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Hepatic and renal mechanisms underlying the osmopressor response

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Abstract

Increased blood pressure (BP) is observed in patients with impaired baroreflexes after water drinking. The stimulus for this effect is low blood osmolality, and it has been termed the osmopressor response (OPR). The BP increase is associated with activation of the sympathetic nervous system and a requirement for transient receptor potential vanilloid 4 (TRPV4) channels. However, the mechanisms underlying the OPR are poorly understood. We tested the hypothesis that hypotonicity is sensed in the portal area to initiate the OPR. Sino-aortic denervated mice were used and BP was monitored for 30 min after fluid infusion while mice were under anesthesia. Infusion of hypotonic fluid (0.45% saline), but not of isotonic 0.9% saline, directly into the portal vein, produced an immediate OPR (increase in BP with saline 0.45%: 15 ± 13 vs. 0.9%: -7 ± 2 mm Hg, $p = 0.003$; AUC: 0.45%: 150 ± 99 , $n = 7$ vs. 0.9%: -74 ± 60 mm Hg · min, $n = 5$, $p = 0.003$). However, 0.45% saline was not able to trigger a similar response in TRPV4^{-/-} mice (BP_{TRPV4}: -2 ± 5 mm Hg, $n = 8$, $p = 0.009$). Hypotonic saline did not raise BP when infused at the same speed and volume into the jugular vein (jugular: -5 ± 6 mm Hg, $p = 0.002$, compared to portal). Denervation of the splanchnic nerve by celiac ganglionectomy (CGX) did not abolish the OPR (CGX: 15 ± 11 vs. Sham: 16 ± 6 mm Hg, $p = 0.34$). Renal denervation diminished the OPR elicited by duodenal water infusion (denervation: 9 ± 4 vs. sham: 31 ± 15 mm Hg, $p = 0.016$). Therefore, hypotonicity in the portal circulation, probably sensed by TRPV4 channels, triggers the OPR and intact renal nerves are needed for the full response.

Keywords

Osmopressor response; Sino-aortic denervation; Portal infusion; Renal denervation

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1. Introduction

Water ingestion has a surprising effect on cardiovascular regulation that was initially missed because of the buffering effects of the baroreflex on blood pressure and heart rate. This effect was unmasked in patients with baroreflex impairment, who experience an increase in systolic blood pressure (BP) averaging about 40 mm Hg (Jordan et al., 1999; Raj et al., 2006), after consumption of 16 oz. of water. In healthy elderly, a significant but much smaller increase of ~10–11 mm Hg occurs (Cariga and Mathias, 2001). The magnitude of the response suggests that water may play a role in an unrecognized mechanism involving cardiovascular regulation. To better understand the mechanism by which water exerts a pressor effect, we have studied a mouse model that mimics human baroreflex dysfunction. These mice, after undergoing sino-aortic denervation, have a vigorous pressor response to water with a time course similar to the water response in humans (McHugh, 2010).

It is not likely that the pressor effect of water is due to an expansion in plasma because the same volume of salt water (0.423% NaCl solution) causes a significantly smaller response when given to patients whose BP increases greatly after drinking water (Raj et al., 2006). Also, mice with impaired baroreflex have no pressor response to normal saline (0.9% NaCl solution, 25 µl/g at 125 µl/min) introduced into the duodenum, in contrast to a robust increase in BP after water (McHugh, 2010). The pressor response to water has been termed the osmopressor response (OPR).

Previous human experiments show significant elevation of plasma norepinephrine but not of renin or vasopressin after drinking water (Jordan et al., 2000; Raj et al., 2006). The OPR is abolished when baroreflex-impaired patients are treated with trimethaphan, a reversible autonomic ganglionic blocker (Jordan et al., 2000) and greatly diminished when mice are treated with prazosin, a specific α -1 adrenergic receptor antagonist. Dopamine beta hydroxylase knockout mice (*Dbh*^{-/-}) lack the OPR (McHugh et al., 2010). These data suggest that an intact sympathetic nervous system is required for the pressor effect of water.

