Endothelin-1 Mediates the Systemic and Renal Hemodynamic Effects of GPR81 Activation

Natalie K. Jones, Kevin Stewart, Alicja Czopek, Robert I. Menzies, Adrian Thomson, Carmel M. Moran, Carolyn Cairns, Bryan R. Conway, Laura Denby, Dawn E.W. Livingstone, John Wiseman, Patrick W. Hadoke, David J. Webb, Neeraj Dhaun, James W. Dear, John J. Mullins, Matthew A. Bailey

Abstract—GPR81 (G-protein-coupled receptor 81) is highly expressed in adipocytes, and activation by the endogenous ligand lactate inhibits lipolysis. GPR81 is also expressed in the heart, liver, and kidney, but roles in nonadipose tissues are poorly defined. GPR81 agonists, developed to improve blood lipid profile, might also provide insights into GPR81 physiology. Here, we assessed the blood pressure and renal hemodynamic responses to the GPR81 agonist, AZ5538. In male wild-type mice, intravenous AZ5538 infusion caused a rapid and sustained increase in systolic and diastolic blood pressure. Renal artery blood flow, intrarenal tissue perfusion, and glomerular filtration rate were all significantly reduced. AZ5538 had no effect on blood pressure or renal hemodynamics in Gpr81−/− mice. Gpr81 mRNA was expressed in renal artery vascular smooth muscle, in the afferent arteriole, in glomerular and medullary perivascular cells, and in pericyte-like cells isolated from kidney. Intravenous AZ5538 increased plasma ET-1 (endothelin 1), and pretreatment with BQ123 (endothelin-A receptor antagonist) prevented the pressor effects of GPR81 activation, whereas BQ788 (endothelin-B receptor antagonist) did not. Renal ischemia-reperfusion injury, which increases renal extracellular lactate, increased the renal expression of genes encoding ET-1, KIM-1 (Kidney Injury Molecule 1), collagen type 1-α1, TNF-α (tumor necrosis factor-α), and F4/80 in wild-type mice but not in Gpr81−/− mice. In summary, activation of GPR81 in vascular smooth muscle and perivascular cells regulates renal hemodynamics, mediated by release of the potent vasoconstrictor ET-1. This suggests that lactate may be a paracrine regulator of renal blood flow, particularly relevant when extracellular lactate is high as occurs during ischemic renal disease. (Hypertension. 2020;75:00-00. DOI: 10.1161/HYPERTENSIONAHA.119.14308.) • Data Supplement

Key Words: blood pressure • hypoxia • ischemia-reperfusion-injury • pericytes • renal blood flow

GPR81 (G-protein-coupled receptor 81), GPR109a, and GPR109b form the hydroxycarboxylic acid receptor subfamily. Encoded by the HCAR1 gene, GPR81 is predominantly expressed in brown and white adipose tissue. L-lactate is the endogenous ligand with an EC50 of ≈5 mmol/L. Lactate, formed from pyruvate during anaerobic glycolysis, has a physiological plasma concentration of 0.5 to 2 mmol/L, rising from 10 to 30 mmol/L during intense exercise or prolonged hypoxia. This suggests that GPR81 is either physiologically quiescent basally or that it is primarily responsive to the local, rather than circulating, concentration of lactate. In adipocytes, activation of GPR81 by L-lactate prevents lipid breakdown and promotes storage of energy-rich metabolites in adipocytes. Synthetic GPR81 agonists are potential therapies for dyslipidemia and inhibit lipolysis in cultured adipocytes and in vivo.

GPR81 is also expressed in brain, kidney, liver, skeletal muscle, and immune cells, but the function in nonadipose tissue is poorly defined and the limited data is somewhat contradictory. In mouse macrophages and human monocytes, GPR81 activation suppresses Toll-like receptor pathways, preventing NLRP3 (NACHT, LRR and PYD domains-containing protein 3) inflammasome activation and cell death. This mechanism appears protective, with receptor activation reducing tissue injury in models of hepatitis, pancreatitis, and colitis. GPR81 inhibition/knockdown has also been shown to be protective, enhancing neuron survival in cerebral ischemia and slowing cancer growth, partly due to effects on the vasculature. For example, GPR81 knockdown in a breast cancer cell line suppressed VEGF (vascular endothelial growth factor) and amphiregulin, retarding angiogenesis. Similarly, in the sensorimotor cortex of GPR81 knockout mice, GPR81 agonists decreased inflammation-related cell death, suggesting a role in protecting neurons in the central nervous system. The exact role of GPR81 in the central nervous system remains to be determined.
knockout mice (Gpr81−/−), the induction of VEGF-A and increased capillary density by either treadmill exercise or subcutaneous lactate injections was absent. Ex vivo lactate infusion in the rat retina decreases capillary lumen diameter, suggesting vasomotor effects of GPR81 activation. Indeed, a small molecule GPR81 agonist, AZ2 (aka AZ:5538), which suppressed in vivo fatty acid levels, also increased blood pressure (BP) when infused intravenously. Nonselective α-adrenoceptor antagonism and endothelin receptor blockade separately reduced this pressor effect.

