Curr Hypertens Rep (2017) 19: 25
DOI 10.1007/s11906-017-0722-5

Microbial Short-Chain Fatty Acids and Blood Pressure Regulation

Jennifer L. Pluznick[1]

Published online: 17 March 2017

Abstract
Purpose of Review Microbial short-chain fatty acids (SCFAs) are byproducts of microbial metabolism which can be absorbed into the bloodstream of the host, where they exert effects on host physiology. SCFAs have been known to influence several aspects of host physiology, including the regulation of blood pressure. In this review, we will consider recent studies linking SCFAs to blood pressure regulation.
Recent Findings Several recent studies have found that changes in blood pressure often coordinate with expected changes in SCFAS. Efforts are now well underway to dissect and better understand this potential connection. One way that SCFAs can influence host cells is by interacting with host GPCRs, including Gpr41 and Olfr78, among others. Intriguingly, mice null for Olfr78 are hypotensive, whereas mice null for Gpr41 are hypertensive, implying that these pathways may be physiologically important links between SCFAs and host blood pressure control.
Summary In sum, these studies demonstrate that there does indeed appear to be a link between SCFAs and blood pressure, which likely involves host GPCRs, at least in part; however, the details and intricacies of these interactions are not yet fully understood and will greatly benefit from further studies.

Keywords Gut microbiome · Blood pressure · SCFAs · Hypertension · Gpr41 · Olfr78

This article is part of the Topical Collection on *Gut Microbiome, Sympathetic Nervous System, and Hypertension*

✉ Jennifer L. Pluznick
jpluznick@jhmi.edu

[1] Department of Physiology, Johns Hopkins University School of Medicine, 725 N Wolfe St, WBSB 205, Baltimore, MD 21205, USA

Introduction

Recent studies have begun to accumulate evidence that the gut microbiota and blood pressure regulation are linked [1••, 2•, 3•, 4••, 5•]. One subset of this evidence is focused on the role of short-chain fatty acids (SCFAs) in modulating blood pressure control. As described below, SCFAs are gut microbial metabolites which can bind to and activate host receptors, thereby acting as a route of "communication" between gut microbial metabolism and host physiology. This review will: discuss SCFAs themselves and the evidence linking them to blood pressure regulation, review the known host receptors for SCFAs, and discuss the blood pressure phenotypes of mice null for SCFA receptors. Finally, this review will attempt to integrate what is currently known into a cohesive view of how SCFAs and their receptors may influence blood pressure control.

Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are metabolites produced by the gut microbiota as a byproduct of the fermentation of dietary fiber. SCFAs are subsequently absorbed into the bloodstream of the host, where they can interact with host proteins in order to influence host physiology. It is worth noting that plasma SCFAs levels in the host are dependent upon microbial production: studies have shown that serum SCFA levels correlate with dietary fiber levels [6], and studies comparing conventional and germ-free animals found that SCFA concentrations in the cecum are enhanced over 100-fold by the presence of gut microbiota [7]. In fact, the three primary short-chain fatty acids (acetate, propionate, and butyrate) are virtually undetectable in germ-free animals [8]. Although acetate is

typically the most abundant SCFA in the plasma (~100 μM on a standard diet [8]), the ratio of acetate/butyrate/propionate can vary, especially with dietary manipulation [6, 9–11].

Lessons from Studying and Manipulating SCFAs

Reports in the clinical literature are supportive of the concept that microbial SCFAs lower blood pressure. For example, manipulations which would be expected to elevated plasma SCFAs (probiotic use, increased dietary fiber intake) also are associated with decreases in blood pressure [12, 13]. Conversely, a study of > 4000 humans found that lowered levels of urinary formate (a SCFA) correlate with increases in blood pressure [14]. Intriguingly, acetate was previously included as a competent of hemodialysis buffers, but this has been largely discontinued because acetate was found to cause hypotension in patients again, consistent with the concept that SCFAs lower blood pressure [15, 16].

