Exp Physiol 101.4 (2016) pp 478–481

Symposium Report

Olfaction in the kidney: 'smelling' gut microbial metabolites

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New Findings

- **What is the topic of this review?**
  This review covers recent findings highlighting roles for renal and vascular sensory receptors that modify blood pressure control in response to changes in gut microbial metabolites.
- **What advances does it highlight?**
  This review highlights the novel roles that G-protein-coupled receptor 41 and olfactory receptor 78 play in blood pressure regulation.

The gut microbiota have recently been recognized as an important component of host physiology and pathophysiology. Our recent studies have shown that a subset of gut microbial metabolites, known as short-chain fatty acids, act as ligands for host G-protein-coupled receptors (G-protein-coupled receptor 41 and olfactory receptor 78). Short-chain fatty acid-mediated activation of G-protein-coupled receptor 41 and olfactory receptor 78 modulates blood pressure control, both by modulating renin secretion and by modulating vascular tone directly. Further studies are needed in order to gain a better understanding of the underlying mechanism by which microbiota and microbial metabolites modulate host physiology and their potential implications in health and disease.

(Received 27 May 2015; accepted after revision 23 July 2015; first published online 2 August 2015)
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Introduction

The role of the gut microbiota in host physiology is a rapidly emerging and expanding field. Until 2010, there were fewer than 1000 papers per year in PubMed with 'microbiota' as a keyword; in 2011–2014 there were 1324, 1900, 2824 and 3735, respectively. The virtual explosion of information about this topic in recent years is the result of a rapidly growing recognition of the important role that the gut microbiota play in host physiology. Indeed, microbial cells outnumber human cells 10:1 (Sekirov *et al.* 2010), and correlations have now been documented showing that changes in the composition of the microbiota are associated with a variety of pathophysiological processes. However, with this newly found, massive database of information at our fingertips, it is critically important that we work not only to document correlations, but also to understand the mechanisms underlying these host–microbe interactions. In our work, we have focused on a role of the gut microbiota in the regulation of blood pressure and we are now focused on understanding both the underlying mechanism and the potential implications of microbial metabolites and microbiome composition in health and disease (i.e. hypertension).

Our accidental interest in the gut microbiota began via studies of novel sensory receptors found in the kidney, which is the primary focus of our group. An emerging pattern in physiology is that 'sensory' receptors (olfactory receptors, taste receptors and other G-protein-coupled receptors) play an important role as specialized chemosensors in a variety of tissues. For example, sour taste receptors sense pH in the tongue to mediate sour taste, but are also expressed in the spinal

DOI: 10.1113/EP085285

canal, where they monitor pH changes in the cerebrospinal fluid (Huang *et al.* 2006). Likewise, bitter taste receptors play roles in ciliary beat frequency and bronchodilatation in the lung (Shah *et al.* 2009; Deshpande *et al.* 2010). Olfactory receptors play roles in the migration of muscle cells (Griffin *et al.* 2009) and the chemotaxis of sperm (Spehr *et al.* 2003), and we have recently shown that olfactory receptors are expressed in the kidney. One of the novel renal G-protein-coupled receptors we are interested in is olfactory receptor 78 (Olfr78; Pluznick *et al.* 2009). Although our original intention was to study the role of Olfr78 in whole-animal physiology, through the course of these studies we came to realize that the functional role of Olfr78 is intimately tied together with short-chain fatty acid (SCFA) metabolites produced by the gut microbiota by breaking down complex dietary polysaccharides (Pluznick *et al.* 2013; Pluznick, 2013).

The major source of SCFAs in the circulation is microbial metabolism, as indicated by Høverstad & Midtvedt (1986). Their analysis of SCFAs in conventional and germ-free animals showed that the magnitude of SCFA production in the gastrointestinal tract is enhanced by up to 100-fold by the presence of gut microbiota. Their study demonstrated the key role of commensal microbiota in SCFA production. In addition, Trompette *et al.* (2014) demonstrated that SCFA levels in the circulation correlated with the amount of fibre in the diet in conventional mice, indicating that microbial production of SCFAs is an extremely influential determinant of serum SCFA concentrations. Trompette *et al.* (2014) demonstrated that suppression of microbial SCFA production using a low-fibre diet lowered caecal SCFAs by ∼50% and lowered serum SCFAs by ∼75% (from ∼1.3 mM on control diet to ∼300 uM on a low-fibre diet). Short-chain fatty acids produced in the colon are then taken up into the circulation by monocarboxylate transporters (Coady *et al.* 2004) and by free diffusion. Thus, although changes in host metabolism or in dietary intake may additionally modulate serum SCFA concentrations, it is clear that gut microbial production is a major determinant of circulating SCFAs.