The concept that lactate could influence blood pressure (BP) through activation of GPR81 is relevant to human health. In the current study, we used AZ:5538 and Gpr81−/− mice to resolve the effects of GPR81 activation on BP and renal hemodynamics in mice and further demonstrated that genetic deletion of the receptor conferred protection against renal ischemia-reperfusion injury.

Methods
See the Data Supplement for detailed methods. The data that support the findings of this study are available in the University of Edinburgh data storage and available from the corresponding author upon reasonable request.

Experiments were performed on adult male C57Bl/6jCrl (Charles River, Paris, France) or adult male Gpr81−/− (wild-type) and Gpr81−/− (knockout) mice on a C57BL/6jOlaHsd background. Experiments were performed under a UK Home Office Licence following ethical review by the University of Edinburgh.

In Vivo Measurement of BP and Renal Function
In anesthetized mice, either AZ:5538 (1 mg/kg bw/min) or 5% α-mannitol vehicle was infused intravenously for 15 minutes. BP was measured via a carotid cannula and renal hemodynamics measured via a Doppler transit time probe, and Doppler flux probes were inserted into the cortex and medulla. Separately, renal artery blood flow (RBF) was measured noninvasively by Pulse-wave Doppler (Vivo770 and 707B 30 MHz ultrasound probe; VisualSonics, Canada). Glomerular filtration rate (GFR) was measured by Fluorescein isothiocyanate-inulin clearance before and after administration of AZ:5538.

In Vivo Blockade of Endothelin Receptors
Anesthetized C57Bl/6jCrl mice had separate intravenous infusion of AZ:5538 and endothelin receptor antagonists or their corresponding vehicles. Bosentan, a mixed endothelin receptor antagonist, was used at 20 and 40 mg/kg; BQ123 (endothelin-A receptor antagonist) and BQ788 (endothelin-B receptor antagonist) were used at 1 and 2 mg/kg.

Ischemia-Reperfusion Injury (IRI)
Male Gpr81−/− and wild-type littermates were subject to 27 minutes of renal pedicle clamping (reperfusion was confirmed visually) followed by nephrectomy of the nonclamped kidney. After 6 days, mice were killed by cervical dislocation and the kidney taken for mRNA extraction and analysis.

RNA Analysis
Polymerase chain reaction (PCR) was used to determine if Gpr81 was expressed in arteries. Subsequently, RNAscope in situ hybridization was used to localize Gpr81 in artery and kidney sections. For quantitative PCR, RNA was extracted from quarter kidneys (RNeasy Mini Kit, Qiagen) and used to assess mRNA abundance by real-time quantitative PCR (Universal Probe Library; Sigma Aldrich). To assess Gpr81 expression in defined populations of renal cells, kidneys from male mice were dissociated into a single-cell suspension and incubated with the following rat anti-mouse antibodies: PDGFRβ (Platelet Derived Growth Factor Receptor Beta), CD31 (cluster of differentiation 31), LITL (Lotus tetragonolobus lectin), and F4/80. Fluorescence-activated cell sorting was performed using the fluorescence-activated cell sorting Aria II (BD Biosciences) using 4,6-diamidino-2-phenylindole to determine live cells. Cells were sorted into lysis buffer and RNA extracted using RNeasy microkit (Qiagen), quality checked by Agilent Bioanalyzer (RNA integrity number >8), and amplified cDNA made from the RNA using Ovation RNA-Seq System V2 (NuGen).

Endothelin-1
ET-1 (endothelin-1) was measured by ELISA (R&D Systems, United Kingdom). ET-1 protein concentrations were normalized to total protein (Pierce BCA assay; Thermo Fisher, United Kingdom).

Statistics
All data are mean±SD. After confirming normal distribution, statistical comparisons (Graphpad Prism 6, La Jolla, CA) were made by using 1-sample t test (comparing against a value of 0), unpaired t test, and 1- or 2-way ANOVA. For 2-way ANOVA, the main effects of the genotype/treatment and time were assessed, and the interaction between them. Planned comparisons were made using Holm-Sidak with a family P value fixed at 0.05.

