionate, respectively [21]. Several mechanisms of vasodilatory activity of BA have been proposed including stimulation of the cyclic AMP second messenger system [1] and increased synthesis of F2 alpha prostaglandins [18].

In this study, we also found a significant, hypotensive response to intravenously administered BA. The hypotensive effect of intravenously administered BA was reduced by the ANT but not by the pretreatment with L-NAME. Previously, it has been shown that BA produces relaxation of rat mesenteric arteries, which was unaffected by endothelial denudation and inhibition of NO synthase with L-NAME [1]. Altogether, it seems that hypotensive effect of BA administered intravenously was produced by vasodilation independent on nitric oxide. Interestingly, the hypotensive effect of BA in the L-NAME-treated rats was similar or even higher than in rats treated with BA alone, which points to the strong vasodilatory and hypotensive actions of BA, and perhaps reduced sympathetic component of baroreflex in the L-NAME-treated rats [26].

Notably, the hypotensive response to BA administered intravenously was significantly shorter than the response to intracolonic administration and was not associated with HR changes. Taking together our findings, we would speculate that the hypotensive effect of intravenously administered BA was dependent on the direct BA-mediated vasodilation. In contrast, the hypotensive effect of BA administered into the colon involved also decreased sympathetic activity. The latter

was produced by the afferent vagus nerve signaling from the colon to the brain (Fig. 4).

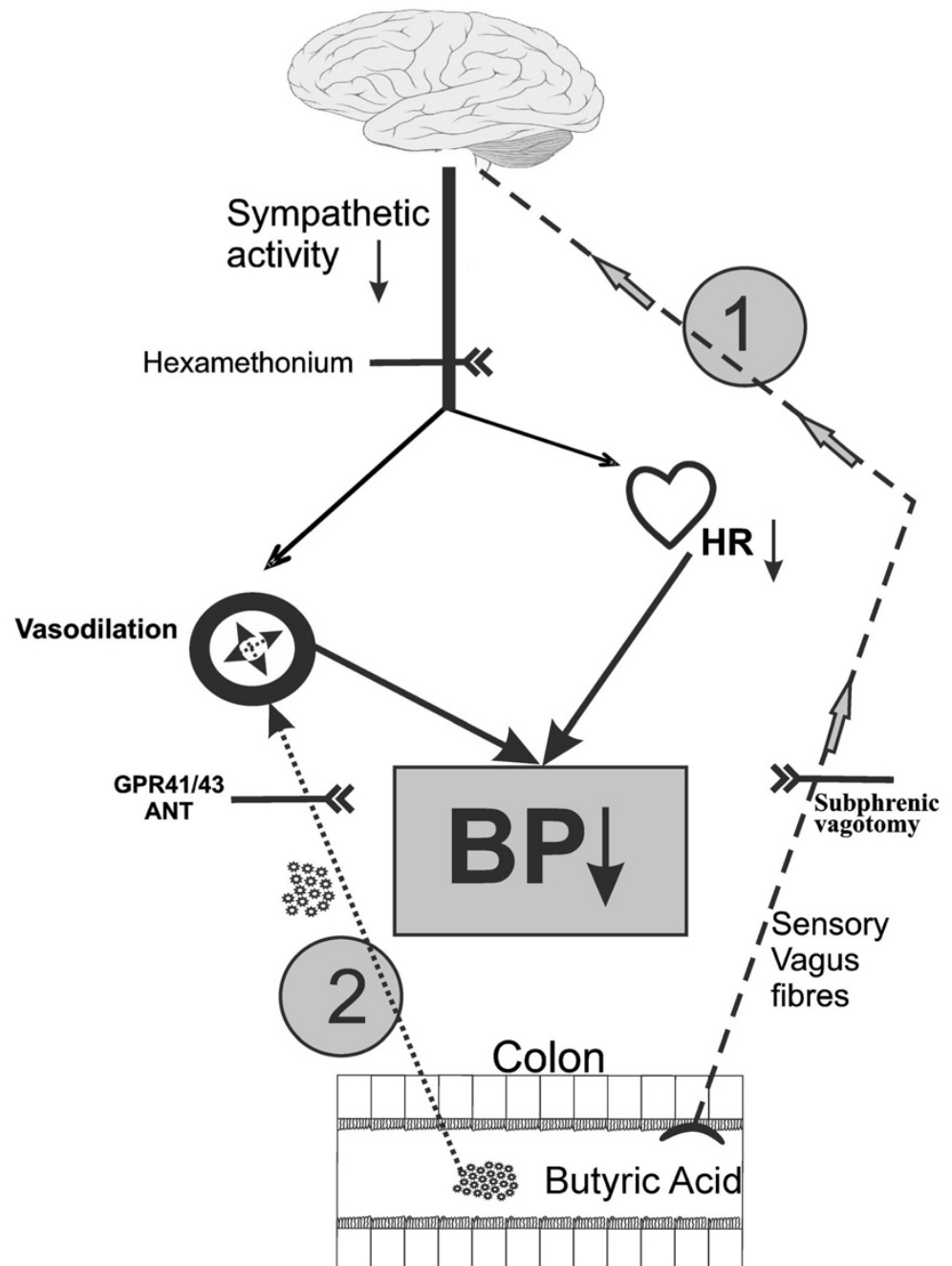
Other mechanisms that may be involved in the circulatory effects of BA but were not the focus of this study include the effect of BA on kidney [31, 32] and cardiac functions [22]. In particular, the effect of BA on diuresis needs further investigation.

A limitation of our study is that we did not evaluate the effect of Olfr78 receptor blockade, which is also thought to be involved in SCFA signaling. This is because biologically

effective Olfr78 receptor blockers are not yet available. Finally, chronic interventional studies are needed to assess the effect of colonic BA on systemic blood pressure.

In conclusion, an increase in the concentration of BA in the colon by 2–3-fold exerts a significant hypotensive effect, which seems to be mediated by the colon afferent nervous signaling and GPR41/43 receptors. It may also involve vasodilation caused by blood-borne BA. Our findings provide evidence that BA is one of the mediators between gut microbiota and the circulatory system.

**Fig. 4** Postulated mechanisms involved in the hypotensive effect of colon-derived butyric acid (BA). **1** BA stimulates the sensory fibers of the vagus nerve that project to the brain centers controlling the circulatory system. This results in decreased tonic sympathetic activity producing a decrease in arterial blood pressure due to a decrease in HR and vasodilation. **2** BA crosses the gut-blood barrier, enters the bloodstream, and produces a direct vasodilation





**Author's contribution** Conception and design of the work: MU and MO. Acquisition, analysis, and interpretation of hemodynamic data: MO, MG, PK, MU. Acquisition, analysis, and interpretation of spectrometry data: ES and MU. Acquisition, analysis, and interpretation of ex vivo experiments MA, AS, EK. Drafting the paper: MO, MA, ES, and MU. All the authors reviewed the manuscript and approved the final version.

**Funding information** This work was supported by the National Science Centre, Poland grant no. UMO-2016/22/E/NZ5/00647.

**Data availability** All data generated or analyzed during this study are included in this published article (and its additional information files).

**Compliance with ethical standards** The experiments were performed according to Directive 2010/63 EU on the protection of animals used for scientific purposes and approved by the I Local Bioethical Committee in Warsaw (permission no 534/2018 and 535/2018).

**Conflict of interest** The authors declare that they have no conflict of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

- Aaronson PI, McKinnon W, Poston L (1996) Mechanism of butyrate-induced vasorelaxation of rat mesenteric resistance artery. *Br J Pharmacol* 117:365–371. <https://doi.org/10.1111/j.1476-5381.1996.tb15200.x>
- Allen LG, Lawrence IE Jr, Burden HW, Hodson CA (1985) Effects of abdominal vagotomy on serum LH concentrations in female rats. *J Reprod Fertil* 74:87–94
- Altschuler SM, Escardo J, Lynn RB, Miselis RR (1993) The central organization of the vagus nerve innervating the colon of the rat. *Gastroenterology* 104:502–509
- Bauer W, Richards DW (1928) A vasodilator action of acetates. *J Physiol* 66:371–378. <https://doi.org/10.1113/jphysiol.1928.sp002534>
- Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF (2011) Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 108:16050–16055. <https://doi.org/10.1073/pnas.1102999108>
- Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ (2003) The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 278:11312–11319. <https://doi.org/10.1074/jbc.M211609200>
- Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, Polson JW, Potts PD, Tagawa T (2002) Central mechanisms underlying short- and long-term regulation of the cardiovascular system. *Clin Exp Pharmacol Physiol* 29:261–268
- den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 54:2325–2340. <https://doi.org/10.1194/jlr.R036012>
- Fleischer J, Bumbalo R, Bautze V, Strotmann J, Breer H (2015) Expression of odorant receptor Olfr78 in enteroendocrine cells of the colon. *Cell Tissue Res* 361:697–710. <https://doi.org/10.1007/s00441-015-2165-0>
- Huc T, Konop M, Onyszkiewicz M, Podsadni P, Szczepanska A, Turlo J, Ufnal M (2018) Colonic indole, gut bacteria metabolite of tryptophan, increases portal blood pressure in rats. *Am J Phys Regul Integr Comp Phys*. <https://doi.org/10.1152/ajpregu.00111.2018>
- Huc T, Nowinski A, Drapala A, Konopelski P, Ufnal M (2018) Indole and indoxyl sulfate, gut bacteria metabolites of tryptophan, change arterial blood pressure via peripheral and central mechanisms in rats. *Pharmacol Res* 130:172–179. <https://doi.org/10.1016/j.phrs.2017.12.025>
- Hulsmann WC (1976) Coronary vasodilation by fatty acids. *Basic Res Cardiol* 71:179–191
- Karaki S, Tazoe H, Hayashi H, Kashiwabara H, Tooyama K, Suzuki Y, Kuwahara A (2008) Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* 39:135–142. <https://doi.org/10.1007/s10735-007-9145-y>
- Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, Kobayashi M, Hirasawa A, Tsujimoto G (2011) Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A* 108:8030–8035. <https://doi.org/10.1073/pnas.1016088108>
- Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashiwara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, Tsujimoto G (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* 4:1829. <https://doi.org/10.1038/ncomms2852>
- Kirby BS, Bruhl A, Sullivan MN, Francis M, Dinunno FA, Earley S (2013) Robust internal elastic lamina fenestration in skeletal muscle arteries. *PLoS One* 8:e54849. <https://doi.org/10.1371/journal.pone.0054849>
- Kmecova J, Klimas J (2010) Heart rate correction of the QT duration in rats. *Eur J Pharmacol* 641:187–192. <https://doi.org/10.1016/j.ejphar.2010.05.038>
- Kristev A, Mitkov D, Lukanov Y (1987) The impact of butyric acid on the cardiovascular system. *Cor Vasa* 29:313–318
- Long X, Li M, Li LX, Sun YY, Zhang WX, Zhao DY, Li YQ (2018) Butyrate promotes visceral hypersensitivity in an IBS-like model via enteric glial cell-derived nerve growth factor. *Neurogastroenterol Motil* 30:e13227. <https://doi.org/10.1111/nmo.13227>
- Mortensen FV, Nielsen H, Mulvany MJ, Hessev I (1990) Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* 31:1391–1394. <https://doi.org/10.1136/gut.31.12.1391>
- Nutting CW, Islam S, Daugirdas JT (1991) Vasorelaxant effects of short chain fatty acid salts in rat caudal artery. *Am J Phys* 261:H561–H567. <https://doi.org/10.1152/ajpheart.1991.261.2.H561>
- Patel BM (2018) Sodium butyrate controls cardiac hypertrophy in experimental models of rats. *Cardiovasc Toxicol* 18:1–8. <https://doi.org/10.1007/s12012-017-9406-2>
- Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JI, Eichmann A, Peti-Peterdi J, Caplan MJ (2013) Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U S A* 110:4410–4415. <https://doi.org/10.1073/pnas.1215927110>
- Ran T, Liu Y, Jiao JZ, Zhou CS, Tang SX, Wang M, He ZX, Tan ZL, Yang WZ, Beauchemin KA (2018) Postnatal differential