Several studies have attempted to purposely manipulate SCFAs in animal models in order to determine whether this can alter blood pressure regulation, and/or to determine whether SCFA levels may change in hypertensive models. In general, it appears that manipulations which are expected to increase SCFAs are associated with lower blood pressure, for example, a recent study found that a high fiber diet in DOCA-salt hypertensive mice increased the abundance of bacteria thought to be acetate-producers and also lowered blood pressure [17]. Although plasma acetate levels were not measured in this study, the authors reported that acetate supplementation had a similar effect on blood pressure. In addition, decreases in bacterial taxa thought to produce short-chain fatty acids have been reported in two different rat models of hypertension [1••, 5•], but plasma levels of SCFAs were not measured directly in these studies. Conversely, another study [4••] (which did measure SCFA levels) found that rats which received a microbial transplant which increased blood pressure had *higher* levels of plasma acetate. Thus, we clearly have much more work to do before we fully understand the connection between SCFAs and blood pressure. In addition, although these studies show correlations between SCFAs and blood pressure, in the future, it will be important to identify whether there is a causative link.

Many studies now take advantage of bioinformatics analysis of microbial sequences in order to "predict" whether SCFA levels would be expected to increase or to decrease (i.e., "acetate-producing" bacteria are increased or decreased with a given treatment). Although this is a logical and valid way to utilize the massive amount of sequencing data that is generated in this field, it is not yet clear to what extent these predictions have been validated. In fact, one recent study [18] showed an increase in acetate-producing bacteria by sequencing analysis, but did not reveal any measurable changes in fecal acetate. There are many potential explanations for such a finding, changes could be transient (peaking after mealtimes?), and fecal levels may not reflect SCFAs levels in other compartments (plasma). In addition, it should also be noted that we understand relatively little about the microbiota—it is possible that a point mutation in an "acetate producing" strain of bacteria could drastically decrease acetate production, without altering the bioinformatics analysis. Clearly, future studies are required in order for us to better understand both the microbes and host, and the nuances of their interactions. In addition, in the future, it will be necessary to carefully elucidate the pathways and mechanisms which underly the correlations seen between SCFAs and blood pressure.

Short-Chain Fatty Acid Receptors

In order to begin to understand the physiological actions of SCFAs, we must address the cellular mechanisms which underlie SCFA signaling. SCFAs are known to mediate effects on the host through a variety of mechanisms, including alterations in histone acetylation and cell proliferation. For the purposes of this review, however, we will concentrate on the role of SCFAs as ligands for host GPCRs. SCFAs are known to be ligands for a number of host GPCRs, including Gpr41, Gpr43, Gpr109a, and Olfr78. In this section, we will briefly review the cell biology of the known SCFA GPCRs.

Gpr41 and Gpr43 were both first described as SCFA receptors by two reports published in 2003 [19, 20]. Gpr41 couples to Gi, whereas Gpr43 couples to both Gi and/or Gq. Propionate is the strongest ligand (μM range) for both Gpr41 and Gpr43, although both receptors also respond to several other compounds including acetate and butyrate. It is worth noting that an additional receptor, Gpr42, is present in humans; however, although Gpr42 is quite homologous to Gpr41, it is as of yet unclear whether it may be a pseudogene [19, 21]. Gpr41 is also known as Free Fatty Acid Receptor 3 (FFAR3), and Gpr43 is also known as Free Fatty Acid Receptor 2 (FFAR2).

Although Gpr41 and Gpr43 have been studied fairly extensively, Gpr109a is not nearly as well studied. Unlike Gpr41 and Gpr43, Gpr109a does not respond to acetate or propionate, but only to butyrate (EC_{50}~1 mM) [22]. Gpr09a also responds to niacin and beta-D-hydroxybutyrate [22–24]. Gpr109a is also known as hydroxycarboxylic acid receptor 2 (HCA2), Niacin receptor 1 (NIACR1), PUMA-G, and HM74a.

An additional SCFA receptor is an olfactory receptor known as Olfr78 in mice, and OR51E2 in humans. Although this receptor is included here as an SCFA receptor, it should be noted that it has been reported to respond to additional ligands as well (summarized in Tables 1 and 2). OR51E2 (the human ortholog) was

Table 1 Reported Ligands for Olfr78

	Mouse receptor: Olfr78			
	Pluznick et al. 2013	Chang et al. 2015	Zhou et al. 2016	Aisenberg et al. 2016
Acetate	YES	YES	YES	YES
Propionate	YES	YES	YES	YES
Lactate		YES	YES	YES
β-ionone	no			