Results
Activation of GPR81 Increases BP and Decreases RBF
In anesthetized male C57BL/6j mice, baseline systolic BP (SBP) was 86±9 mm Hg, diastolic BP (DBP) was 69±11 mm Hg, and heart rate was 28±38 bpm. AZ:5538 increased SBP (Figure 1A; ANOVA effect of treatment P=0.0013; effect of time and interaction P=0.0001) and DBP (Figure 1B; ANOVA effect of treatment P=0.0094; effect of time and interaction P=0.0001). The peak BP increases were 13±4 mm Hg and 11±3 mm Hg for SBP and DBP, respectively (Figure 1C). Heart rate fell with infusion of AZ:5538 compared with 5% mannitol vehicle (Figure 1D). All mice received a second infusion of AZ:5538, 35 minutes after the first administration. The pressor response to the second administration was attenuated, particularly in DBP (Figure 1 in the Data Supplement).

RBF was measured in 2 different groups of mice by direct Doppler ultrasound with a probe around the right renal artery (baseline RBF =60.6±0.23 mL/min) and by pulse-wave Doppler (baseline velocity =275±21 mm/s). AZ:5538 infusion significantly decreased RBF, by ≈50% measured by flow probe (Figure 1E; ANOVA effect of treatment P=0.027; effect of time and interaction P=0.0001) and by ≈30% by pulse-wave Doppler (Figure S2; peak decrease of −186±26 mm/s; ANOVA drug treatment P=0.0002, time and interaction P=0.0001). In another experiment, GFR was measured, and the mice were then randomized to receive continued intravenous vehicle (n=8) or AZ:5538 (n=8). There was no significant change in GFR with vehicle but AZ:5538 infusion caused GFR to fall significantly (Figure 1F).

The Cardiovascular Effects of AZ:5538 Are Mediated Via Gpr81
BP and heart rate were comparable in Gpr81−/− mice and wild-type littermates (Table S3). In wild types, AZ:5538 significantly increased SBP (Figure 2A; P<0.0001 for main effects of genotype and time and for interaction) and DBP (Figure 2B; P<0.0001 for main effects of genotype and
time and for interaction) and decreased heart rate (Figure S3; ANOVA genotype $P=0.025$, time $P=0.003$, and interaction $P<0.0001$), consistent with our data in C57BL/6J mice. AZ'5538 did not increase BP in Gpr81−/− mice.

RBF decreased significantly with AZ'5538 infusion in wild-type mice but remained unchanged in Gpr81−/− mice (Figure 2C; ANOVA genotype $P=0.008$ interaction and time $P<0.0001$). Renal vascular resistance did not differ between genotypes, although the interaction was significantly different (Figure 2D; ANOVA genotype $P=0.151$, time $P=0.022$, and interaction $P=0.0002$). AZ'5538 reduced renal cortical perfusion but only in wild-type mice (Figure 2E; genotype $P=0.0003$ and time interaction $P<0.0001$). Medullary flux also fell in wild-type mice but not in the Gpr81−/− (Figure 2F; genotype $P=0.070$, time and interaction $P<0.0001$). Baseline GFR was not different between genotype. AZ'5538 reduced GFR in wild-type mice (Figure 2G; $\Delta$GFR=−125±48 µL/min;
Expression of Gpr81 in Arteries and Kidney

Gpr81 was expressed in aorta, renal, and mesenteric arterioles (Figure 3A). Separately, quantitative PCR was used to assess Gpr81 expression in fluorescence-activated cell sorting–isolated renal cell populations (Figure 3B; n=4 mice). Gpr81 mRNA was highly expressed in PDGFRβ+ cells (pericytes) from all 4 samples, identified in only one of 4 samples of CD31+ cells (endothelial) and was undetectable in LTL (renal tubules) and F4/80 (macrophage) cell populations. Using in situ hybridization on whole kidney sections, Gpr81 was localized in the cortex and medulla of wild-type but not Gpr81−/− mice (Figures 4A through 4D). The staining in the cortex was localized mainly to the glomeruli (Figure 4A and Figure S4), particularly at the vascular pole, consistent with localization in arterioles. Gpr81 was also expressed in the medulla (Figure 4C) and did not co-localize with the nuclear stain. We examined Gpr81 expression in aorta and renal artery (Figure 4E and 4F), observing positive staining in the medial layer, indicating localization to vascular smooth muscle cells. No staining was visible in arteries from Gpr81−/− mice (Figure S5).