- expression of chemoreceptors of free fatty acids along the gastrointestinal tract of supplemental feeding v. grazing kid goats. *Animal* 1–9. <https://doi.org/10.1017/S1751731118001581>
25. Skrzypecki J, Zera T, Ufnal M (2018) Butyrate, a gut bacterial metabolite, lowers intraocular pressure in normotensive, but not in hypertensive rats. *J Glaucoma*. <https://doi.org/10.1097/IJG.0000000000001025>
  26. Souza HC, De Araujo JE, Martins-Pinge MC, Cozza IC, Martins-Dias DP (2009) Nitric oxide synthesis blockade reduced the baro-reflex sensitivity in trained rats. *Auton Neurosci* 150:38–44. <https://doi.org/10.1016/j.autneu.2009.04.007>
  27. Tanida M, Takada M, Kato-Kataoka A, Kawai M, Miyazaki K, Shibamoto T (2016) Intragastric injection of *Lactobacillus casei* strain Shirota suppressed spleen sympathetic activation by central corticotrophin-releasing factor or peripheral 2-deoxy-d-glucose in anesthetized rats. *Neurosci Lett* 619:114–120. <https://doi.org/10.1016/j.neulet.2016.03.016>
  28. Tazoe H, Otomo Y, Karaki S, Kato I, Fukami Y, Terasaki M, Kuwahara A (2009) Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomed Res* 30:149–156
  29. Topping DL, Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 81:1031–1064. <https://doi.org/10.1152/physrev.2001.81.3.1031>
  30. Toth A, Czikora A, Pasztor ET, Dienes B, Bai P, Csemoch L, Rutkai I, Csato V, Manyine IS, Porszasz R, Edes I, Papp Z, Boczan J (2014) Vanilloid receptor-1 (TRPV1) expression and function in the vasculature of the rat. *J Histochem Cytochem* 62:129–144. <https://doi.org/10.1369/0022155413513589>
  31. Wang L, Zhu Q, Lu A, Liu X, Zhang L, Xu C, Liu X, Li H, Yang T (2017) Sodium butyrate suppresses angiotensin II-induced hypertension by inhibition of renal (pro)renin receptor and intrarenal renin-angiotensin system. *J Hypertens* 35:1899–1908. <https://doi.org/10.1097/HJH.0000000000001378>
  32. Wang S, Lv D, Jiang S, Jiang J, Liang M, Hou F, Chen Y (2019) Quantitative reduction in short-chain fatty acids, especially butyrate, contributes to the progression of chronic kidney disease. *Clin Sci (Lond)* 133:1857–1870. <https://doi.org/10.1042/CS20190171>
  33. Wretling A (1957) Effect of tributyrin on circulation and respiration. *Acta Physiol Scand* 40:59–74. <https://doi.org/10.1111/j.1748-1716.1957.tb01477.x>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## PUBLIKACJA nr 3

*Valeric acid lowers arterial blood pressure in rats*

Maksymilian Onyszkiewicz

Marta Gawryś-Kopczyńska

Maciej Sałagaj

Marta Aleksandrowicz

Aneta Sawicka

Ewa Koźniewska

Emilia Samborowska

Marcin Ufnal



## Full length article

## Valeric acid lowers arterial blood pressure in rats

Maksymilian Onyszkiewicz<sup>a</sup>, Marta Gawrys-Kopczynska<sup>a</sup>, Maciej Sałagaj<sup>a</sup>,  
Marta Aleksandrowicz<sup>b</sup>, Aneta Sawicka<sup>b</sup>, Ewa Koźniewska<sup>b</sup>, Emilia Samborowska<sup>c</sup>,  
Marcin Ufnal<sup>a,\*</sup>

<sup>a</sup> Department of Experimental Physiology and Pathophysiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland

<sup>b</sup> Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

<sup>c</sup> Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland



## ARTICLE INFO

## Keywords:

Valeric acid  
Blood pressure  
SCFA  
Bacterial metabolites

## ABSTRACT

Valeric acid (VA) is a short-chain fatty acid produced by microbiota and herbs such as *Valeriana officinalis*. Moreover, VA is released from medicines such as estradiol valerate by esterases. We evaluated the concentrations of endogenous VA in male, 14-week-old rats in the liver, heart, brain, kidneys, lungs, blood and in the colon, a major site of microbiota metabolism, using liquid chromatography coupled with mass spectrometry. In addition, the tissue distribution of VA D9-isotope (VA-D9) administered into the colon was assessed. Finally, we investigated the effect of exogenous VA on arterial blood pressure (BP) and heart rate (HR) in anesthetized rats, and the reactivity of mesenteric (MA) and gracilis muscle (GMA) arteries *ex vivo*. Physiological concentration of VA in the colon content was  $\approx 650 \mu\text{M}$ ,  $\approx 0.1\text{--}1 \mu\text{M}$  in the investigated tissues, and  $\approx 0.4 \mu\text{M}$  in systemic blood. VA-D9 was detected in the tissues 5 min after the administration into the colon. The vehicle did not affect BP and HR. VA produced a dose-dependent decrease in BP, and at higher doses lowered HR. The hypotensive effect of VA was inhibited by 3-hydroxybutyrate, an antagonist of GPR41/43-receptors but not by the subphrenic vagotomy. Hexamethonium prolonged the hypotensive effect of VA while atropine did not influence the hypotensive effect. VA dilated GMA and MA. In conclusion, the exogenous VA produces vasodilation and lowers BP. The colon-derived VA rapidly penetrates to tissues involved in the control of BP. Further studies are needed to evaluate the effects of endogenous and exogenous VA on the circulatory system.

## 1. Introduction

Valeric acid (VA), or pentanoic acid is present in *Valeriana officinalis* which is commonly used in herbal medicine as sedative, anxiolytic and blood pressure lowering agent (Caron and Riedlinger, 1999; Chevalier, 1996; Sellappan et al., 2010; Wichtl, 2004). Furthermore, valerate has been administered as estradiol valerate for many years for various indications showing a hypotensive effect (Hassager et al., 1987; Seely et al., 1999; Zacharieva et al., 2002). For example, oral administration of estradiol valerate in postmenopausal women lowered arterial blood pressure (Cannoletta and Cagnacci, 2014; Davis et al., 2019). Although the hypotensive effect is likely to be dependent on estradiol (Cannoletta and Cagnacci, 2014; Davis et al., 2019), the contribution of the released valerate to the hypotensive action of estradiol valerate cannot be excluded.

Finally, VA is one of short-chain fatty acids (SCFAs) produced by gut microbiota as a byproduct of the fermentation of dietary fiber (Adam

et al., 2014; Anderson and Bridges, 1984; Tap et al., 2015). Notably, there is ample evidence that SCFAs including acetic and butyric acid exert significant effect on arterial blood pressure by interacting with host G-protein-coupled receptors (GPCRs), such as Gpr41 and Olfr78 (Brown et al., 2003; Fleischer et al., 2015; Kimura et al., 2011, 2013; Pluznick et al., 2013; Skrzypecki et al., 2018). However, to the best of our knowledge, hemodynamic effects of VA have not yet been evaluated.

We hypothesized that VA may exert hypotensive effect via GPCRs receptors and/or gut-vagus nerve signaling as those pathways were previously described for other SCFAs.

## 2. Materials and methods

## 2.1. Animals

The experiments were performed according to Directive 2010/63

\* Corresponding author. Department of Experimental Physiology and Pathophysiology Medical University of Warsaw Banacha 1B, 02-097, Warsaw, Poland.  
E-mail address: [mufnal@wum.edu.pl](mailto:mufnal@wum.edu.pl) (M. Ufnal).

<https://doi.org/10.1016/j.ejphar.2020.173086>

Received 1 January 2020; Received in revised form 19 March 2020; Accepted 25 March 2020

Available online 29 March 2020

0014-2999/ © 2020 Elsevier B.V. All rights reserved.



EU on the protection of animals used for scientific purposes and approved by the I Local Bioethical Committee in Warsaw (permission no 103/2016 and 474/2017). Experiments were performed on 14–16-week-old, male Wistar rats (300–350 g), (Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland) fed a standard laboratory diet (Labofeed B standard, Kcynia, Poland), food and water ad libitum. Rats were housed in groups (3–4) in polypropylene cages with environmental enrichment, 12 h light/12 h dark cycle, temperature 22–23 °C, humidity 45–55%. Rats were randomly taken from cages for experiments, rats from one cage were assigned to different experimental series. However, there was no specific randomization method.

## 2.2. VA in body fluids and tissues

### 2.2.1. Evaluation of VA concentration in body fluids and tissues

Rats ( $n = 9$ ) were maintained for 2 days in metabolic cages to evaluate 24 h water and food balance and to collect urine for VA. Data from the second day were analyzed. Next, rats were anaesthetized with urethane (i.p. 1.5 g/kg b.w.) and were implanted with polyurethane catheters inserted into the portal vein and into the inferior vena cava as we previously described (Huc et al., 2018b). After the blood taking, rats were killed by decapitation. A 7–8 cm long segment of the colon (a middle part between the cecum and the rectum) was closed with sutures and removed. A sample of 0.5 ml of stools was collected from the removed colon, weighed and homogenized with 1 ml of 0.9% NaCl in a closed 2 ml laboratory tube by vortexing it for 5 min. Afterwards, the sample was centrifuged for 5 min at 1800  $\times$  g (RCF), and 1 ml of the obtained supernatant was transferred to a laboratory tube and again centrifuged for 5 min. All procedures were performed at the temperature of 2–5 °C. The supernatant was collected into Eppendorf tubes and frozen at -20 °C. VA concentration in the colon content was calculated as VA concentration in the supernatant multiplied by a factor of 3 (as described above, 1 ml of saline was added to 0.5 ml of colon content to prepare supernatant for analysis).

In a separate series of experiments rats ( $n = 5$ ) were killed by decapitation and tissue samples of the liver, the heart, the brain, kidneys and lungs were collected. Tissue samples were weighted and 100 mg of tissue was homogenized in 0.5 ml of homogenizing mixture using Precellys Cryolys Evolution tissue homogenizer (Bertin Instruments). All tissues were homogenized using 10% ethanol. After homogenization samples were stored in -80 °C until analysis. During analysis, samples were thawed and derivatized as described below.

### 2.2.2. Distribution of VA after the intracolonic administration

Intracolonic infusions (IC) were performed under general anesthesia with urethane at a dose of 1.5 g/kg b.w. ( $n = 6$ ) by means of a pediatric Foley catheter (10 F) inserted into the colon, 8 cm from the anus as we previously described (Huc et al., 2018b). Valeric acid-D9 was administered into colon at a dose of 0.3 mmol/kg. Rats were killed by decapitation and tissues were collected 5 min after the treatment. All homogenates were prepared using 10% ethanol as described above. After homogenization samples were stored in -80 °C until analysis.

### 2.2.3. Changes in VA concentration in portal and systemic blood after the IC administration of VA

In a separate series of experiments blood samples from the portal vein and vena cava were collected at baseline, 2 and 25 min after the IC administration of VA at a dose of 0.15 mmol/kg ( $n = 6$ ). The selected time points corresponded to the maximal hypotensive effect and return of blood pressure to baseline according to our findings from the hemodynamic part of the study.

## 2.3. Hemodynamic studies

### 2.3.1. Surgical preparation of animals

Before the measurements rats were implanted with a polyurethane arterial catheter which was inserted through the femoral artery into the abdominal aorta under general anesthesia with urethane at a dose of 1.5 g/kg b.w. For intravenous treatment, a polyurethane catheter was implanted into the femoral vein. For electrocardiogram (ECG) recordings, standard needle electrodes were used.

**2.3.1.1. Vagotomy.** The surgical bilateral abdominal (subdiaphragmatic) truncal vagotomy was performed as previously described (Allen et al., 1985) with modification. In short, after cutting skin and muscles from the xiphoid to the navel, the liver was stabilized with ligatures to visualize the subdiaphragmatic vagus nerves. Saline solution of methylene blue (0.4 %) was applied on tissues for better visualization of nerves. The nerves were cut with vascular scissors. After the procedure the wound was stitched.

It needs to be mentioned, that in contrast to humans, in rats all regions of the colon, except the rectum, are innervated by the branches of the vagus nerve (Altschuler et al., 1993).