Localization of Olfr78 and ligands

Olfactory receptor 78, like most olfactory receptors, was an orphan receptor with no identified ligand when we began our studies. Although we knew that Olfr78 was expressed in the murine kidney by RT-PCR, its cell-specific localization was unknown. Initially, therefore, we worked to identify the cell type of expression as well as the ligand profile for the receptor. Using a reporter-gene mouse model, in which β-galacatosidase is driven by the native Olfr78 reporter (Bozza *et al.* 2009), we found that Olfr78 localizes to the renal afferent arteriole, the primary site where renin is stored and secreted in the kidney (Pluznick *et al.* 2013). In addition, we localized Olfr78 to vascular resistance beds in a variety of tissues beyond the kidney (skeletal muscle, skin, diaphragm, etc.; Pluznick *et al.* 2013). As both of the identified tissue types – the peripheral vasculature (both conduit vessels and resistance beds) and the renal afferent arteriole – that express Olfr78 are classically associated with blood pressure regulation, we began to consider whether this receptor might play a role in blood pressure regulation. Short-chain fatty acids, upon absorption from the colon, reach the peripheral vasculature (Trompette *et al.* 2014) and potentially also the renal circulation, both sites where they can activate Olfr78; this led us to hypothesize that Olfr78 may modulate blood pressure in response to changes in gut microbial metabolites.

Furthermore, we performed a ligand screen for Olfr78 and determined that it is activated by two compounds, acetate and propionate, which are two- and three-carbon SCFAs (Pluznick *et al.* 2013). This surprising result is what first led us to consider a potential role for Olfr78 in mediating host–microbiome interactions, because the primary source of SCFAs in the plasma is the metabolic production by the gut microbiota (Hoverstad & Midtvedt, 1986; Bugaut, 1987). In the colon, the key site of SCFA production, SCFA concentrations range up to 100 mM (Bugaut, 1987). Sodium-coupled monocarboxylate transporters absorb SCFAs from the colon into the plasma (Natarajan & Pluznick, 2014), where they have been reported to be in the 0.1–10 mM range (Le Poul *et al.* 2003; Samuel & Gordon, 2006; Maslowski *et al.* 2009; Trompette *et al.* 2014). We found that Olfr78 had an EC_{50} of 920 μM for propionate and 2.35 mM for acetate. The identification of Olfr78 as an SCFA receptor localized to the afferent arteriole and vascular resistance beds led us to hypothesize that Olfr78 may modulate blood pressure in response to changes in gut microbial metabolites.

Function of Olfr78 in the afferent arteriole

To assay a potential role of Olfr78 in renin secretion in the afferent arteriole, glomeruli from Olfr78 wild-type and knockout (WT and KO) mice were studied *ex vivo* and labelled with quinacrine. Quinacrine is a dye which naturally accumulates in acidic granules; therefore, it labels renin-containing granules, and the change in quinacrine fluorescence over time is an indicator of renin release. Using this method, it was found that propionate (an SCFA) induced renin release in WT but not KO mice. In agreement with this, Olfr78 KO mice were found to have lower plasma renin and lower baseline blood pressure (Pluznick *et al.* 2013). Thus, Olfr78 in the afferent arteriole mediates renin release in response to SCFAs.