Study of OPR in mice suggests that osmolality reduction in the portal region is the key stimulus, as water but not saline infusion lowered portal osmolality relative to systemic osmolality and produced the osmopressor response (McHugh et al., 2010). Studies by Adachi et al. previously showed that hepatic osmoreceptors exist in the rat as hepatic vagotomy caused the animals to lose the ability to respond to osmotic changes when Ringer solution was perfused directly into the portal vein. Also, portal infusion of water resulted in significant reduction of water intake compared to portal infusion of normal saline or hypertonic solution of 1.8% saline, showing that osmoreceptors in the portal region directly affect the behavior of water drinking in animals (Adachi and Nijima, 1982; Adachi et al., 1976). TRPV4 is a non-selective Ca²⁺-entry channel that is sensitive to stretch, temperature and hypo-osmolality (Lechner et al., 2011; Liedtke and Friedman, 2003; Vriens et al., 2009; Watanabe et al., 2012) and is found in the mesenteric vessels, dorsal root ganglia, along the gastrointestinal tract (GI), in the kidneys, in the portal vein and in peripheral portal neurons that sense portal vein osmolality (Bichet, 2012a; Brierley et al., 2008; Lechner et al., 2011; Vergnolle et al., 2010). TRPV4 channels play an important functional role in cellular and systemic osmoregulation (Bossus et al., 2011; Lechner et al., 2011). Mice that lack TRPV4

do not have increased BP after water is infused into the duodenum (McHugh et al., 2010). Therefore, we hypothesized that the reduction of osmolality might be detected by TRPV4 channels in the portal region to initiate the OPR.

Moreover, we are also attempting to define the afferent pathway by which hypotonicity is sensed and the efferent pathway that increases sympathetic nervous activity to produce the OPR. Blood vessels serving the splanchnic organs (stomach, small intestine, large intestine, colon, liver, spleen and pancreas) make up the splanchnic circulation and contain about 25% of total blood volume (Verbrugge et al., 2013). Because of its powerful capacitance for blood storage, the splanchnic circulation plays a major role in blood pressure regulation. There are two mechanisms by which the splanchnic circulation could affect systemic blood pressure: 1) constriction of splanchnic arteries can dramatically increase BP and total peripheral resistance and 2) cardiac preload increases because of venous constriction (King et al., 2007). Therefore, we hypothesize that the splanchnic nerves are essential in both the afferent and efferent arms of the response. Increased sympathetic activities of the splanchnic nerves might cause vasoconstriction in the splanchnic circulation, leading to increased BP.

Another important system in BP regulation is the renal system. Afferent renal nerves feed directly into the dorsal root ganglia (DRG) and the efferent nerves run through both the celiac and mesenteric ganglia (Johns, 2013). Decreased gastric osmolality has been shown to increase sympathetic renal nerve activity (Johns, 2013; Pedersen et al., 2011). Therefore, renal sympathetic nerves might play an important role as the efferent arm of the OPR. A better understanding of how portal osmolality, TRPV4 channels and increased sympathetic nerve activities interact to produce the OPR after water drinking would contribute to our knowledge of fundamental mechanisms for cardiovascular regulation and perhaps suggest novel treatment options.

2. Materials and methods

All protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee (IACUC) and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011). All surgeries were performed using aseptic techniques. Mice were anesthetized with 4% isoflurane and maintained at 2% during surgery.

2.1. Mice

Wild type C57BL/6J mice (n = 67; in-house breeding) were used in all experiments unless otherwise noted. TRPV4^{-/-} mice on a C57BL/6J background (n = 8) were provided by Wolfgang Liedtke, MD of Duke University (Liedtke and Friedman, 2003). Animals were all male, 3–5 months old and age-matched at the time of all experiments.

2.2. Sino-aortic Denervation (SAD) mouse model

Sino-aortic denervation was used to produce a mouse model of baroreflex impairment. A ventral midline incision of ~1 cm was made in the neck of an anesthetized mouse to allow access to both carotid bifurcations as previously described (Guo et al., 1982; McHugh, 2010; Schreihöfer and Sved, 1994). The submandibular glands were carefully separated to expose

the carotid arteries and the afferent components of the baroreflex. All connective tissue associated with the carotid sinus region was removed. The superior cervical ganglia, as well as the carotid sinus nerve, were isolated and removed. To ensure complete denervation, the adventitia in the bifurcation area was also stripped. Lack of bradycardia after injection of phenylephrine (20 µg/Kg, 10 µl/30 g body weight-BW) was used to confirm baroreflex impairment in SAD mice, as described before by our laboratory (McHugh, 2010).