Cardiorenal Effects of GPR81 Activation Are Endothelin-1 Dependent

AZ′5538 infusion significantly increased plasma ET-1 concentration (Figure 5A) without changing the amount in aorta or whole kidney homogenates (Figure S6); renal expression of Edn1 was lower in mice that had received a 15-minute infusion of AZ′5538 (Figure S6). To assess functional crosstalk between GPR81 and the endothelin system, wild-type mice were pretreated with bosentan, before infusion of AZ′5538. Bosentan pretreatment at 40 mg/kg did not change the peak pressor response to AZ′5538 but significantly blunted the sustained effect on BP (Figure 5B; treatment P=0.024, time P<0.0001, and interaction P=0.064); similar actions were also observed at the 20 mg/kg bosentan. Next, we used pretreatment with BQ123 and, separately, BQ788. Baseline BP was
not changed by either BQ123 or BQ788 (Figure S7). BQ123 at 2 mg/kg largely prevented the pressor effect of GPR81 activation, and there was no sustained BP rise in this group (Figure 5C and Figure S8; BQ123 treatment \( P = 0.044 \), time \( P < 0.0001 \), and interaction \( P = 0.988 \)). BQ788 did not change the BP response to AZ′5538 (Figure 5D and Figure S8).

Gpr81−/− Mice Are Protected From Renal Ischemia-Reperfusion Injury

In wild-type mice, renal ischemia significantly increased the mRNA expression of the tubule injury marker KIM-1 (Kidney Injury Molecule 1) (Figure 6A), the fibrosis marker collagen 1α1 (Figure 6B), the inflammatory cytokines TNF-α (Tumor necrosis factor alpha; Figure 6C), MCP-1 (monocyte chemotactic protein 1; Figure 6D), CXCL-1 (C-X-C Motif Chemokine Ligand-1; Figure 6E), CXCL-10 (C-X-C Motif Chemokine Ligand-10; Figure S9A), and the pan-macrophage marker F4/80 (Figure S9B). ET-1, assessed by renal expression of Edn1 mRNA, was also increased by injury (Figure 6F). The transcriptional response to IRI was significantly blunted in Gpr81−/− mice. Injury did not affect endothelin-A receptor expression in either genotype (Figure S9C); endothelin-B receptor expression was reduced in both genotypes by IRI (Figure S9D).

Discussion

A decade ago, the orphan receptor GPR81 was shown to be activated with low affinity by L-lactate and \( \alpha \)- and \( \gamma \)-hydroxybutyrate.\(^6\) GPR81 mRNA was enriched in mouse and human adipocytes and receptor activation by lactate inhibited lipolysis.\(^6\) GPR81 mRNA expression was also found in nonadipose tissue, with expression in the highly vascularized tissues of heart, skeletal muscle, and kidney being \( \approx 10\% \) of that in adipocytes.\(^6\) Here, we detected GPR81 mRNA in whole kidney homogenates. In each of 3 artery types examined, GPR81 localized to the smooth muscle layer. Transgenic fluorescent-reporter mice show GPR81 expression in the vessel wall of pial arteries.\(^16\) Single-cell RNA sequencing detected GPR81 in cerebral and lung vascular smooth muscle cells.\(^15\) GPR81 has been identified in cultured human umbilical vein endothelial cells,\(^19\) but we suggest this is not a major expression site, finding discernable levels in only 1 of 4 isolated renal endothelial cell samples and no in situ endothelial GPR91 mRNA expression in large arteries. Similarly, GPR81 does not co-localize with endothelial cell markers in the cerebral vasculature.\(^16\) Within the kidney, GPR81 was additionally expressed in glomerular arterioles, with a previous study reporting arteriolar localization in dog and mouse.\(^3\) We detected GPR81 in perivascular cells, particularly, in the renal medulla and in isolated PDGFRβ+ cells. GPR81 also colocalizes with PDGFRβ-expressing cells and leptomeningeal cells in the cerebral microcirculation.\(^16\)

This expression profile suggests that GPR81 is intimately involved in vascular/microvascular function, as described for other metabolic GPCRs. For example, activation of GPR109a by niacin reduces reactive oxygen production in arterial endothelial cells\(^20\) and promotes vasodilation by stimulating prostaglandin production.\(^21\) Succinate, which activates GPR91, acutely increases circulating Ang II (angiotensin II) and BP in rats.\(^22\) Like GPR81, GPR91 is also coupled to \( G_i \) and...
succinate has direct effects on arterial contractility. For lactate/GPR81, however, functional data are limited. Increasing lactate concentration in the brain, either through exercise or by exogenous administration, promotes angiogenesis. This effect is GPR81-dependent since lactate-induced angiogenesis does not occur in Gpr81−/− mice. Lactate also constricts retinal microvessels, but it is not known whether this is GPR81–mediated. Infusion of very high concentrations of lactate increases BP in rats, most likely reflecting panic-induced sympathoexcitation, rather than activation of GPR81.