Two SCFA receptors and blood pressure regulation

As noted above ("Localization of Olfr78 and ligands"), Olfr78 was also localized to resistance beds in the peripheral vasculature. Several reports in the literature indicate that SCFAs induce vasorelaxation in *ex vivo* preparations (Mortensen *et al.* 1990; Nutting *et al.* 1991, 1992); therefore, we set out to determine whether there was an *in vivo* consequence to this *ex vivo* effect and, if so, the role of Olfr78. Our studies showed that an intravenous dose of propionate caused a rapid, reproducible and dose-dependent drop in blood pressure in anaesthetized mice (Pluznick *et al.* 2013). Surprisingly, we found that Olfr78 KO mice were hypersensitive to lower doses of propionate, and Olfr78 KO mice exhibited an exaggerated blood pressure response. Therefore, we concluded that Olfr78 modifies this response but is not the primary mediator. Further investigations revealed that the hypotensive response to propionate is primarily mediated by G-protein-coupled receptor 41 (Gpr41; Pluznick *et al.* 2013), another SCFA receptor (Brown *et al.* 2003; Le Poul *et al.* 2003), which had previously been studied for its role in modulating host metabolism (Xiong *et al.* 2004; Samuel *et al.* 2008). Currently, we are working to elucidate the role of Gpr41 in blood pressure regulation via microbial metabolites and are investigating ways in which we may be able to manipulate this novel pathway for therapeutic benefit.

Reflections

It may well seem paradoxical that Olfr78 increases blood pressure in response to SCFAs and Gpr41 mediates a decrease in blood pressure in response to the same ligand. However, we believe that this apparent paradox is explained by the rather different EC_{50} values for these two receptors. While plasma SCFAs range between 0.1 and 10 mM (Le Poul *et al.* 2003; Samuel & Gordon, 2006; Maslowski *et al.* 2009; Trompette *et al.* 2014), Gpr41 has an EC_{50} of 100–300 μM for propionate (Brown *et al.* 2003; Le Poul *et al.* 2003), whereas Olfr78 has an EC_{50} of 0.9 mM for propionate (Pluznick *et al.* 2013). Thus, we contemplate that Gpr41 is likely to be at least partly active in basal conditions, whereas Olfr78 would be activated only by higher plasma SCFA levels. Therefore, we hypothesize that Gpr41 serves as a tonic input to lower blood pressure in response to SCFAs (this would imply that Gpr41 KO mice are hypertensive at baseline, a hypothesis we are now testing). Activation of Gpr41 would increase with an increase in plasma SCFA concentration and further decrease blood pressure, up to a point. When plasma SCFA levels reach the upper bound of the range, Olfr78 will also be activated, acting as a 'brake' on this pathway to prevent inappropriate hypotension.

Moreover, we have often wondered why gut microbes and blood pressure would be interrelated. One possibility is that, at a local level, SCFAs serve to mediate local vasodilatation of vessels around the colon in order to ensure efficient nutrient absorption from the gut. The elevated SCFAs produced by the gut microbiota during digestion could act much like intestinal vasopeptides, leading to local vasodilatation via Gpr41 and ensuring that nutrients are not lost in the stool. Although it is not yet fully understood why the gut microbiota would be tied to systemic blood pressure – or, for that matter, to other aspects of host physiology – there is evidence that systemic blood pressure may be affected by microbial activity *in vivo*; postprandial hypotension is fairly common in the elderly (Van Orshoven *et al.* 2010), and the use of acetate as a buffer for haemodialysis is associated with hypotensive responses in patients (Keshaviah, 1982; Pagel *et al.* 1982). An ideal experiment to confirm the relationship between microbial metabolites and blood pressure regulation would be to examine blood pressure in germ-free mice. Unfortunately, owing to inherent technical difficulties of handling and experimenting with germ-free mice, to date these experiments have not been performed to our knowledge.

Finally, with regard to potential translation of this work, it will be interesting to determine whether being colonized with a microbial strain that produces high (or low) amounts of SCFAs may alter baseline blood pressure. Can manipulating gut microbiota – by diet (prebiotics, probiotics) or antibiotic treatment – alter long-term blood pressure control? Clearly, there is much that remains to be uncovered and understood with regard to these novel pathways. In the future, we hope that better understanding of the interactions between SCFAs, host receptors and blood pressure will lead to novel insight into physiology, as well as the potential for future therapeutics.

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Additional information

Competing interests

None declared.

Author contributions

NN and JLP outlined, drafted, edited and revised the manuscript. Both NN and JLP approved the final manuscript.

Funding

This work was supported by funding from the American Heart Association (Mid-Atlantic Affiliate Maggie Wimsatt Memorial Predoctoral Fellowship, 14PRE20090006, to N.N.) and the Hopkins Digestive Diseases Basic and Translational Research Core Center (to J.L.P.).

Acknowledgements

We are grateful to members of the Pluznick Laboratory for helpful discussions.