2.3. Continuous blood pressure recordings

Mice were maintained under 1% isoflurane in oxygen during measurements. An isothermal pad (Braintree Scientific, Inc.) was used to keep body temperature constant at 36 °C to 37 °C. All drugs were administered via a venous catheter in the left jugular vein. BP was measured through a left femoral artery catheter (PE-20, Micro-Renathane, Braintree Scientific Inc.) connected to a pressure transducer (DTX Plus-6), which was then connected to a carrier amplifier (Gould Instruments). ECG leads were inserted under the skin for heart rate (HR) measurement. BP and HR signals were recorded using a WINDAQ data acquisition system (DI720, DATAQ, Akron, OH) and analyzed by Physiowave software written by Dr. Diedrich in PV-Wave (Visual Numerics, Boulder, Co.).

2.4. Duodenal cannulation

A Kocher subcostal incision ~1 cm was made on the left side to expose the stomach. Blunt forceps were used to puncture the fundus and for access of the PE-50 catheter to the stomach. The catheter was passed beyond the pyloric sphincter into the duodenum and secured in place by silk suture. This would also prevent any reflux of GI fluids. Water was infused into the duodenum at 125 µl/min at a volume of 25 µl/g of body weight.

2.5. Portal vein cannulation

This procedure was similar to previously described methods of portal vein cannulation (Chueh et al., 2006) but applied in an acute setup. A midline laparotomy of ~2.5 cm was made and the intestine was moved aside with saline-soaked Q-tips to expose the portal vein. A catheter constructed by connecting Silastic (0.025 in. OD) tubing to a 3 mm tip of PE-10 was used to puncture the portal vein and anchored by topical tissue adhesive (World Precision Instruments, Inc.). The abdominal incision was closed with suture and secured by the same adhesive. Either half normal saline or normal saline was infused directly into the portal vein at 100 µl/min (10% of the venous blood flow speed) at a volume of 12.5 µl/g of BW. Infusion of 0.45% saline would not cause hemolysis yet should significantly alter the osmolality of the portal circulation.

2.6. Celiac ganglionectomy (CGX)

To study the role of the great splanchnic nerve in the OPR, celiac ganglionectomy surgery was performed. The splanchnic nerve has neuron cell bodies in the celiac and dorsal root ganglia (DRG) that project into the spinal cord. Therefore, by damaging the celiac ganglion, the splanchnic nerve is severed. The procedure was adapted from a method previously described by Li et al. (Li et al., 2010). A midline laparotomy incision ~2 cm exposed the celiac ganglion area. All visible nerves on the aorta, celiac artery and mesenteric artery, as

well as the celiac plexus, were removed by stripping with blunt forceps. The abdominal wall was closed in two layers with silk suture. In sham animals, the ganglion plexus was exposed for ~5 min but otherwise untouched. All mice were given Cefazolin (1.6 mg/g BW) intramuscularly (IM) for 3 days post-op and allowed to recover for one week before the water study in which water was infused into the duodenum as described above.

Completeness of the CGX surgery was confirmed by the significantly lower level of norepinephrine in the spleen, liver and small intestine at the end of the water study.

2.7. Bilateral renal denervation (RD)

Bilateral renal denervation was performed to assess the need for intact renal sympathetic efferents for the OPR. Similar to procedures previously described (Wang et al., 2002), incisions of ~0.5 cm were made bilaterally in the back to expose the kidneys and their renal arteries. Silk sutures (4.0) soaked in a solution composed of 10% phenol and 70% ethanol was used to wrap around and rub on the arteries. Sham mice underwent the same procedure without perturbation of the arteries to eliminate any friction-related damage to the nerve. All animals were given Cefazolin (1.6 mg/g BW) IM for three days post-op and allowed at least 7 days to recover before the water study. Renal denervation was confirmed by reductions in norepinephrine in the kidneys at the end of the water experiment.

2.8. Catecholamine measurements

Tissue samples (including kidneys, heart, spleen, liver and small intestine) were collected and immediately frozen in dry ice before being stored at -80°C . During preparation for the catecholamine assay, the tissues were kept on ice and in 0.1 N perchloric acid. All tissues were homogenized by an Omni International TH homogenizer with disposable probes. The samples were sonicated for a minimum of 20 s then centrifuged twice at 3000 rpm for 20 min. Supernatant aliquots were centrifuged at 9500 rpm for 5 min and a clear liquid was collected for catechol analysis using HPLC (Keller et al., 2004). Plasma was collected by cardiac puncture and catechols were assayed using the method previously described (Eisenhofer, 1986). Briefly, samples were extracted with alumina, and 3,4-dihydroxybenzylamine was added as an internal standard. Eluate was run on an ESA HPLC system (Thompson Instruments, Clearbrook, Va.). Plasma controls from Biorad Inc. were included.