The recent development of potent GPR81 agonists to treat dyslipidemia provides tools to probe the cardiovascular physiology of GPR81. One study has examined this, finding that structurally distinct GPR81 agonists increased SBP by ≈15 mm Hg in rats and dogs when given intravenously and by ≈5 mm Hg in mice when given orally. Our studies confirm and extend this work, unequivocally demonstrating that one of these compounds, AZ5538, increases BP dependent on the expression of GPR81. We further show that GPR81 activation reduces renal artery flow, cortical and medullary perfusion, and GFR. The rapid increase in BP makes it difficult to unambiguously interpret these renal hemodynamic effects of AZ5538. However, RBF and cortical perfusion normally autoregulate when BP increases, and our data most likely reflect direct vasoconstriction of the renal artery and preferential constriction of the afferent over efferent arteriole, accounting for the GFR reduction. Furthermore, expression of GPR81 in renal PDGFRβ cells is consistent with localization in pericytes, contractile cells which regulate vasa recta blood flow, independent of changes in total or cortical blood flow. Thus, pericyte constriction may contribute to reduced intrarenal perfusion following GPR81 activation, but this was not demonstrated directly.

The cellular mechanism underpinning GPR81-mediated vasoconstriction is not fully known. In adipocytes, GPR81 couples to G1, downregulating cAMP production and protein
kinase A signaling. Activation of G_i pathways in vascular smooth muscle cells, by GPCR kinase 5, for example, lowers intracellular cAMP (Cyclic adenosine monophosphate), enhances vasoconstriction, and causes sustained hypertension. Similarly, activation of A1 receptors by adenosine constricts the renal afferent arteriole by a G_i-mediated cascade involving activation of phospholipase C. Reciprocally, agents that increase cAMP promote arterial vasorelaxation. However, our data do not support a direct vasomotor effect of GPR81 activation and instead indicate dependency on ET-1 release and subsequent endothelin-A receptor activation. Notably, ET-1 is synthesized by arterial myocytes, a cell that expresses GPR81. Further, a reduction in cAMP stimulates the production of ET-1 by myocytes, as does vascular disease and injury. The hemodynamic response to AZ 5538 is also consistent with this sequence: ET-1, via endothelin-A receptors, induces a stronger constriction of the afferent than efferent arteriole and also causes pericyte contraction, reducing vasa recta blood flow, both of which were found in our study.

In the final series of experiments, renal IRI was induced as this is known to increase intrarenal extracellular lactate. We selected a panel of transcriptional markers to capture cardinal features of IRI, tubular injury (KIM-1), increased matrix deposition (collagen 1a1), enhanced intrarenal cytokine production (TNF-α, CXCL-1, CXCL-10), and increased monocyte/macrophage infiltration (F4/80).

In wild-type mice, ischemia-reperfusion increased expression of these markers, as anticipated. In contrast, this transcriptional response to ischemia-reperfusion was absent or blunted in Gpr81−/− mice. Similar outcomes are reported in cerebral ischemic injury: 3-hydroxy-butyrate, which antagonizes GPR81, prevents lactate-induced injury in primary cultured neurons and is neuroprotective in mice exposed to cerebral artery occlusion. In contrast, overexpression of GPR81 amplifies sensitivity to hypoxic injury in a neuronal cell line. We cannot unequivocally establish the mechanism of renoprotection in Gpr81−/− mice, but these animals did not display the sustained post-ischemic increase in renal ET-1 expression that occurred in wild-type mice. This is likely to be important for at least 2 reasons. First, endothelin-A receptor–dependent vasoconstriction may contribute to progressive renal injury, which has a strong hemodynamic component. Second, ET-1, via endothelin-B receptors, can drive epithelial-mesenchymal transition and promote renal fibrosis. Gpr81−/− mice did not respond to ischemia-reperfusion with an increase in TNF-α or collagen 1a1 production, and the disconnection between extracellular lactate and ET-1 may account for this.