### 2.3.2. Hemodynamic recordings

Blinking was provided. The investigated compounds were prepared and labelled with a group code. Unblinding was performed after statistical analysis. The arterial catheter was connected to the BP and HR recording system, BIOPAC MP160. Since rats are active during dark phase and show significant diurnal variation in hemodynamic parameters (Lemmer, 2017) the experiments were performed at the same time (10.00–14.00) in the middle of the dark phase. The measurements were started 40 min after the induction of anesthesia, and 10 min after connecting the arterial and venous catheters.

Hemodynamic studies comprised the following experimental series performed on separate groups of rats:

### 2.3.3. Experimental series

#### 2.3.3.1. Administrations into the colon

1. Administration of a vehicle (10% DMSO solution, 0.25 ml/30s,  $n = 5$ ), 10% DMSO solution of VA at a dose of 0.15 ( $n = 5$ ), 0.3 ( $n = 5$ ) and 0.6 mmol/kg ( $n = 5$ ).
2. Administration of VA at a dose of 0.15 mmol/kg after the pretreatment with the 3-hydroxybutyrate (ANT, a nonspecific antagonist of SCFA receptors GPR41/43) at a dose of 0.15 mmol/kg ( $n = 5$ ) and the ANT alone ( $n = 5$ ).
3. Administration of VA at a dose of 0.15 mmol/kg after the vagotomy ( $n = 5$ ) and vagotomy alone ( $n = 5$ ).
4. Administration of VA at a dose of 0.15 mmol/kg after the intravenous pretreatment with atropine (Atropinum Sulfuricum WZF) at a dose of 1 mg/kg, ( $n = 5$ ).
5. Administration of VA at a dose of 0.15 mmol/kg during the treatment with hexamethonium, an autonomic ganglia blocker (bolus 15 mg/kg, followed by continuous infusion at a rate of 1.5 mg/kg/min), ( $n = 5$ ).

#### 2.3.3.2. Intravenous administrations

1. Intravenous bolus administration of a vehicle (10% DMSO solution 0.2 ml/2 min,  $n = 5$ ), 10% DMSO solution of VA at a dose of 0.15 ( $n = 5$ ), 0.3 ( $n = 5$ ), and 0.6 mmol/kg ( $n = 5$ ).
2. Intravenous continuous administration (2ml/60min) of a vehicle (10% DMSO solution,  $n = 5$ ), 10% DMSO of VA at a dose of 0.15 mmol/kg ( $n = 5$ ) with an infusion pump.

After hemodynamic studies rats were killed by decapitation.



**Table 1**

Concentration of valeric acid (VA,  $\mu\text{M}$ ), in body fluids, daily urine excretion ( $\mu\text{moles}/24\text{ h}$ ) ( $n = 9$ ) and tissues ( $n = 5$ ). Presented data are means  $\pm$  S.E.M.

| VA concentrations/urine excretion                       |                    |
|---------------------------------------------------------|--------------------|
| Colon content ( $\mu\text{M}$ )                         | $668.58 \pm 55.98$ |
| Portal blood ( $\mu\text{M}$ )                          | $8.08 \pm 0.65$    |
| Systemic blood ( $\mu\text{M}$ )                        | $0.37 \pm 0.11$    |
| Brain ( $\mu\text{M}$ )                                 | $0.12 \pm 0.02$    |
| Liver ( $\mu\text{M}$ )                                 | $1.01 \pm 0.11$    |
| Kidneys ( $\mu\text{M}$ )                               | $0.24 \pm 0.02$    |
| Heart ( $\mu\text{M}$ )                                 | $0.52 \pm 0.03$    |
| Lungs ( $\mu\text{M}$ )                                 | $0.22 \pm 0.03$    |
| Urine ( $\mu\text{M}$ )                                 | $7.07 \pm 3.76$    |
| Daily urine excretion ( $\mu\text{moles}/24\text{ h}$ ) | $0.132 \pm 0.07$   |

blood concentration of VA was approximately three orders of magnitude lower, whereas systemic blood concentration of VA was approximately four orders of magnitude lower than the concentration of VA in the colon content. The physiological concentration of endogenous VA in the investigated tissues was in the range of 0.1–1.0  $\mu\text{M}$ .

### 3.2. Distribution of valeric acid-D9 after intracolonic administration

5 min after the intracolonic administration of VA-D9, the signal of the tracer was detected in the brain, the liver, the kidneys, the heart and systemic blood plasma in all investigated animals ( $n = 6$ ). Exemplary chromatograms of analyzed samples are presented in Fig. 1. The presence of exogenous VA (VA-D9) in homogenates confirms that VA penetrated to the tissues from the colon.

### 3.3. Hemodynamic effects of VA administered into the colon

There were no significant differences in hemodynamic parameters between experimental series at baseline (Table 2).

The vehicle, vagotomy and the ANT did not affect significantly MABP and HR (Figs. 2–4).

VA administered IC produced a dose dependent decrease in MABP which lasted for about 5 min (Fig. 2A). This was associated with a

significant decrease in HR in 0.6 mmol/kg series (Fig. 2B), but no significant change in QTc, a marker of drug cardiotoxicity (Table 3).

The pretreatment with the ANT significantly reduced the hypotensive effect of VA (Fig. 3). In contrast, the size of hypotensive response to VA was not affected by subphrenic vagotomy (Fig. 4) however, vagotomized rats showed longer decrease in MABP.

In comparison to the rats treated with VA alone, the rats pre-treated with hexamethonium showed longer hypotensive responses which were accompanied by decreases in HR. (Fig. 5A and B). In contrast, the pretreatment with atropine did not affect significantly the hypotensive response to VA, however, this group showed a stronger rebound of MABP (Fig. 5A and B).

### 3.4. Changes in VA level in portal and systemic blood after IC infusion of VA

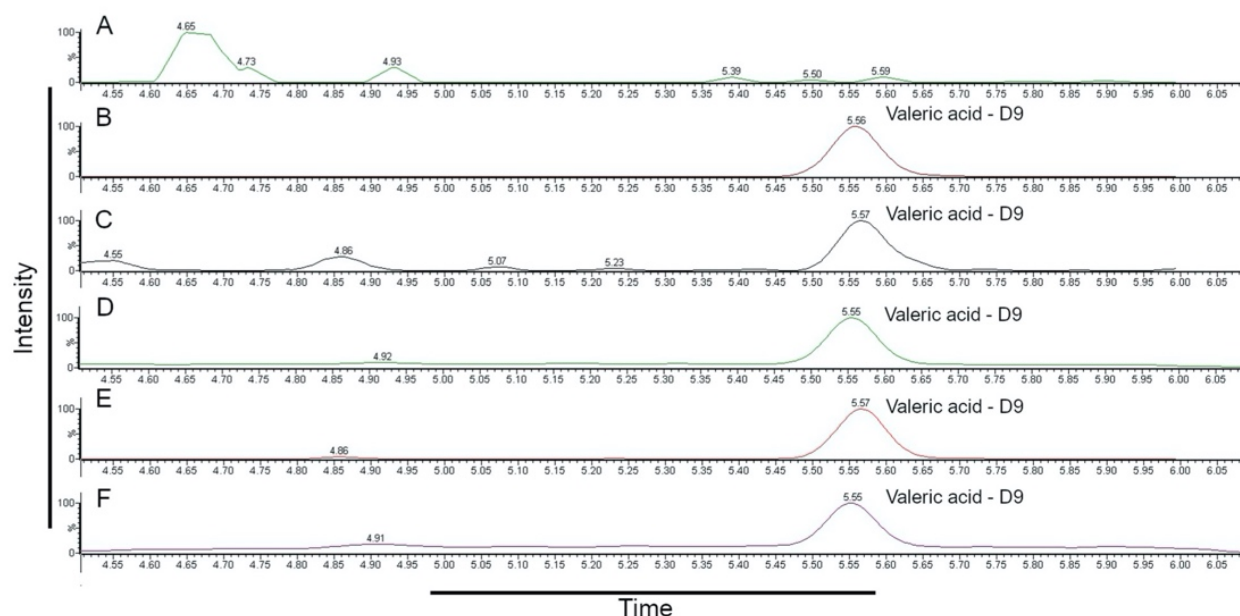
Concentration of VA at baseline and after the administration of VA into the colon are presented in Table 4. In general, systemic blood concentration of VA was approximately 10-fold lower than in portal blood. Intracolonic treatment with VA at a dose which produced hypotensive response was associated with approximately 15-fold (after 2 min) and 3-fold (after 25 min) increase in systemic plasma VA concentration from baseline.

### 3.5. Reactivity studies

At 50 mmHg, the mean internal diameter of MA branches was  $295 \pm 9\text{ }\mu\text{m}$  ( $n = 10$ ) and at 80 mmHg the mean internal diameter of GMA was  $240 \pm 3\text{ }\mu\text{m}$  ( $n = 10$ ). All arteries were pre-constricted with phenylephrine (0.5  $\mu\text{M}$ ), which decreased vessels diameter by about 50%.

#### 3.5.1. Effect of VA on MA branches and GMA diameter

VA at the concentration close to physiological one in systemic blood (1  $\mu\text{M}$ ) did not change the diameter of MA and GMA. A significant relaxation of MA (by  $17 \pm 2\%$ ) was observed at the threshold concentration of 5  $\mu\text{M}$ . The response of the GMA to VA was shifted to the right, i.e. dilation of the GMA by  $8 \pm 2\%$  was observed at the concentration of 10  $\mu\text{M}$ . MA showed a tendency to a greater dilation than

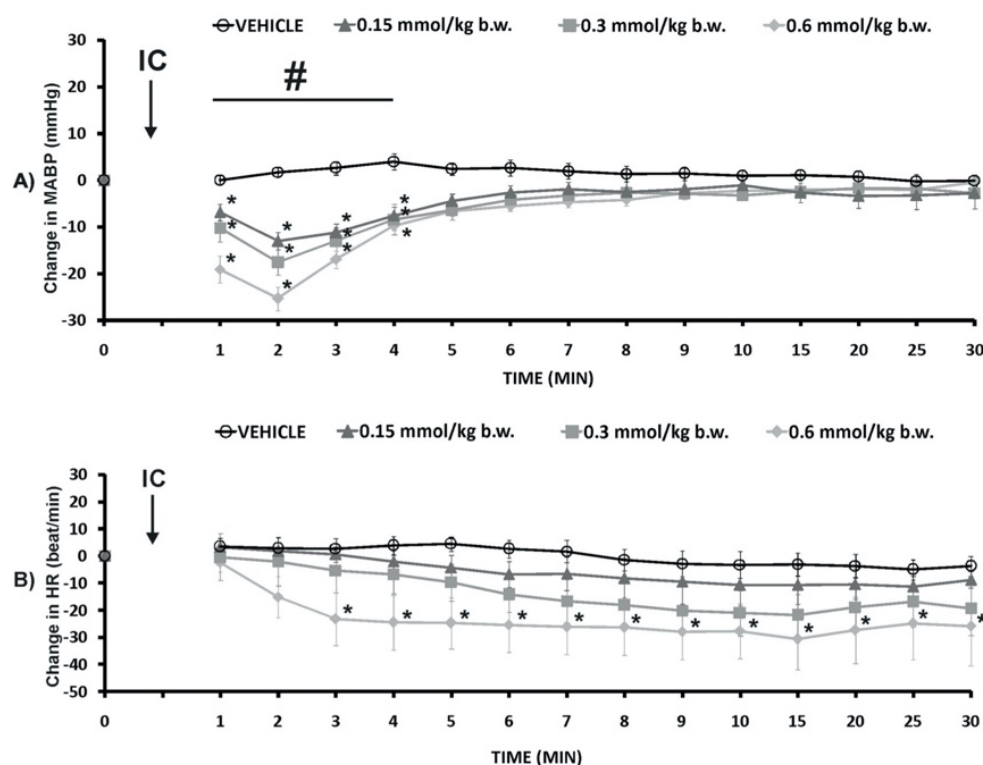


**Fig. 1.** Chromatograms for valeric acid-D9 of blank sample (A), plasma (B), homogenate samples: liver (C), kidney (D), brain (E), heart (F). After the intracolonic administration of VA-D9 the signal specific for VA-D9 was present in plasma and investigated tissues homogenates confirming penetration of VA-D9 from the colon to the tissues.