2.9. Drugs

Phenylephrine hydrochloride (Sigma) (20 $\mu\text{g/kg}$ dose) was dissolved in saline and given intravenously to confirm completeness of sino-aortic denervation (McHugh, 2010). Saline (0.9% NaCl) was diluted 50:50 with distilled water to make a 0.45% NaCl solution. The osmolality of 145 mOsm was confirmed with Advanced Instrument Micro-Os-mometer Model 3320.

2.10. Water study protocols

BP was recorded continuously from the left femoral catheter until the end of the experiment. Baseline BP was recorded for 10 min before infusion. SAD procedure was performed right after baseline recording and lasted approximately 10 min. After SAD, BP was allowed to

stabilize before baseline after SAD recordings and water infusion (20–40 min). Infusion lasted ~6 min if into the duodenum and ~3 min if directly into the portal vein. The post-infusion recording interval was 30 min. Phenylephrine was given before SAD and at the end of the experiment to confirm sino-aortic denervation.

2.11. Statistics

A response-feature approach was used to avoid complex longitudinal models for our repeated-measures data (Dupont, 2009). Average change in BP for each mouse was derived using this formula: $BP = BP_{pi} - BP_b$, in which BP_{pi} was the BP post intervention and BP_b was the baseline BP. Baseline BP was recorded for 10 min before the start of infusion, from T_0 to T_{10} . Area under the curve (AUC) was calculated from T_0 to T_{35} where T_{35} indicated the end of the infusion. Phenylephrine was given at T_{35} and BP was recorded for another 5 min. The on-rate slope was calculated from the beginning of infusion to the maximum change in BP. Mann-Whitney U test was used to assess the difference in BP response between separate treatments or genetic groups and to examine the difference in the AUC and the slopes. This test was chosen to eliminate the influence of unusual outliers. Data were presented as Mean $\pm$ SD. Prism 5.0 was used for all statistical analyses.

3. Results

3.1. Sensing of osmolality change in the liver is the key to OPR initiation

To investigate whether hypotonicity was sensed in the portal area to initiate the pressor effect of water, either saline or half normal saline (12.5 μ l/g of BW at 100 μ l/min) was infused directly into the portal vein of mice. Infusion of half normal saline but not saline triggered a robust and rapid increase of BP (Fig. 1A). Area under the curve (AUC) (0.45%: 150 ± 99 , $n = 7$ vs. 0.9%: -74 ± 60 mm Hg $\cdot$ min, $n = 5$, $p = 0.003$) and the maximum response after fluid infusion (0.45%: 15 ± 13 , $n = 7$ vs. 0.9%: -7 ± 2 mm Hg, $n = 5$, $p = 0.003$) differed between saline and 0.45% saline (Fig. 1B and C). Mean baseline BP was not significantly different between two groups (0.45%: 68 ± 18 , $n = 7$ vs. 0.9%: 86 ± 7 mm Hg, $n = 5$, $p = 0.16$). To minimize the number of mice used in our experiments, the data described here for the 0.45% saline infusion ($n = 7$) served as the comparison and were also included in Figs. 2 and 3 for the following protocols.

To test the hypothesis that hypo-osmolality was sensed specifically in the portal region and was not a systemic effect, the same volume of 0.45% saline was given directly into the jugular vein of WT mice at the same speed as into the portal vein. Jugular vein infusion did not produce a pressor response (Fig. 2A). The maximum change in BP after infusion (portal: 15 ± 13 , $n = 7$ vs. jugular: -5 ± 6 mm Hg, $n = 7$, $p = 0.002$) and the area under the curve (portal: 150 ± 99 , $n = 7$ vs. -59 ± 47 mm Hg $\cdot$ min, $n = 7$, $p = 0.0006$) were significantly different between portal vein and jugular vein infusions (Fig. 2B and C), while baseline mean BP was similar between two groups (portal: 68 ± 18 , $n = 7$ vs. jugular: 82 ± 16 mm Hg, $n = 7$, $p = 0.14$).