"YES" indicates the compound was found to be a ligand for the receptor and "no" indicates it was not. A blank space indicates that the compound was not tested in this study

first deorphanized by two groups in 2009: one reported that OR51E2 was a receptor for β-ionone and other androgens [25] and the second group reported that OR51E2 is a receptor for propionate [26]. A 2013 paper by an independent group confirmed that OR51E2 responded to propionate as well as acetate, but did not detect action of OR51E2 by β-ionone [3•]. Similarly, it was found that Olfr78 (the murine ortholog) responded to acetate and propionate, but not β-ionone [3•]. However, a study in 2016 did show evidence that β-ionone is a functional ligand: this study demonstrated that cells natively expressing OR51E2 exhibited an increase in intracellular calcium in response to β-ionone which was negated by transfection of siRNA for OR51E2 [27]. In late 2015, it was reported that Olfr78 responded to lactate in addition to acetate and propionate [28], and this was confirmed by two other groups in 2016 [29, 30]. Notably, microbial metabolism also contributes to circulating lactate levels. Lactate, however, is a partial agonist of Olfr78 (acetate/propionate are full agonists) and is a ligand for (murine) Olfr78 but not for (human) OR51E2 [29]. The reported EC50 of lactate has varied between different reports, from reported EC50's of ~4 mM [28] vs. ~21 mM [29]. Therefore, although the ligand profile of this receptor has been fairly well studied, there still is not a clear consensus on which ligands are most physiologically relevant. For the purpose of this review, we will primarily consider this receptor as an SCFA receptor.

Lessons from Olfr78 Null Mice

To better understand how Olfr78 may influence blood pressure regulation, it can be useful to consider the phenotype of Olfr78 null animals. First of all, it is important to note that Olfr78 localizes to at least two tissues which might be expected to influence blood pressure regulation: (a) the renal afferent arteriole and (b) vascular smooth muscle cells in the peripheral vasculature [3•]. The renal afferent arteriole is a particularly intriguing place to find Olfr78 localization, as this is the primary location where renin is stored and secreted from. In agreement with this, it was found that Olfr78 null mice had lowered plasma renin levels and lowered blood pressure. However, it is reasonable to assume that the expression of Olfr78 in vascular smooth muscle cells in peripheral vessels may also influence baseline blood pressure in Olfr78 KO. In the future, it will be necessary to use tissue-specific KOs in order to conclusively determine to what extent expression at each site influences blood pressure regulation.

Lessons from Gpr41 Null Mice

An initial study indicated that Gpr41 and Olfr78 likely played opposing roles in the regulation of blood pressure [3•]. In agreement with this, a subsequent study reported that Gpr41 null mice are hypertensive (the "opposite" phenotype of the hypotensive Olfr78 null animals) [2•]. Intriguingly, these mice have isolated

Table 2 Reported ligands for OR51E2

	Human receptor: OR51E2				
	Neuhaus et al. 2009	Saito et al. 2009	Pluznick et al. 2013	Gelis et al. 2016	Aisenberg et al. 2016
Acetate			YES		YES
Propionate		YES	YES		YES
Lactate					no
β-ionone	YES		no	YES	

"YES" indicates the compound was found to be a ligand for the receptor and "no" indicates it was not. A blank space indicates that the compound was not tested in this study

systolic hypertension, a hypertension of "vascular" origin (as opposed to the hypotension in Olfr78 null mice, which seems to be largely renin-driven). In agreement with a vascular form of hypertension in the Gpr41 KOs, Gpr41 was found to localize to the vascular endothelium. Gpr41 KO mice have increased pulse pressure (in older animals); however, vessels studied ex vivo do not appear to be "stiffer" [2•]. Therefore, if the vessels "behave" stiffer in vivo, but not ex vivo, it may be that there is an increase in intrinsic vascular tone in these animals which drives the hypertension. Intriguingly, SCFAs are known to induce vasorelaxation [31–34], so it may be that SCFAs acting on Gpr41 in the vascular endothelium help to set vascular tone.