**Table 2**

Baseline mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats/min) in Wistar rats. Means  $\pm$  S.E.M. are presented. <sup>a</sup> - After the pre-treatment with atropine and before administration of VA. <sup>b</sup> - After the pre-treatment with hexamethonium and before administration of VA.

| Series                          | MABP                                          | HR                                     |
|---------------------------------|-----------------------------------------------|----------------------------------------|
| Vehicle                         | 95.5 $\pm$ 5.0                                | 342 $\pm$ 14                           |
| ANT                             | 96.1 $\pm$ 1.9                                | 346 $\pm$ 13                           |
| VA 0.6 mmol/kg                  | 98.2 $\pm$ 4.8                                | 359 $\pm$ 25                           |
| VA 0.3 mmol/kg                  | 101.3 $\pm$ 0.7                               | 358 $\pm$ 6                            |
| VA 0.15 mmol/kg                 | 99.4 $\pm$ 3.2                                | 379 $\pm$ 32                           |
| ANT + VA 0.15 mmol/kg           | 104.5 $\pm$ 6.7                               | 375 $\pm$ 43                           |
| Vagotomy + VA 0.15 mmol/kg      | 94.5 $\pm$ 2.2                                | 330 $\pm$ 26                           |
| Vagotomy                        | 97.9 $\pm$ 5.4                                | 349 $\pm$ 21                           |
| Atropine + VA 0.15 mmol/kg      | 102.5 $\pm$ 5.6/102.1 <sup>a</sup> $\pm$ 4.14 | 358 $\pm$ 21/403 <sup>a</sup> $\pm$ 18 |
| Hexamethonium + VA 0.15 mmol/kg | 99 $\pm$ 3.9/61.96 <sup>b</sup> $\pm$ 2       | 368 $\pm$ 14/288 <sup>b</sup> $\pm$ 4  |



**Fig. 2.** [A] Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and [B] heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the intracolonic administration (IC) of either a vehicle (10% DMSO solution, (n = 5) or valeric acid (VA) at a dose of 0.15 (n = 5), 0.3 (n = 5) and 0.6 (n = 5) mmol/kg, \* -  $P < 0.05$  - vs baseline, # -  $P < 0.05$  - 0.15, 0.3 and 0.6 mmol/kg VA series vs the vehicle. Means  $\pm$  S.E.M. are presented.

GMA in response to VA, however significant differences between both arteries appeared only at concentrations of 100  $\mu$ M and 500  $\mu$ M, (Fig. 6 A).

### 3.5.2. Effect of 3-hydroxybutyrate (ANT) on MA branches and GMA diameter

ANT regardless of the applied concentration did not affect the MA and GMA diameters (Fig. 6 B). Studying the influence of the ANT on the vasodilating effect of VA, it turned out that ANT 1 mM does not affect the dilation of GMA in response to VA, but partially inhibits the dilation in MA (Fig. 6 C vs 6 A).

### 3.6. Hemodynamic effects of VA administered intravenously

There were no significant differences in hemodynamic parameters between experimental series at baseline (Table 5).

Administration of the vehicle did not produce a significant change in MABP (Fig. 7 A). VA at a dose of 0.15, 0.3 and 0.6 mmol/kg produced a significant, transient (4–5 min long) decrease in MABP. The hypotensive response was not associated with significant changes in HR

(Fig. 7 B).

Continuous administration of the vehicle did not produce a significant change in MABP (Fig. 8 A). In contrast, continuous administration of VA at dose of 0.15 mmol/kg/60 min produced hypotensive response that lasted through the experiment (Fig. 8 B).

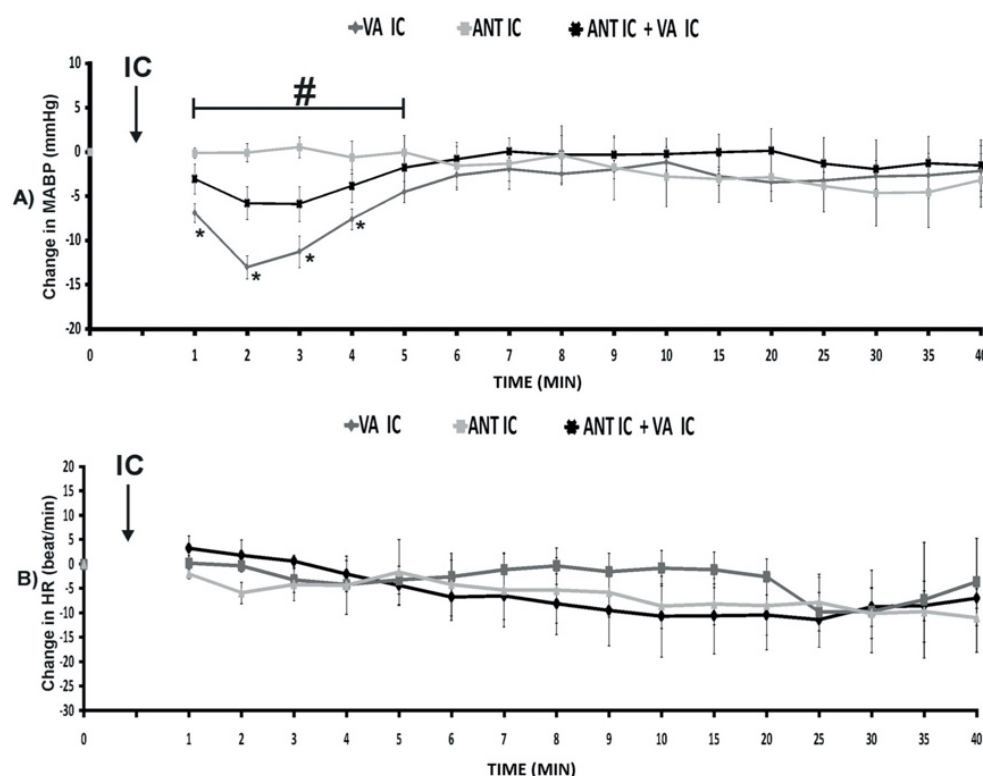
## 4. Discussion

A new finding of our study is that exogenous VA lowers arterial blood pressure in rats. The hypotensive effect involves vasodilation and is diminished by an inhibitor of GPR41/43 receptors. Finally, we showed that VA rapidly penetrates from the colon, a major site of microbiota metabolism, to vital organs.

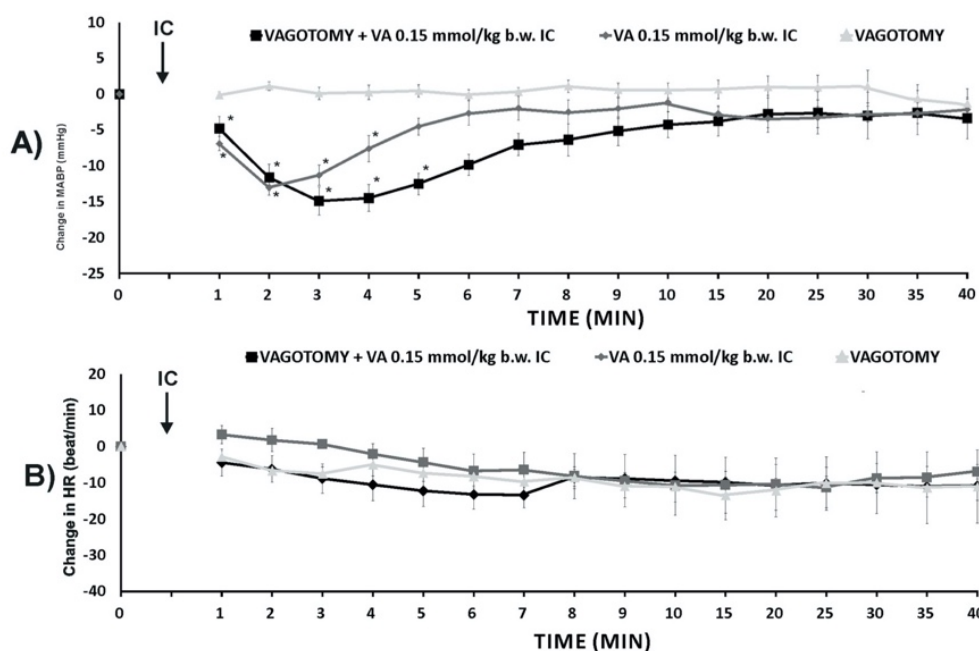
VA is a colorless, oily, short-chain fatty acid produced by gut microbiota and rhizomes of *Valeriana officinalis*, a sedative herb. Finally, esterases free VA from estradiol valerate, a hormonal medicine used for various indications (Caron and Riedlinger, 1999; Chevalier, 1996; Hassager et al., 1987; Seely et al., 1999; Wichtl, 2004; Zacharieva et al., 2002).

Notably, treatment with *Valeriana officinalis* extracts (Rosecrans





**Fig. 3.** [A] Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and [B] heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the intracolonic administration (IC) of valeric acid at a dose of 0.15 mmol/kg (VA,  $n = 5$ ), or 3-hydroxybutyrate, a non-specific antagonist of GPR41/43 receptors at a dose of 0.15 mmol/kg (ANT,  $n = 5$ ) or VA after the pre-treatment with ANT (ANT + VA,  $n = 5$ ). \* $-P < 0.05$  - vs baseline, #  $-P < 0.05$  - the VA series vs the ANT + VA series. Means  $\pm$  S.E.M. are presented.



**Fig. 4.** [A] Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and [B] heart rate ( $\Delta$ HR, beats/min) in intact Wistar rats ( $n = 5$ ) and in vagotomized rats ( $n = 5$ ) after the administration of VA into the colon (IC) at a dose of 0.15 mmol/kg b.w. \* $-P < 0.05$  - vs baseline. Means  $\pm$  S.E.M. are presented.

et al., 1961; ZHOU et al., 2009) and estradiol valerate (Hassager et al., 1987; Seely et al., 1999; Zacharieva et al., 2002) has been associated with blood pressure lowering effects. The administration of estradiol valerate in postmenopausal women lowered arterial blood pressure, which was suggested to be dependent on the action of estradiol (Cannoletta and Cagnacci, 2014; Davis et al., 2019; Zacharieva et al., 2002), however, the contribution of VA to the hypotensive effect cannot be excluded. Besides, estradiol valerate was found to reduce the hypertensive response to mental stress (Komesaroff et al., 1999), dilate

coronary vessels (Enderle et al., 2000) and reduce sympathetic nervous activity (Sudhir et al., 1997). However, there are also studies showing no significant effect of estradiol valerate on blood pressure (Goretzlehner et al., 1996; Mueck et al., 2001).

Nevertheless, to the best of our knowledge, hemodynamic effects of VA alone, have not yet been reported.

In our study, intravenous bolus administration of VA produced a short-lasting hypotensive effect, which is comparable to those observed after the administration of hypotensive agents such as nitric oxide

Table 3

Electrocardiographic parameters in rats (n = 5) at baseline, 2 and 20 min after the intracolonic administration of VA (IC VA) at a dose of 0.15 mmol/kg b.w. \*P < 0.05 - vs baseline. Means ± S.E.M. are presented.

| ECG parameters     | Baseline     | 2 min after IC VA | 20 min after IC VA |
|--------------------|--------------|-------------------|--------------------|
| RR (ms)            | 177.4 ± 13.7 | 177.1 ± 11.6      | 180.7 ± 14.3       |
| QT (ms)            | 78 ± 7.9     | 79.4 ± 7.3        | 80.6 ± 7.3         |
| QTc (ms)           | 71.4 ± 4.7   | 72.9 ± 4.8        | 73.3 ± 4.2         |
| PR (ms)            | 63.3 ± 2.4   | 62.4 ± 1.9        | 62.7 ± 2.5         |
| QRS width (ms)     | 24.4 ± 0.9   | 23.9 ± 0.4        | 22.7 ± 2.5         |
| QRS amplitude (mV) | 2.8 ± 0.4    | 2.8 ± 0.4         | 2.8 ± 0.4          |

donors (Page et al., 1955; Verner, 1974). The transient character of hemodynamic response to blood pressure lowering agents may be caused by activation of counteracting nervous and humoral mechanism or/and a rapid metabolic breakdown of the molecule and relatively short half-life of VA i.e. 6-12 min (Sampath et al., 2012). The latter notion is supported by our experiments showing that the continuous intravenous infusion of VA produced a hypotensive response that lasted through the whole experiment i.e. 60 min. These findings suggest a possible long-lasting blood pressure lowering effect of VA which could be accomplished by developing slow-releasing VA-donors.