3.2. TRPV4^{-/-} mice did not display the OPR after hypo-osmotic fluid infusion into the portal circulation

We determined whether we could bypass the need for TRPV4 channels by infusing 0.45% saline directly into the portal vein of TRPV4^{-/-} mice. Baseline mean BP was measured for 10 min after recovery from SAD surgery. There was no significant difference between baseline mean BP in WT and TRPV4^{-/-} mice (BP_{WT}: 68 ± 18, n = 7 vs. BP_{TRPV4}: 82 ± 12 mm Hg, n = 8, p = 0.15). Infusion in TRPV4^{-/-} mice did not provoke an increase in BP

(BP_{TRPV4}: -2 ± 5 mm Hg, n = 8, p = 0.009; AUC_{TRPV4}^{-/-}: -74 ± 60 mm Hg · min, n = 8, p = 0.003; Fig. 3).

3.3. Role of the celiac ganglion plexus in the OPR

There are two afferent nerves that innervate the liver and the portal vein region: the vagal nerve and the greater splanchnic nerve (Berthoud, 2004). Wild-type mice that had either a celiac ganglionectomy (CGX) or sham surgery were used in this experiment. After 7-days of recovery from CGX or sham surgery, mice underwent sino-aortic denervation before water was given into the duodenum. The baseline mean BP was significantly higher in sham control mice compared to CGX mice (CGX: 62 ± 12, n = 11 vs. Sham: 79 ± 8 mm Hg, n = 12, p = 0.005). The OPR was retained in the CGX mice; maximum BP was similar to that in the sham controls (CGX: 15 ± 11, n = 11 vs. Sham: 16 ± 6.0 mm Hg, n = 12, p = 0.34) (Fig. 4A and B). However, the response did not last as long as it did in the sham animals. At 20 min after the start of infusion, BP dropped back to baseline value for the CGX mice while remaining elevated for sham mice (CGX: 2 ± 8, n = 11 vs. Sham: 13 ± 6 mm Hg, n = 12, p = 0.02) (Fig. 4C). The AUC for sham mice was also significantly higher than for the CGX mice (CGX: 4 ± 162 n = 11 vs. Sham: 176 ± 145 mm Hg · min, n = 12, p = 0.02) (Fig. 4D). The off-rate slope calculated from the maximum BP to the end of the experiment (T = 35 min) was calculated for each individual mouse. The off-rate slopes in CGX mice were significantly steeper compared to the sham controls, indicating a faster normalization of BP (CGX: -3.0 ± 1, n = 11 vs. Sham: -0.4 ± 0.3 mm Hg/min, n = 12, p < 0.0001) (Fig. 4E). The completeness of the CGX surgery was confirmed by the significant reduction of NE content in the duodenum (CGX: 24 ± 12, n = 12 vs. Sham: 264 ± 119 ng/g tissue, n = 14, p < 0.0001) and spleen (CGX: 64 ± 39, n = 12 vs. Sham: 715 ± 235 ng/g tissue, n = 14, p < 0.0001) (Fig. 4F).

3.4. Bilateral renal denervation significantly abolished the OPR

To test the hypothesis that renal sympathetic nerves play an important role in the OPR, the water study was carried out in mice following bilateral renal denervation (RD) or sham surgery. After 7 days of recovery from the renal denervation surgery, baseline mean BP was similar between sham and renal denervated mice (RD: 77 ± 4, n = 5 vs. Sham: 71 ± 10, n = 5, p = 0.53). The OPR was significantly diminished in mice with bilateral renal denervation (BP_{RD}: 9 ± 4 vs. BP_{Sham}: 29 ± 14 mm Hg, n = 5, p = 0.008; AUC_{RD}: 4 ± 45 vs. AUC_{Sham}: 277 ± 129 mm Hg · min, n = 5, p = 0.008) (Fig. 5A, B and C). The completeness of the renal denervation surgery was confirmed by a significant reduction in the NE but not epinephrine content of the kidneys (RD_{NE}: 40 ± 28 vs. Sham_{NE}: 306 ± 44 ng/g tissue, p < 0.0001; RD_{Epi}: 7 ± 3 vs. Sham_{Epi}: 12 ± 8 ng/g tissue, p = 0.08) (Fig. 5D and E). However,

norepinephrine in the plasma and in 24-h urine was similar between the two groups (plasma: RD: 2761 ± 619 vs. Sham: 2758 ± 1637 pg/ml, $n = 7$, $p = 0.54$; Urine: RD: 404 ± 162 vs. Sham: 407 ± 135 pg/ml, $n = 7$, $p = 0.9$).