A limitation of our work is that all of the studies were performed on male mice. Although recent studies show that sex does not influence afferent arteriolar reactivity to ET-1, other literature indicates sex differences in the renal actions of ET-1, which might be particularly relevant in an injury context.
For example, post ischemia-reperfusion, male rats display an exaggerated early increase in renal vascular resistance and then a more pronounced decline in renal function and lower survival rate than do female rats. Furthermore, IRI increased ET-1 expression in male rats and prophylactic endothelin-A receptor blockade improved survival. In marked contrast, endothelin-A receptor blockade in females was detrimental, worsening post-ischemic survival rates, suggesting a protective role of ET-1/endothelin-A receptors activation. Overall, these data indicate that female Gpr81\(^{-/-}\) may not show the post-ischemic renoprotection of male animals identified by the current study.

Perspectives

In summary, our study indicates that GPR81 activation regulates macro- and microvascular perfusion within the kidney, dependent on ET-1 signaling. The physiological requirement for a system that would lead to vasoconstriction of regions with high anaerobic cellular metabolism is not readily apparent, given the injurious effect of hypoxia. However, earlier work in skeletal muscle associated exercise-induced accumulation of extracellular lactate with the release of ET-1 and proposed that constraining vasodilation of the skeletal vasculature would help maintain systemic BP during exercise. It may be that GPR81 activation similarly constrains the propensity to increase flow into relatively ischemic areas of the kidney and thereby mitigates against hyperemic damage. Additionally, the ET-1 release may contribute to vascular remodeling with sustained hypoxia. Overall, our data suggest that blockade of the GPR81/ET-1 system could offer beneficial vascular support in the post-injury phase.

---

Figure 6. Gpr81\(^{-/-}\) mice have reduced injury following renal ischemia-reperfusion. Renal ischemia-reperfusion injury or a sham operation was performed on Gpr81\(^{-/-}\) (n=4/6) and wild-type mice (n=6/4). One week later, the renal expression of the following genes was measured by quantitative polymerase chain reaction: (A) Havcr1 (encoding KIM-1 [kidney injury molecule 1]); (B) Col1a1 (encoding collagen type 1 α1); (C) Trif (encoding TNF-α [tumor necrosis factor-α]); (D) Ccl2 (encoding MCP-1 [monocyte chemotactic protein 1]); (E) Cxcl1 (encoding C-X-C motif chemokine ligand 1); and (F) Edn1 (encoding preproendothelin-1). Expression is normalized to housekeepers; individual data points and group mean±SD are shown. Statistical comparisons were made by 1-way ANOVA with Holm-Sidak test for planned comparisons with P values as indicated.
Acknowledgments
We thank the University of Edinburgh The Queen’s Medical Research Institute Flow Cytometry and Cell Sorting Facility and acknowledge Kristina Wallenius and Robert Unwin (AstraZeneca R&D Gothenburg) for data discussions and for providing us with samples of kidney tissue from the IRI experiment.

Sources of Funding
This work was funded by a PhD studentship from the British Heart Foundation (BHF) (FS/15/63/32033) and an Intermediate Fellowship from Kidney Research United Kingdom (PDF6/2012). Abstracts of this work have been presented at Experimental Biology 2017, Europhysiology 2018 and Experimental Biology 2019, with travel support to N.K. Jones from the Physiological Society and the BHF Centre of Research Excellence Edinburgh (RE/13/3/30183).

Disclosures
M.A. Bailey discloses prior research funding from AstraZeneca (Research Agreement 10028531). AZ GPR81-selective agonist that suppresses lipolysis in mice without therapeutic index for GPR109A agonists. J Pharmacol Toxicol Methods. 2007;56:308–316. doi: 10.1016/j.jptm.2007.05.007

References
1. Ahmed K, Tunaru S, Offermanns S. GPR109A, GPR109B and GPR81, a family of hydroxy-carboxylic acid receptors. Trends Pharmacol Sci. 2009;30:557–562. doi:10.1016/j.tips.2009.09.001
2. Lauritzen KH, Morland C, Puchades M, Holm-Hansen S, Hagelin EM, Rinholm JE, Palibrk V, Diget EH, Kennedy LH, et al. Comparison of rat and dog models of vasodilatation and lipolysis for the calculation of a therapeutic index for GPR109A agonists. J Pharmacol Toxicol Methods. 2007;56:308–316. doi: 10.1016/j.jptm.2007.05.007
3. Cai TQ, Ren N, Jin L, Cheng K, Kashi S, Chen R, Wright SD, Taggart AK, Waters MG. Role of GPR81 in lactate-mediated reduction of adipose lipolysis. Biochim Biophys Acta. 2008;1787:987–991. doi: 10.1016/j.bbaplyp.2008.10.088
4. Chudalla R, Baerwalde S, Schneider G, Maassen N. Local and systemic effects on blood lactate concentration during exercise with small and large muscle groups. Pflugers Arch. 2005;459:691–697. doi: 10.1007/s00424-005-0882-5
5. Goodwin ML, Harris JE, Hernández A, Gladden LB. Blood lactate measurements and analysis during exercise: a guide for clinicians. J Diabetes Sci Technol. 2007;1:558–569. doi: 10.1172/jdiabsctechnol.7001000144
6. Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J, Sutton SW, Li X, Yun SJ, Mirzadegan T, et al. Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. J Biol Chem. 2009;284:2811–2822. doi: 10.1074/jbc.M806409200
7. Geyer M, Baus JA, Fjellström O, Wellner E, Gustafsson L, Tacke R. Knockout mouse for this study were provided free of charge by AstraZeneca. R.I. Menzies is now employed by AstraZeneca (BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden). The other authors report no conflicts.