Our study shows that at physiological conditions VA is present in the colon content, portal and systemic blood as well as in vital organs. Considering that physiological concentration of VA in the colon content (stool masses) is three orders of magnitude higher than in portal blood, and four orders of magnitude higher that in systemic blood and tissues, we think that blood and tissue VA originates from the colon. This is also supported by the fact that the exogenous VA (VA-D9) administered into the colon was rapidly distributed to distant tissues including the liver, kidneys, the heart and the brain. The rapid gut-to-systemic organs penetration of VA suggests that even transient changes in colonic VA level may be translated to systemic effects. Finally, administration of VA into the colon produced a significant, dose-dependent decrease in arterial blood pressure.

Increasing research shows that metabolites produced in the colon by the microbiota affect the control of arterial blood pressure (Bauer and Richards, 1928; Skrzypecki et al., 2018; Wang et al., 2017). Firstly, bacterial metabolites may stimulate sensory fibers of the enteric

Table 4

A. VA concentration in portal blood and systemic blood at baseline, 2 and 25 min after the intracolonic administration (IC) of VA at a dose of 0.15 mmol/kg b.w. (n = 6). The selected time points correspond to the maximal hypotensive effect and return of BP to baseline according to our findings from the hemodynamic part of the study. B. Liver clearance of VA calculated as a  $\frac{CP-CH}{CP}$ , where CP is concentration of a VA in portal blood, and CH concentration of VA in systemic blood. Means ± S.E.M. are presented.

|                                                                                                    |              |
|----------------------------------------------------------------------------------------------------|--------------|
| A. VA (μM) concentration before (baseline) and after administration of VA at a dose of 0.15mmol/kg |              |
| Portal vein blood                                                                                  |              |
| Baseline                                                                                           | 2.19 ± 0.22  |
| 2 min after administration                                                                         | 23.82 ± 3.76 |
| 25 min after administration                                                                        | 6.35 ± 1.49  |
| Systemic vein blood                                                                                |              |
| Baseline                                                                                           | 0.26 ± 0.06  |
| 2 min after administration                                                                         | 7.55 ± 1.3   |
| 25 min after administration                                                                        | 1.28 ± 0.22  |
| B. Liver clearance of VA at baseline and after IC administration of VA at a dose of 0.15 mmol/kg   |              |
| Baseline                                                                                           | 0.87 ± 0.03  |
| 2 min after administration                                                                         | 0.52 ± 0.21  |
| 25 min after administration                                                                        | 0.84 ± 0.04  |

nervous system which communicate with cardiovascular centers located in the brain (Bravo et al., 2011; Onyszkiewicz et al., 2019). Secondly, bacterial metabolites penetrate through the gut-blood barrier and as a blood-borne agents may affect function of organs and tissues involved in the regulation of the circulatory system (Huc et al., 2018a; Jaworska et al., 2018; Tomasova et al., 2016).

Several papers indicate that metabolites of gut bacteria affect the host homeostasis via nervous gut-brain axis (Bienenstock et al., 2015; Cryan and Dinan, 2012; Yang and Zubcevic, 2017). Recently, we found that colon-derived butyric acid, a SCFA, may affect the circulatory system via nervous gut-brain signaling (Onyszkiewicz et al., 2019). In the present study, the subphrenic vagotomy had no significant influence on the size of hypotensive effect of VA administered into the colon. This suggests the lack of involvement of the nervous gut signaling in hemodynamic effects of the colon-derived VA. The rapid gut-to-blood penetration of VA and the vasodilatory action of VA in ex vivo experiments indicate that the hypotensive effect was dependent on

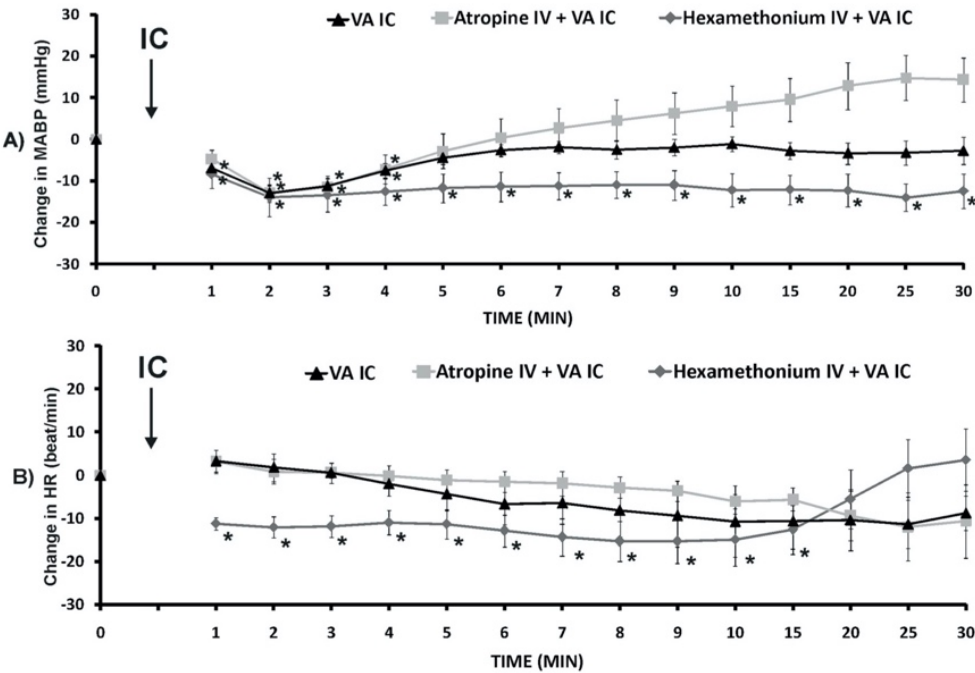
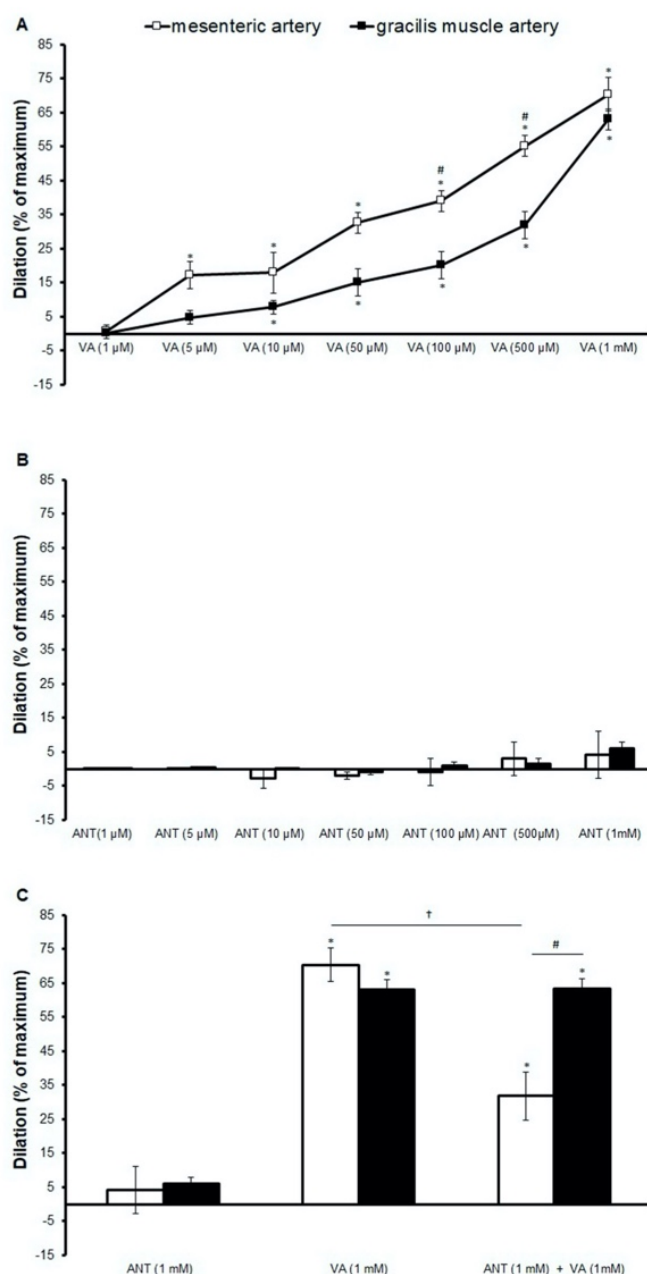


Fig. 5. [A] Changes in mean arterial blood pressure (ΔMABP, mmHg) and [B] heart rate (ΔHR, beats/min) in Wistar rats after the intracolonic administration of VA (VA IC) at a dose of 0.15 mmol/kg in rats pre-treated with atropine (atropine IV + VA IC), (n = 5) or hexamethonium (hexamethonium IV + VA IC), (n = 5). \*P < 0.05 - vs baseline. Means ± S.E.M. are presented.





**Fig. 6.** Response of the pre-constricted with PE mesenteric artery branches and gracilis muscle arteries to: **[A]** increasing concentration of valeric acid (VA, from 1  $\mu$ M up to 1 mM,  $n = 10$ ); **[B]** increasing concentration of 3-hydroxybutyrate (from 5  $\mu$ M to 1 mM,  $n = 10$ ) with VA (1  $\mu$ M) in the background **[C]** the effect of 3-hydroxybutyrate (ANT, 1 mM) on vasorelaxant responses of mesenteric artery branches ( $n = 5$ ) and gracilis muscle artery ( $n = 5$ ) to VA (1 mM). Dilation is expressed as a percentage of maximum diameter (0 Ca<sup>2+</sup>, EGTA 3 mM). Values are means  $\pm$  S.E.M. of  $n$  arteries. \* $-P < 0.05$ : significant vasorelaxation; #  $-P < 0.05$ : significant difference between the MA vs GMA response to the VA or ANT; †††  $-P < 0.05$  significant effect of ANT (1 mM) on the vasodilation evoked by VA (1 mM).

vasodilation produced by colon-derived, blood-borne VA.

Specifically, in *ex vivo* experiments, VA produced a significant, dose-dependent vasodilation in GMA and MA arteries. Interestingly, the vasodilatory effect of VA was significantly reduced by the GPR41/43 blocker in MA but not in GMA. In addition, a dose-response relation revealed greater sensitivity of MA than GMA to VA and a greater relaxation of MA than GMA in response to 100 mM and 500 mM VA. This suggests that relaxation of GMA and MA may be mediated by different

**Table 5**

Baseline mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats/min) in Wistar rats. Means  $\pm$  S.E.M. are presented.

| Series                                 | MABP            | HR           |
|----------------------------------------|-----------------|--------------|
| Vehicle (bolus administration)         | 96.2 $\pm$ 2.9  | 312 $\pm$ 26 |
| VA 0.6 mmol/kg (bolus administration)  | 100.3 $\pm$ 5.2 | 353 $\pm$ 1  |
| VA 0.3 mmol/kg (bolus administration)  | 97.9 $\pm$ 4.4  | 368 $\pm$ 12 |
| VA 0.15 mmol/kg (bolus administration) | 104.4 $\pm$ 5.3 | 371 $\pm$ 16 |
| Vehicle (continuous infusion)          | 99.5 $\pm$ 3.2  | 338 $\pm$ 8  |
| VA (continuous infusion)               | 104.8 $\pm$ 3.0 | 337 $\pm$ 15 |

mechanisms. In this regard, the relaxation of various resistance blood vessels *ex vivo* in response to other SCFAs is well documented (Aaronsen et al., 1996; Mortensen et al., 1990). However, the mechanisms seem to be complex and are not fully elucidated (Aaronsen et al., 1996; Daugirdas and Nawab, 1987; Knock et al., 2002; Mortensen et al., 1990; Natarajan et al., 2016; Nutting et al., 1991).