An Integrated View of SCFAs, SCFA Receptors, and Blood Pressure Regulation

An experiment which highlights the dual roles of these two receptors in blood pressure regulation was a study in which blood pressure was measured in wild-type and Olfr78 KO mice before and after treatment with a mixture of antibiotics [3•]. Antibiotic treatment dramatically reduced fecal microbiota and also differentially affected blood pressure. Whereas wild-type mice had a mild increase in blood pressure on antibiotics, Olfr78 KO mice had a much more dramatic increase. This is thought to be due to the fact that Olfr78 and Gpr41 normally act in physiologically opposite roles in response to the same stimulus (SCFAs). Therefore, in wild-type mice, it has been proposed that the two receptors balance each other out to avoid "wild swings" in blood pressure. Thus, when antibiotics are given (and thus the amount of SCFA ligands are decreased), both receptors decrease their signaling in parallel in wild-type animals. However, when one receptor is absent (as in Olfr78 KO mice), the effect of the antibiotics on SCFAs is solely affecting Gpr41 signaling. Therefore, antibiotic treatment reveals a clear unidirectional change in blood pressure in the Olfr78 KO mice.

It may seem puzzling that Olfr78 and Gpr41 are activated by the same stimulus, yet work in opposition to one another with regards to physiology. However, the explanation for this may be the very different $EC_{50's}$ for these two receptors. Gpr41 has a relatively low EC_{50} and is likely to be at least partially activated at basal concentrations of SCFAs, and therefore would favor decreasing blood pressure. In contrast, Olfr78 has a much higher EC_{50} and would only be activated when SCFA concentrations rise significantly. Thus, Olfr78 may act as a safety "brake" on Gpr41-mediated decreases in blood pressure, to prevent inappropriate levels of hypotension when SCFA levels rise [35].

Conclusions

Clearly, the role of SCFAs in blood pressure regulation is multi-faceted, involves at least two different SCFA receptors, multiple species of bacteria, and multiple host tissues. In the future, it will be important to explore how we can use both genetics (tissue-specific knockouts) and pharmacology (agonists and antagonists) to manipulate these pathways—this will allow us both to better understand the physiology—and perhaps, to explore potential opportunities to leverage these pathways to manipulate blood pressure in a purposeful way.

Acknowledgements I am grateful for support from the NIH NIDDK (R01DK-107726), NIH NHLBI (R01HL-128512), and the AHA (16IRG27260265), and to members of the Pluznick Lab, especially Dr. Niranjana Natarajan, for helpful discussions.

Compliance with Ethical Standards

Conflict of Interest Dr. Pluznick reports grants from NIH NIDDK (R01DK-107,726), NIH NHLBI (R01HL-128,512), and the AHA (16IRG27260265).

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.•• Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, et al. Gut dysbiosis is linked to hypertension. Hypertension. 2015;65(6):1331–40. **This paper showed that changes in the gut microbiota, including changes in SCFA-producing bacteria, are associated with hypertension in the SHR model; in addition, this study provides evidence that gut dysbiosis occurs in human hypertension**

2.• Natarajan N, Hori D, Flavahan S, Steppan J, Flavahan NA, Berkowitz DE, et al. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G-protein coupled receptor 41. Physiol Genomics. 2016;physiolgenomics 00089 2016. **This paper demonstrated that Gpr41 null animals have a phenotype including isolated systolic hypertension, further indicating a role for SCFAs and SCFA receptors in blood pressure regulation**.

3.• Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, et al. 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. 2013;110(11):4410–5. **This paper demonstrated that Olfr78 null animals have a phenotype including lowered plasma renin, indicating a role for SCFAs and SCFA receptors in blood pressure regulation**

4.•• Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, et al. Evidence for a link between gut microbiota and hypertension in the

Dahl rat model. Physiol Genomics. 2015;47(6):187–97. **This paper provided evidence for a link between gut microbiota and blood pressure in the Dahl salt-sensitive rat model of hypertension, including measurement of plasma SCFA levels**

5.• Durgan DJ, Ganesh BP, Cope JL, Ajami NJ, Phillips SC, Petrosino JF, et al. Role of the gut microbiome in obstructive sleep apnea-induced hypertension. Hypertension. 2016;67(2):469–74. **This paper demonstrated changes in the gut microbiota in the rat obstructive sleep apnea/high fat diet model of hypertension, including changes in SCFA-producing microbiota**

6. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20(2):159–66.
7. Hoverstad T, Midtvedt T. Short-chain fatty acids in germfree mice and rats. J Nutr. 1986;116(9):1772–6.
8. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. Nature. 2016;534(7606):213–7.
9. Cummings JH, Hill MJ, Bone ES, Branch WJ, Jenkins DJ. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. Am J Clin Nutr. 1979;32(10):2094–101.
10. 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(9):2325–40.
11. Levrat MA, Remesy C, Demigne C. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J Nutr. 1991;121(11):1730–7.
12. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension. 2014;64(4):897–903.
13. Whelton SP, Hyre AD, Pedersen B, Yi Y, Whelton PK, He J. Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. J Hypertens. 2005;23(3):475–81.
14. Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, et al. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature. 2008;453(7193):396–400.
15. Keshaviah PR. The role of acetate in the etiology of symptomatic hypotension. Artif Organs. 1982;6(4):378–87.
16. Pagel MD, Ahmad S, Vizzo JE, Scribner BH. Acetate and bicarbonate fluctuations and acetate intolerance during dialysis. Kidney Int. 1982;21(3):513–8.
17. Marques FZ, Nelson EM, Chu PY, Horlock D, Fiedler A, Ziemann M, et al. High fibre diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in DOCA-salt hypertensive mice. Circulation. 2016.
18. Adnan S, Nelson JW, Ajami NJ, Venna VR, Petrosino JF, Bryan RM, Jr., et al. Alterations in the gut microbiota can elicit hypertension in rats. Physiol Genomics. 2016;physiolgenomics 00081 2016.
19. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, et al. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003;278(13):11312–9.
20. Le PE, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem. 2003;278(28):25481–9.
21. Liaw CW, Connolly DT. Sequence polymorphisms provide a common consensus sequence for GPR41 and GPR42. DNA Cell Biol. 2009;28(11):555–60.
22. Thangaraju M, Cresci GA, Liu K, Ananth S, Gnanaprakasam JP, Browning DD, et al. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res. 2009;69(7):2826–32.
23. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014;40(1):128–39.
24. Taggart AK, Kero J, Gan X, Cai TQ, Cheng K, Ippolito M, et al. (D)-beta-hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. J Biol Chem. 2005;280(29):26649–52.
25. Neuhaus EM, Zhang W, Gelis L, Deng Y, Noldus J, Hatt H. Activation of an olfactory receptor inhibits proliferation of prostate cancer cells. J Biol Chem. 2009;284(24):16218–25.
26. Saito H, Chi Q, Zhuang H, Matsunami H, Mainland JD. Odor coding by a mammalian receptor repertoire. Sci Signal. 2009;2(60):ra9. 1-14
27. Gelis L, Jovancevic N, Veitinger S, Mandal B, Arndt HD, Neuhaus EM, et al. Functional characterization of the odorant receptor 51E2 in human melanocytes. J Biol Chem. 2016;291(34):17772–86.
28. Chang AJ, Ortega FE, Riegler J, Adison DVM, Krasnow MA. Oxygen regulation of breathing through an olfactory receptor activated by lactate. Nature. 2015;527(7577):240–4.
29. Aisenberg WH, Huang J, Zhu W, Rajkumar P, Cruz R, Santhanam L, et al. Defining an olfactory receptor function in airway smooth muscle cells. Sci Rep. 2016;6:38231.
30. Zhou T, Chien MS, Kaleem S, Matsunami H. Single cell transcriptome analysis of mouse carotid body glomus cells. J Physiol. 2016;594(15):4225–51.
31. Nutting CW, Islam S, Daugirdas JT. Vasorelaxant effects of short chain fatty acid salts in rat caudal artery. Am J Phys. 1991;261(2 Pt 2):H561–H7.
32. Mortensen FV, Nielsen H, Mulvany MJ, Hessov I. Short chain fatty acids dilate isolated human colonic resistance arteries. Gut. 1990;31(12):1391–4.
33. Nutting CW, Islam S, Ye MH, Batlle DC, Daugirdas JT. The vasorelaxant effects of acetate: role of adenosine, glycolysis, lyotropism, and pHi and Cai2+. Kidney Int. 1992;41(1):166–74.
34. Knock G, Psaroudakis D, Abbot S, Aaronson PI. Propionate-induced relaxation in rat mesenteric arteries: a role for endothelium-derived hyperpolarising factor. J Physiol. 2002;538(Pt 3):879–90.
35. Pluznick J. A novel SCFA receptor, the microbiota, and blood pressure regulation. Gut Microbes. 2014;5(2):202–7.