Data
17. Yamanishi S, Katsuruma K, Kobayashi T, Puro DG. Extracellular lactate as a dynamic vasoactive signal in the rat renal microcirculation. Am J Physiol Heart Circ Physiol. 2006;290:H929–H934. doi: 10.1152/ajpheart.00120.2005
20. Ganji SH, Qin S, Zhang Y, Kandi A, Del Gaudio F, Gao J, Chen JL, Tian H, Ling L. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. J Med Chem. 2007;50:4681–4689. doi: 10.1021/jm0620985
22. He W, Miao JT, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H, Ling L. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. J Med Chem. 2007;50:4681–4689. doi: 10.1021/jm0620985
23. Leite LN, Gonzaga NA, Simplicio JA, do Vale GT, Carballido JM, Alves-Filho JC, Tirapelli CR. Pharmacological characterization of the mechanisms underlying the vascular effects of succinate. Eur J Pharmacol. 2019;839:334–343. doi: 10.1016/j.ejphar.2016.07.045
24. Wikander I, Roos T, Ståkkedal A, Eriksson E. Sodium lactate elicits a rapid increase in blood pressure in Wistar rats and spontaneously hypertensive rats. Effect of pretreatment with the antiplatelet drugs clopidogrel and alprazolam. Neuropeuropharmacology. 1995;12:245–250. doi:10.1006/nphe.1995.0082
25. Dvorak CA, Liu C, Shelton J, Kuei C, Sutton SW, Lovenberg TW, Carruthers NI. Identification of hydroxybenzoic acids as selective lactate receptor (GPR81) agonists with antilipolytic effects. ACS Med Chem Lett. 2012;3:637–639. doi: 10.1021/ml3000676
26. Culshaw GJ, Costello HM, Binnie D, Stewart KR, Czopek A, Dhaun N, Parton L, Sykes A, Mack S, Bousba S, et al. Identification of a novel receptor (GPR81) agonists with antilipolytic effects. ACS Med Chem Lett. 2012;3:637–639. doi: 10.1021/ml3000676
27. Matsson DL, Lu S, Roman RJ, Cowley AW Jr. Relationship between renal perfusion pressure and blood flow in different regions of the kidney. Am J Physiol. 1993;264(3 pt 2):R578–R583. doi: 10.1152/ajprenal.1993.264.3.R578
28. Shaw I, Rider S, Mallinson J, Hughes J, Pericic S, Currarah RS, jeep Meek. Nephrol Dial Transplant. 2015;30:271–279. doi: 10.1093/ndt/gfu033
on Gi-mediated signaling. Circulation. 2005;112:1145–1153. doi: 10.1161/CIRCULATIONAHA.104.531657

32. Hansen PB, Castrop H, Briggs J, Schnermann J. Adenosine induces vasorelaxation through Gi-dependent activation of phospholipase C in isolated perfused afferent arterioles of mice. J Am Soc Nephrol. 2003;14:2457–2465. doi: 10.1097/01.asn.0000086474.80845.25

33. Karsten AJ, Derouet H, Ziegler M, Eckert RE. Involvement of cyclic nucleotides in renal artery smooth muscle relaxation. Urol Res. 2003;30:367–373. doi: 10.1007/s00240-002-0281-2

34. Kawanabe Y, Takahashi M, Jin X, Abdul-Majeed S, Nauli AM, Sari Y, Kanse SM, Takahashi K, Warren JB, Perera T, Porta M, Ghatei M, Nishimura J, van Breemen C. Direct regulation of smooth muscle contraction and proliferation. PLoS One. 2012;7:e44476. doi: 10.1371/journal.pone.0044476