A limitation of our study is that we did not evaluate the effect of Olfr78 receptor blockade, which is also thought to be involved in SCFA signaling. This is because, biologically effective Olfr78 receptor blockers are not yet available. Besides, chronic studies are needed to assess the effect of VA on arterial blood pressure. Regarding hypotensive effect of gut microbiota-produced VA it needs to be stressed that at physiological conditions VA is produced with many other bacterial products that may have similar or the opposite hemodynamic effects.

In conclusion, we found that VA produces vasodilatory and hypotensive effects in rats. Under physiological conditions concentration of VA in the colon is four orders of magnitude higher than that in systemic blood. VA rapidly penetrates from the colon to distant organs including the brain. Further studies are needed to evaluate the effects of endogenous and exogenous VA on the circulatory system as it may be an interesting target for developing blood pressure lowering agents.

## Funding

This work was supported by the National Science Centre, Poland grant no. UMO-2016/22/E/NZ5/00647. The equipment used was sponsored by the Centre for Preclinical Research and Technology (CePT), a project co-sponsored by European Regional Development Fund and Innovative Economy, The National Cohesion Strategy of Poland (POIG.02.02.00-14-024/08-00). We would like to thank Foundation of Polish Science TEAM TECH CORE FACILITY/2016-2/2 Mass Spectrometry of Biopharmaceuticals - improved methodologies for qualitative, quantitative and structural characterization of drugs, proteinaceous drug targets and diagnostic molecules.

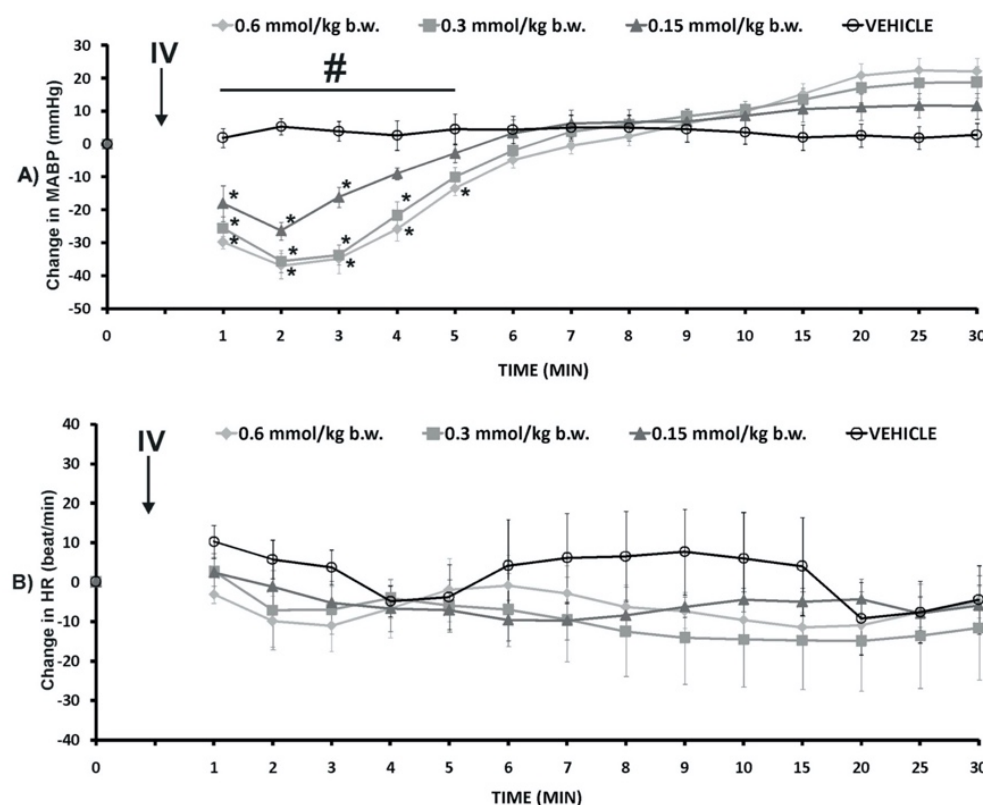
## CRediT authorship contribution statement

**Maksymilian Onyszkiewicz:** Conceptualization, Investigation, Formal analysis, Writing - original draft. **Marta Gawrys-Kopczynska:** Investigation, Formal analysis, Data curation. **Maciej Sałagaj:** Investigation, Formal analysis, Data curation. **Marta Aleksandrowicz:** Investigation, Formal analysis, Writing - original draft. **Aneta Sawicka:** Investigation, Formal analysis. **Ewa Koźniewska:** Funding acquisition, Formal analysis, Writing - review & editing. **Emilia Samborowska:** Investigation, Formal analysis, Data curation. **Marcin Ufnal:** Conceptualization, Funding acquisition, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.

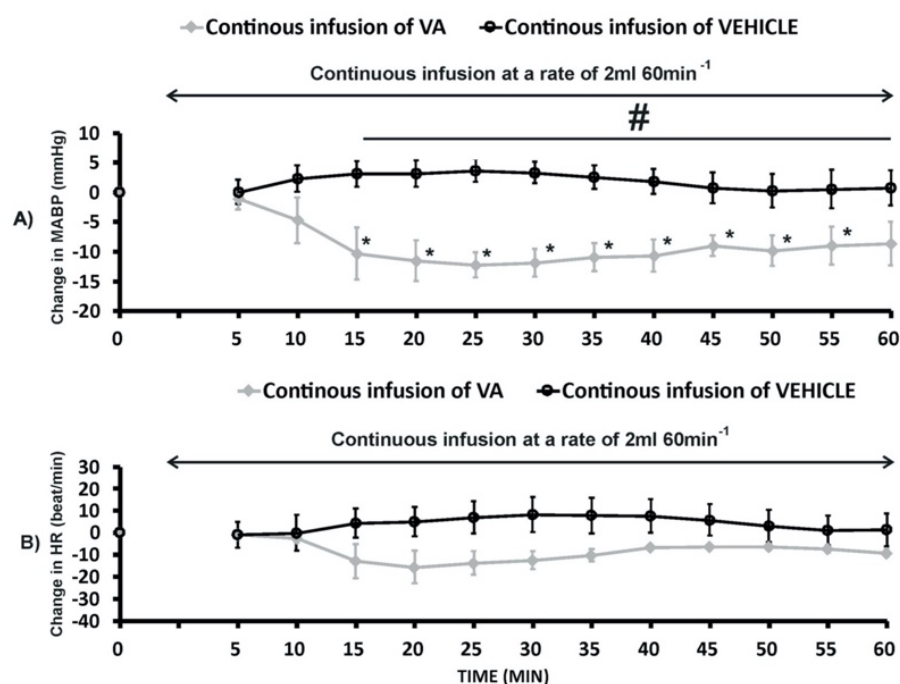
## Declaration of competing interest

The authors declare no conflict of interest.





**Fig. 7.** [A] Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and [B] heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the intravenous administration (IV) of either a vehicle (10% DMSO solution 0.2 ml/2min), or valeric acid (VA) at a dose of 0.15, 0.3 and 0.6 mmol/kg, \* $P < 0.05$  - vs baseline, # -  $P < 0.05$ : 0.15, 0.3 and 0.6 mmol/kg VA series vs the vehicle. Means  $\pm$  S.E.M. are presented.



**Fig. 8.** [A] Changes in mean arterial blood pressure ( $\Delta$ MABP, mmHg) and [B] heart rate ( $\Delta$ HR, beats/min) in Wistar rats after the chronic intravenous administration (IV) via an infusion pump of either a vehicle (10% DMSO solution 0.2 ml/60 min) or VA at dose of 0.15 mmol/kg/60 min, \* $P < 0.05$  - vs baseline, # -  $P < 0.05$ : VA vs the vehicle. Means  $\pm$  S.E.M. are presented.

## References

- Aaronson, P.I., McKinnon, W., Poston, L., 1996. Mechanism of butyrate-induced vasorelaxation of rat mesenteric resistance artery. *Br. J. Pharmacol.* 117, 365–371.
- Adam, C.L., Williams, P.A., Dalby, M.J., Garden, K., Thomson, L.M., Richardson, A.J., Gratz, S.W., Ross, A.W., 2014. Different types of soluble fermentable dietary fibre decrease food intake, body weight gain and adiposity in young adult male rats. *Nutr. Metab.* 11, 36.
- Aleksandrowicz, M., Kozniowska, E., 2013. Disturbed regulation of the isolated middle cerebral artery in acute hyponatremia. *Folia Neuropathol.* 51, 227–234.
- Allen, L.G., Lawrence Jr., I.E., Burden, H.W., Hodson, C.A., 1985. Effects of abdominal vagotomy on serum LH concentrations in female rats. *J. Reprod. Fertil.* 74, 87–94.
- Altschuler, S.M., Escardo, J., Lynn, R.B., Miselis, R.R., 1993. The central organization of the vagus nerve innervating the colon of the rat. *Gastroenterology* 104, 502–509.
- Anderson, J.W., Bridges, S.R., 1984. Short-chain fatty acid fermentation products of plant fiber affect glucose metabolism of isolated rat hepatocytes. *Proc Soc Exp Biol Med* 177, 372–376.
- Bauer, W., Richards, D.W., 1928. A vasodilator action of acetates. *J. Physiol.* 66, 371–378.
- Bienenstock, J., Kunze, W., Forsythe, P., 2015. Microbiota and the gut-brain axis. *Nutr. Rev.* 73 (Suppl. 1), 28–31.
- Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., Cryan, J.F., 2011. Ingestion of Lactobacillus strain regulates



- emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16050–16055.
- Brown, A.J., Goldsworthy, S.M., Barnes, A.A., Eilert, M.M., Tcheang, L., Daniels, D., Muir, A.I., Wigglesworth, M.J., Kinghorn, I., Fraser, N.J., Pike, N.B., Strum, J.C., Steplewski, K.M., Murdock, P.R., Holder, J.C., Marshall, F.H., Szekeres, P.G., Wilson, S., Ignar, D.M., Foord, S.M., Wise, A., Dowell, S.J., 2003. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278, 11312–11319.
- Cannoletta, M., Cagnacci, A., 2014. Modification of blood pressure in postmenopausal women: role of hormone replacement therapy. *Int J Womens Health* 6, 745–757.
- Caron, M.F., Riedinger, J.E., 1999. Valerian: a practical review for clinicians. *Nutr. Clin. Care* 2, 250–257.
- Chevalier, 1996. *The Encyclopedia of Medicinal Plants*, first ed. Dorling Kindersley, London.
- Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712.
- Daugirdas, J.T., Nawab, Z.M., 1987. Acetate relaxation of isolated vascular smooth muscle. *Kidney Int.* 32, 39–46.
- Davis, G.K., Newsome, A.D., Cole, A.B., Ojeda, N.B., Alexander, B.T., 2019. Chronic estrogen supplementation prevents the increase in blood pressure in female intrauterine growth-restricted offspring at 12 Months of age. *Hypertension* 73, 1128–1136.
- Enderle, M.D., Sayer, R., Balletshofer, B., Meisner, C., Muck, A.O., Haasis, R., Haering, H.U., Pfohl, M., 2000. Acute improvement of peripheral endothelial function in postmenopausal women with coronary artery disease after single oral intake of 17beta-estradiol valerate. *Exp. Clin. Endocrinol. Diabetes* 108, 382–385.
- Fleischer, J., Bumbalo, R., Bautze, V., Strotmann, J., Breer, H., 2015. Expression of odorant receptor Olfr78 in enteroendocrine cells of the colon. *Cell Tissue Res.* 361, 697–710.
- Goretzlehner, G., Nikschick, S., Zimmermann, T., 1996. [Effect of estradiol valerate and chlormadinone acetate in hormone replacement therapy in postmenopause on Kupperman index, body weight, blood pressure, lipids, enzymes and electrolytes]. *Zentralblatt für Gynäk.* 118, 420–425.
- Han, J., Lin, K., Sequeira, C., Borchers, C.H., 2015. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* 854, 86–94.
- Hassager, C., Riis, B.J., Strom, V., Guyene, T.T., Christiansen, C., 1987. The long-term effect of oral and percutaneous estradiol on plasma renin substrate and blood pressure. *Circulation* 76, 753–758.
- Huc, T., Konop, M., Onyszkiewicz, M., Podsadni, P., Szczepanska, A., Turlo, J., Ufnal, M., 2018a. Colonic indole, gut bacteria metabolite of tryptophan, increases portal blood pressure in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 315, R646–R655.
- Huc, T., Konop, M., Onyszkiewicz, M., Podsadni, P., Szczepanska, A., Turlo, J., Ufnal, M., 2018. Colonic indole, gut bacteria metabolite of tryptophan, increases portal blood pressure in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 315, R646–R655. <https://doi.org/10.1152/ajpregu.00111.2018>.
- Jaworska, K., Huc, T., Gawrys, M., Onyszkiewicz, M., Samborowska, E., Ufnal, M., 2018. An in vivo method for evaluating the gut-blood barrier and liver metabolism of microbiota products. *JoVE* 140, 58456. <https://doi.org/10.3791/58456>.
- Jaworska, K., Konop, M., Bielinska, K., Hutsch, T., Dziekiewicz, M., Banaszekiewicz, A., Ufnal, M., 2019. Inflammatory bowel disease is associated with increased gut-to-blood penetration of short-chain fatty acids: a new, non-invasive marker of a functional intestinal lesion. *Exp. Physiol.* 104, 1226–1236.
- Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., Kobayashi, M., Hirasawa, A., Tsujimoto, G., 2011. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc. Natl. Acad. Sci. U. S. A.* 108, 8030–8035.
- Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., Terasawa, K., Kashiwara, D., Hirano, K., Tani, T., Takahashi, T., Miyauchi, S., Shioi, G., Inoue, H., Tsujimoto, G., 2013. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4, 1829.
- Kmecova, J., Klimas, J., 2010. Heart rate correction of the QT duration in rats. *Eur. J. Pharmacol.* 641, 187–192.
- Knock, G., Psaroudakis, D., Abbot, S., Aaronson, P.I., 2002. Propionate-induced relaxation in rat mesenteric arteries: a role for endothelium-derived hyperpolarising factor. *J. Physiol.* 538, 879–890.
- Komesaroff, P.A., Esler, M.D., Sudhir, K., 1999. Estrogen supplementation attenuates glucocorticoid and catecholamine responses to mental stress in perimenopausal women. *J. Clin. Endocrinol. Metab.* 84, 606–610.
- Lemmer, B., 2017. Signal transduction and chronopharmacology of regulation of circadian cardiovascular rhythms in animal models of human hypertension. *Heart Fail. Clin.* 13, 739–757.
- Mortensen, F.V., Nielsen, H., Mulvany, M.J., Hessov, I., 1990. Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* 31, 1391–1394.
- Mueck, A.O., Seeger, H., Ludtke, R., Graser, T., Wallwiener, D., 2001. Effect on biochemical vasoactive markers during postmenopausal hormone replacement therapy: estradiol versus estradiol/dienogest. *Maturitas* 38, 305–313.
- Natarajan, N., Hori, D., Flavahan, S., Stepan, J., Flavahan, N.A., Berkowitz, D.E., Pluznick, J.L., 2016. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiol. Genom.* 48, 826–834.
- Nutting, C.W., Islam, S., Daugirdas, J.T., 1991. Vasorelaxant effects of short chain fatty acid salts in rat caudal artery. *Am. J. Physiol.* 261, H561–567.
- Onyszkiewicz, M., Gawrys-Kopczynska, M., Konopelski, P., Aleksandrowicz, M., Sawicka, A., Kozniowska, E., Samborowska, E., Ufnal, M., 2019. Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors. *Pflügers Archiv* 471, 1441–1453.
- Page, I.H., Corcoran, A.C., Dustan, H.P., Koppanyi, T., 1955. Cardiovascular actions of sodium nitroprusside in animals and hypertensive patients. *Circulation* 11, 188–198.
- Pluznick, J.L., Protzko, R.J., Gevorgyan, H., Peterlin, Z., Sipos, A., Han, J., Brunet, I., Wan, L.X., Rey, F., Wang, T., Firestein, S.J., Yanagisawa, M., Gordon, J.I., Eichmann, A., Peti-Peterdi, J., Caplan, M.J., 2013. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4410–4415.
- Rosecrans, J.A., Defeo, J.J., Youngken Jr., H.W., 1961. Pharmacological investigation of certain *Valeriana officinalis* L. extracts. *J. Pharmacol. Sci.* 50, 240–244.
- Sampath, C., Haug, K., Thane, S., Hamburger, M., Derendorf, H., Frye, R., Butterweck, V., 2012. Pharmacokinetics of valeric acid in rats after intravenous and oral administrations. *Planta Med.* 78, 575–581.
- Seely, E.W., Walsh, B.W., Gerhard, M.D., Williams, G.H., 1999. Estradiol with or without progesterone and ambulatory blood pressure in postmenopausal women. *Hypertension* 33, 1190–1194.
- Sellappan, M., Mvnl, C., Dhanabal, S.P., Suresh, B., Chandrasekar, R., Nanjan, M.J., 2010. Estimation of n-Valeric Acid Content in *Valeriana officinalis* Linn by HPTLC Technique.
- Skrzypecki, J., Zera, T., Ufnal, M., 2018. Butyrate, a gut bacterial metabolite, lowers intraocular pressure in normotensive, but not in hypertensive rats. *J. Glaucoma.*
- Sudhir, K., Elser, M.D., Jennings, G.L., Komesaroff, P.A., 1997. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. *Hypertension* 30, 1538–1543.
- Tap, J., Furet, J.P., Bensaada, M., Philippe, C., Roth, H., Rabot, S., Lakhdari, O., Lombard, V., Henrissat, B., Corthier, G., Fontaine, E., Dore, J., Leclerc, M., 2015. Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ. Microbiol.* 17, 4954–4964.
- Tomasova, L., Dobrowolski, L., Jurkowska, H., Wrobel, M., Huc, T., Ondrias, K., Ostaszewski, R., Ufnal, M., 2016. Intracolonic hydrogen sulfide lowers blood pressure in rats. *Nitric Oxide* 60, 50–58.
- Verner, I.R., 1974. Sodium nitroprusside: theory and practice. *Postgrad. Med.* 50, 576–581.
- Wang, L., Zhu, Q., Lu, A., Liu, X., Zhang, L., Xu, C., Liu, X., Li, H., Yang, T., 2017. Sodium butyrate suppresses angiotensin II-induced hypertension by inhibition of renal (pro) renin receptor and intrarenal renin-angiotensin system. *J. Hypertens.* 35, 1899–1908.
- Wichtl, M., 2004. *Herbal Drugs and Phytopharmaceuticals*, third ed. Med Pharm Scientific Publishers.
- Yang, T., Zubcevic, J., 2017. Gut-brain Axis in regulation of blood pressure. *Front. Physiol.* 8, 845.
- Zacharieva, S., Kirilov, G., Kalinov, K., Shigarginova, R., Nachev, E., Orbetzova, M., Atanassova, I., 2002. Effect of different hormone replacement therapy regimens on circadian blood pressure profile and active renin in postmenopausal women. *Gynecol. Endocrinol.* 16, 461–467.
- Zhou, X.-z., Kang, L., Tang, Y., Li, L., Xiong, S.-h., 2009. Effect of *Valeriana officinalis* var *latifolia* miq on heart rat and arterial blood pressure of rabbits. *J. Liaoning Univ. Trad. Chin. Med.* 109.

## Podsumowanie i wnioski

Wyniki pracy pozytywnie zweryfikowały hipotezę badawczą zakładającą, że kwas masłowy oraz kwas walerianowy, które są wytwarzane przez bakterie jelitowe, wywierają istotny wpływ na ciśnienie tętnicze.

Zrealizowano wszystkie zaplanowane cele główne i szczegółowe, tj. zbadano wpływ kwasu masłowego oraz kwasu walerianowego na ciśnienie tętnicze u szczurów po podaniu dookreślonym i dożylnym. Oznaczono fizjologiczne stężenie kwasu masłowego i walerianowego w tkankach oraz oceniono stopień przenikania badanych związków z jelita do układu krążenia. Zbadano także mechanizmy działania hipotensyjnego kwasu masłowego i walerianowego oraz oceniono kardiotoksyczność obu związków.

### Najważniejsze wyniki badania to:

- 2-3 krotne zwiększenie stężenia kwasu masłowego w jelicie grubym w stosunku do warunków fizjologicznych powoduje długotrwałe obniżenie ciśnienia tętniczego.
- Efekt hipotensyjny wywołany przez dookreślone podanie roztworu kwasu masłowego związany jest z pobudzeniem receptorów dla krótkołańcuchowych kwasów tłuszczowych (GPR41/43) oraz aferentnych włókien nerwu błędnego unerwiających jelito grube.
- Działanie hipotensyjne kwasu masłowego jest częściowo związane z efektem naczyniorozszerzającym.
- Kwas walerianowy wywołuje krótkotrwały efekt hipotensyjny po podaniu dożylnym oraz po podaniu dookreślonym.
- Krótki czas działania hipotensyjnego kwasu walerianowego wynika prawdopodobnie z krótkiego okresu półtrwania kwasu walerianowego we krwi.
- Efekt hipotensyjny wywołany przez dookreślone podanie roztworu kwasu walerianowego związany jest z pobudzeniem receptorów dla krótkołańcuchowych kwasów tłuszczowych (GPR41/43).
- Odpowiedź hipotensyjna uzyskana po podaniu kwasu walerianowego jest związana z działaniem naczyniorozszerzającym, bez zaangażowania aferentnej impulsacji przywspółczulnej z jelita grubego.
- Efekt hipotensyjny spowodowany przez kwas masłowy i walerianowy w zastosowanych dawkach nie był związany z działaniem kardiotoksycznym.



### **Najważniejsze wnioski:**

- W warunkach fizjologicznych stężenie kwasu masłowego i kwasu walerianowego jest najwyższe w jelicie grubym, które jest głównym habitatem mikrobioty.
- Kwas masłowy i kwas walerianowy przedostają się z jelita grubego do układu krążenia powodując istotny efekt hipotensyjny, oddziałując zarówno poprzez układ nerwowy, jak również bezpośrednio na naczynia.
- Kwas masłowy i kwas walerianowy są ważnymi mediatorami oddziaływania pomiędzy mikrobiotą przewodu pokarmowego a układem krążenia.

## **Opinia Komisji Etycznej**

### **UCHWAŁA NR 535/2018**

z dnia 21 marca 2018 r.

I Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach w Warszawie

#### **§ 1**

Na podstawie art. 48 pkt. 1 ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266) zwanej dalej „ustawą” po rozpatrzeniu wniosku pt. **Wpływ kwasu masłowego na ciśnienie tętnicze u szczurów** z dnia **09.03.2018 r.** złożonego przez **Warszawski Uniwersytet Medyczny, Wydział Lekarsko-Dentystyczny ul. Banacha 1B, 02-091 Warszawa** zaplanowanego przez **dr hab. Marcina Ufnala** lokalna komisja etyczna

#### **WYRAŻA ZGODĘ**

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

#### **§ 2**

W wyniku rozpatrzenia wniosku o którym mowa w § 1, Lokalna Komisja Etyczna ustaliła, że:

1. Wniosek należy przypisać do kategorii: **DOŚWIADCZENIA NA ZWIERZĘTACH (A)**
2. Najwyższy stopień dotkliwości proponowanych procedur to: **TERMINALNA**
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków:  
**Szczur, szczep WKY 12 tygodni 81 os.,**  
**Szczur, szczep SHR 12 tygodni 81 os.**
4. Doświadczenia będą przeprowadzane przez: **Ufnal Marcin, Maksymilian Onyszkiewicz**
5. Doświadczenie będzie przeprowadzane w terminie<sup>1</sup> **01.04.2018 –01.04.2023 r.**
6. Doświadczenie będzie przeprowadzone w ośrodku
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w:
8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez
9. Doświadczenie zostanie poddane ocenie retrospektywnej w terminie **do 3 miesięcy** od dnia przekazania przez użytkownika dokumentacji, mającej stanowić podstawę dokonania oceny retrospektywnej. Użytkownik jest zobowiązany do przekazania ww. dokumentacji niezwłocznie, tj. w terminie, o którym mowa w art. 52 ust. 2 ustawy.

---

<sup>1</sup> Nie dłużej niż 5 lat

#### § 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1

##### **I LOKALNA KOMISJA ETYCZNA**

ds. Doświadczeń na Zwierzętach

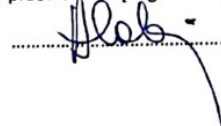
przy Wydziale Biologii UW

ul. Ilji Miecznikowa 1, 02-096 Warszawa

tel. 022 5541028, e-mail: lke1waw@biol.uw.edu.pl

(Pieczęć lokalnej komisji etycznej)

Podpisy przewodniczącego komisji



##### Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowania, decyzja staje się ostateczna i prawomocna.

##### Otrzymuje:

- 1) Użytkownik,
- 2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
- 3) a/a

##### Użytkownik kopie przekazuje:

- Osoba planująca doświadczenie
- Zespół ds. dobrostanu



## **UCHWAŁA NR 474/2017**

z dnia 21 lutego 2018 r.

**I Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach w Warszawie**

### **§ 1**

Na podstawie art. 48 pkt. 1 ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266) zwanej dalej „ustawą” po rozpatrzeniu wniosku pt. **Rola metabolitów bakterii jelitowych oraz bariery jelito-krew w regulacji ciśnienia tętniczego oraz rozwoju nadciśnienia tętniczego** z dnia 15.02.2018 r. złożonego przez **Warszawski Uniwersytet Medyczny, Wydział Lekarsko-Dentystyczny ul. Banacha 1B, 02-091 Warszawa** zaplanowanego przez **dr hab. Marcina Ufnala** lokalna komisja etyczna

### **WYRAŻA ZGODĘ**

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

### **§ 2**

**W wyniku rozpatrzenia wniosku o którym mowa w § 1, Lokalna Komisja Etyczna ustaliła, że:**

1. Wniosek należy przypisać do kategorii: **DOŚWIADCZENIA NA ZWIERZĘTACH (A)**
2. Najwyższy stopień dotkliwości proponowanych procedur to: **TERMINALNA**
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków:  
**Szczur, szczep Wistar Kyoto 12 tygodni 135 os.**
4. Doświadczenia będą przeprowadzane przez: **Ufnal Marcin, Artur Nowiński**
5. Doświadczenie będzie przeprowadzane w terminie<sup>1</sup> **10.03.2018 – 01.10.2022 r.**
6. Doświadczenie będzie przeprowadzone w ośrodku
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w:
8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez
9. Doświadczenie nie zostanie poddane ocenie retrospektywnej

---

<sup>1</sup> Nie dłużej niż 5 lat

#### § 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1

**I LOKALNA KOMISJA ETYCZNA**

ds. Doświadczeń na Zwierzętach

przy Wydziale Biologii UW

ul. Ilji Miecznikowa 1, 02-096 Warszawa

tel. 022 5541028, e-mail: lke1waw@biol.uw.edu.pl

(Pieczęć lokalnej komisji etycznej)

Podpisy przewodniczącego komisji



Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowania, decyzja staje się ostateczna i prawomocna.

Otrzymuje:

- 1) Użytkownik,
- 2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
- 3) a/a

Użytkownik kopie przekazuje:

- Osoba planująca doświadczenie
- Zespół ds. dobrostanu

# Oświadczenia wszystkich współautorów publikacji

## PUBLIKACJA nr 1

*Short chain fatty acids and methylamines produced  
by gut microbiota as mediators and markers in the  
circulatory system*

Maksymilian Onyszkiewicz

Kinga Jaworska

Marcin Ufnal



Lek. Kinga Jaworska

Warszawa, 27.04.2020

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system” oświadczam iż mój wkład merytoryczny w formie publikacji to:

Współudział w przygotowaniu manuskryptu. (20%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji.

przygotowaniu manuskryptu oraz wykonaniu figur zawartych w pracy. (60%)

*Kinga Jaworska*

(podpis współautora)

Prof. dr hab. Marcin Ufnal

Warszawa,

(Stopień/tytuł, imię, nazwisko)

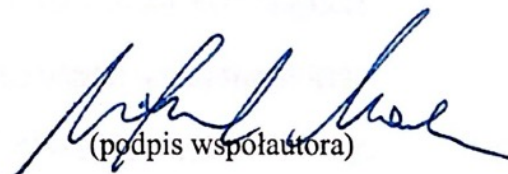
### OŚWIADCZENIE

Jako współautor pracy pt. „Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system” oświadczam iż mój wkład merytoryczny w formie publikacji to:

Współudział w przygotowaniu manuskryptu. (20%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji. przygotowaniu manuskryptu oraz wykonaniu figur zawartych w pracy (60%).



(podpis współautora)

## PUBLIKACJA nr 2

*Butyric acid, a gut bacteria metabolite, lowers  
arterial blood pressure via colon-vagus nerve  
signaling and GPR41/43 receptors*

Maksymilian Onyszkiewicz

Marta Gawryś-Kopczyńska

Piotr Konopelski

Marta Aleksandrowicz

Aneta Sawicka

Ewa Koźniewska

Emilia Samborowska

Marcin Ufnal



Mgr inż. Marta Gawryś – Kopczyńska

Warszawa, 27.06.2020

(Stopień/tytuł, imię, nazwisko)

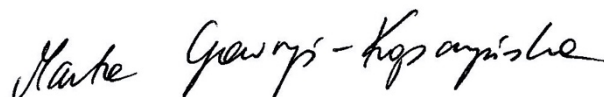
### OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przeprowadzeniu części eksperymentów. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy. (50%)



(podpis współautora)

Lek. Piotr Konopelski

Warszawa, 28.04.2020 r.

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam, iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przeprowadzeniu części eksperymentów. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy. (50%)

  
(podpis współautora)

Dr n. med. Marta Aleksandrowicz

Warszawa, 27.04.2020

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie, interpretacji eksperymentów przeprowadzonych w warunkach ex vivo oraz przygotowanie manuskryptu. (10%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy. (50%)

Marta Aleksandrowicz  
(podpis współautora)



mgr Aneta Sawicka

Warszawa, 04.05.2020r.

(Stopień/tytuł, imię, nazwisko)

## OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów przeprowadzonych w warunkach ex vivo. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy. (50%)

Aneta Sawicka

(podpis współautora)

Prof. dr hab. Ewa Koźniewska  
Instytut Medycyny Doświadczalnej i Klinicznej  
im. M. Mossakowskiego PAN

Warszawa, 27.04.2020 r.

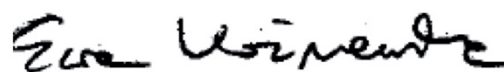
## OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów przeprowadzonych w warunkach ex vivo. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i rycin zawartych w tej pracy. (50%)



Mgr Emilia Samborowska

Warszawa, 30.04.2020

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów spektrometrycznych oraz przygotowanie manuskryptu. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymasłanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy. (50%)

Emilia Samborowska

(podpis współautora)



(Stopień/tytuł, imię, nazwisko)

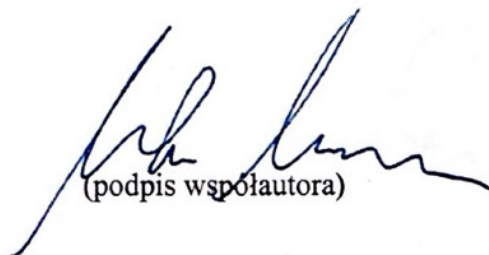
## OŚWIADCZENIE

Jako współautor pracy pt. „Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w planowaniu doświadczeń, metodologii badań, formułowaniu wniosków oraz przygotowanie manuskryptu. Współudział w przygotowaniu, analizie i interpretacji eksperymentów spektrometrycznych. (15%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu masłowego w tkankach, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu masłowego, 3-hydroksymaślanu, L-NAME, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych, wykonał większość wykresów i figur zawartych w tej pracy (50%).



(podpis współautora)

## PUBLIKACJA nr 3

*Valeric acid lowers arterial blood pressure in rats*

Maksymilian Onyszkiewicz

Marta Gawryś-Kopczyńska

Maciej Sałagaj

Marta Aleksandrowicz

Aneta Sawicka

Ewa Koźniewska

Emilia Samborowska

Marcin Ufnal

Mgr inż. Marta Gawryś – Kopczyńska

Warszawa, 24.04.2020

(Stopień/tytuł, imię, nazwisko)

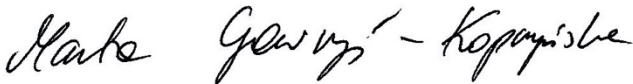
### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przeprowadzeniu części eksperymentów. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreśnionym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)

  
(podpis współautora)



Maciej Sałagaj-student

Warszawa, 28.04.2020 r.

(Stopień/tytuł, imię, nazwisko)

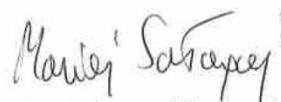
### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przeprowadzeniu części eksperymentów. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreźniczym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)

  
(podpis współautora)

Dr n. med. Marta Aleksandrowicz

Warszawa, 27.04.2020

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie, interpretacji eksperymentów przeprowadzonych w warunkach ex vivo oraz przygotowanie manuskryptu. (10%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreśnionym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)

Marta Aleksandrowicz  
(podpis współautora)

mgr Aneta Sawicka

Warszawa, 04.05.2020r.

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów przeprowadzonych w warunkach ex vivo. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreślonym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)

Aneta Sawicka

(podpis współautora)



Prof. dr hab. Ewa Koźniewska  
Instytut Medycyny Doświadczalnej i Klinicznej  
im. M. Mossakowskiego PAN

Warszawa, 27.04.2020 r.

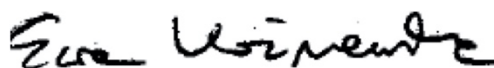
### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów przeprowadzonych w warunkach ex vivo. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookrężniczym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i rycin zawartych w tej pracy. (50%)



Mgr Emilia Samborowska

Warszawa, 30.04.2020

(Stopień/tytuł, imię, nazwisko)

### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w przygotowaniu, analizie i interpretacji eksperymentów spektrometrycznych oraz przygotowanie manuskryptu. (5%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreślonym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)

*Emilia Samborowska*

(podpis współautora)

Prof. dr hab. Marcin Ufnal

Warszawa,

(Stopień/tytuł, imię, nazwisko)

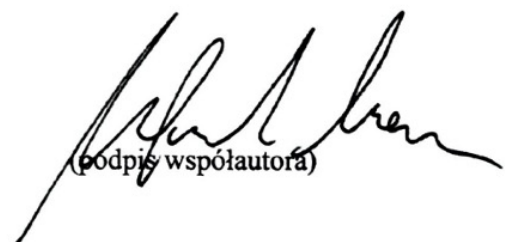
### OŚWIADCZENIE

Jako współautor pracy pt. „Valeric acid lowers arterial blood pressure in rats.” oświadczam iż mój wkład merytoryczny w przeprowadzenie badań, opracowanie wyników oraz ich przedstawienie w formie publikacji to:

Współudział w planowaniu doświadczeń, metodologii badań, formułowaniu wniosków oraz przygotowanie manuskryptu. Współudział w przygotowaniu, analizie i interpretacji eksperymentów spektrometrycznych. (15%)

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Maksymiliana Onyszkiewicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część niniejszej pracy wykazuje indywidualny wkład lek. Maksymiliana Onyszkiewicza w opracowaniu koncepcji, metodologii badań, formułowaniu wniosków, określeniu stężenia kwasu walerianowego w tkankach, określeniu dystrybucji kwasu walerianowego po podaniu dookreźniczym, przeprowadzeniu badań hemodynamicznych z użyciem: kwasu walerianowego, 3-hydroksymaślanu, atropiny oraz hexamethonium. Ponadto lek. Maksymilian Onyszkiewicz przeprowadził analizę statystyczną wyników z badań hemodynamicznych oraz wykonał większość wykresów i figur zawartych w tej pracy. (50%)



(podpis współautora)