35. Woods M, Wood EG, Mitchell JA, Warner TD. Cyclic AMP regulates endothelin-induced smooth muscle constriction and proliferation. PLoS One. 2012;7:e44476. doi: 10.1371/journal.pone.0044476

36. Wort SJ, Mitchell JA, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Lerman A, Dong Q, Van Breemen C. ET-1 (endothelin-1) and activation of the endothelin-A receptor. The systemic and renal hemodynamic effects are mediated by release of ET-1 in male mice. Ischemia-reperfusion injury in rats: possible role of endothelin. Kidney Int. 2002;62:1364–1371. doi: 10.1111/j.1523-1755.2002.kid590.x

37. Woods M, Wood EG, Mitchell JA, Warner TD. Cyclic AMP regulates endothelin-induced smooth muscle constriction and proliferation. PLoS One. 2012;7:e44476. doi: 10.1371/journal.pone.0044476

38. Kawanabe Y, Takahashi M, Jin X, Abdul-Majeed S, Nauli AM, Sari Y, Kanse SM, Takahashi K, Warren JB, Perera T, Porta M, Ghatei M, Nishimura J, van Breemen C. Direct regulation of smooth muscle contraction and proliferation. PLoS One. 2012;7:e44476. doi: 10.1371/journal.pone.0044476

39. Nauli SM. Cilostazol prevents endothelin-induced smooth muscle constriction and proliferation. Atherosclerosis. 2005;176(suppl 1):S404–S406. doi: 10.1016/j.atherosclerosis.2003.06.051

40. Ihling C, Szombathy T, Bohrmann B, Brockhaus M, Schaefer HE, Loeflner BM. Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. Circulation. 2001;104:864–869. doi: 10.1161/01.cir.3031.094742

41. Inscho EW, Imig JD, Cook AK, Pollock DM. ETA and ETB receptors differentially modulate afferent and efferent arteriolar responses to endothelin. Br J Pharmacol. 2005;146:1019–1026. doi: 10.1038/sj.bjp.07006412

42. Kennedy-lydon T, Crawford C, Wildman SM, Pappas-Wildman CM. Nonsteroidal anti-inflammatory drugs alter vasa recta diameter via pericytes. Am J Physiol Renal Physiol. 2015;309:F648–F657. doi: 10.1152/ajprenal.00199.2015

43. Zager RA, Johnson AC, Anderson D, Becker K. Progressive endothelin-1 gene activation initiates chronic/early-stage renal disease following experimental ischemic/reperfusion injury. Kidney Int. 2013;84:703–712. doi: 10.1038/kj.2013.157

44. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011;121:4210–4221. doi: 10.1172/JCI45161

45. Seccia TM, Carocci B, Gioco F, Piazza M, Bucella V, Guidolin D, Guerzoni E, Montini B, Petrelli L, Pagnin E, et al. Endothelin-1 drives epithelial-mesenchymal transition in hypertensive nephroangiosclerosis. J Am Heart Assoc. 2016;5:e003588. doi: 10.1161/JAHA.116.003588

46. Gohar EY, Cook AK, Pollock DM, Inscho EW. Afferent arteriole responsiveness to endothelin receptor activation: does sex matter? Biol Sex Differ. 2019;10:1. doi: 10.1186/s13293-018-0218-2

47. Kittiukulsh W, Sullivan JC, Pollock DM. ET-1 actions in the kidney: evidence for sex differences. Br J Pharmacol. 2013;168:318–326. doi: 10.1111/j.1476-5381.2012.01922.x

48. Müller V, Losonczy G, Heemann U, Vannay A, Fekete A, Reusz G, Tulassay T, Szabó AJ. Sexual dimorphism in renal ischemia-reperfusion injury in rats: possible role of endothelin. Kidney Int. 2002;62:1364–1371. doi: 10.1111/j.1523-1755.2002.kid590.x

49. Barrett-O’Keefe Z, Ives SJ, Trinity JD, Morgan G, Rossman MJ, Donato AJ, Runnels S, Morgan DE, Gmelch BS, Bledsoe AD, et al. Taming the “sleeping giant”: the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise. Am J Physiol Heart Circ Physiol. 2013;304:H162–H169. doi: 10.1152/ajpheart.00199.2015

50. Matejovic M, Ince C, Chawla LS, Blantz R, Molitoris BA, Rosner MH, Okusa MD, Kellum JA, Ronco C; ADQI XIII Work Group. Renal hemodynamics in AKI in search of new treatment targets. J Am Soc Nephrol. 2016;27:49–58. doi: 10.1681/ASN.2015030234