4. Discussion

The effect of water drinking on BP in patients and mice with compromised baroreflex function has provided us with insights into fundamental components of autonomic cardiovascular regulation. In this study, we aimed to further characterize mechanisms underlying this effect. Our data suggested that: 1) the decrease in osmolality after water drinking was sensed and the OPR was initiated in the portal region; 2) the OPR was absent in TRPV4^{-/-} mice not only when water was given into the duodenum but also when hypo-osmolality was induced directly in the liver; 3) the greater splanchnic nerve did not contribute to the initiation of the pressor response, but was essential for maintaining the OPR; and 4) the renal nerves contributed significantly to the OPR.

A profound pressor response (~40 mm Hg increase) occurs after drinking water in patients with impaired baroreflex function (Jordan et al., 1999). However, this response is significantly smaller when these patients consume the same volume of hypotonic fluid (0.42% NaCl) (Raj et al., 2006). The OPR can also be observed in healthy elderly consistent with their compromised baroreflex function (Jordan et al., 2000; Oberman et al., 2000). The pressor response to water can be replicated in sino-aortic denervated mice when water is infused into the stomach or the duodenum. When an isotonic solution (0.9% NaCl) is infused into mice, the response is absent (McHugh, 2010). These data indicate that the increase in BP after water consumption is not in response to a mechanical stimulus (McHugh et al., 2010; Raj et al., 2006) and that osmolality could be the triggering signal for the OPR. A decrease in portal osmolality relative to systemic osmolality after water infusion but not after saline infusion (McHugh et al., 2010) led us to hypothesize that detection of hypo-osmolality in the portal region initiates the OPR.

As shown in Fig. 1, when infused directly into the portal vein, hypo-osmotic fluid, but not saline, stimulated an immediate pressor effect. These results were consistent with hypo-osmolality being the stimulus of the OPR and suggested that the liver sensed this signal and triggered the response. The slight decrease in BP following normal saline infusion directly into the portal vein was surprising because adding ~8.5% of the blood volume to the circulation might be expected to increase BP. This decrease in BP could have been a compensatory response to counteract the sudden volume increase especially in the portal region. A tendency toward a decrease in BP was also apparent after infusion of 0.45% NaCl into the jugular vein (Fig. 2). By initiating the OPR, hypo-osmotic fluid into the portal vein, but not the jugular vein, was able to overcome this compensatory effect. Our findings indicated that the OPR was not related to an increase in blood volume and that the hypo-osmolality signal was specific to the portal region and not a systemic effect. In human studies, patients with a liver transplant do not exhibit an OPR after drinking water (May et al., 2011), suggesting that liver innervation is required for transducing the portal hypo-osmolality signal in the OPR.

One attractive candidate for the molecular mediator of the OPR in the liver is TRPV4. These channels are highly expressed in the liver and sensitive to hypo-osmotic stimuli (Benfenati et al., 2011; Chen et al., 2009; Lechner et al., 2011). The absence of OPR in TRPV4^{-/-} mice after water infusion into the duodenum (McHugh et al., 2010) or after 0.45% NaCl infusion directly into the portal vein (Fig. 3) indicated that TRPV4 channels were needed for sensing the osmolality change that initiates the OPR. Even with hypo-osmolality induced directly in the portal region, global expression of TRPV4 channels were still required to initiate the OPR. Further experiments with site-specific TRPV4 knockout mouse models, in which TRPV4 is deleted only in the portal region would provide insight into the exact location where TRPV4 channels are needed for the OPR. It was demonstrated previously that osmolality in the portal vein is significantly lower than systemic osmolality in both TRPV4^{-/-} and WT mice after water infusion into the duodenum (McHugh, 2010). Also baseline portal vein and systemic osmolality do not differ between TRPV4^{-/-} and WT mice (Mai, 2014). On the other hand, Lechner et al. showed that activation of hepatic afferent nerves by hypotonicity is absent in TRPV4 knockout mice (Lechner et al., 2011). These data suggest that a defect in the osmolality sensing mechanism due to an absence of TRPV4 channels, rather than an abnormality in osmolality, was responsible for the lack of response to hypo-osmotic stimulus in TRPV4 knockout mice.

There are two main sensory nerves that receive information from the liver and convey it to the CNS: the vagal and the splanchnic nerves. The vagus transmits signals through the nodose ganglia to the solitary tract nucleus, and the splanchnic nerve goes through the celiac and dorsal root ganglia (DRG) that project into the spinal cord (McBride et al., 2001). While the hepatic sensory neurons that run through the celiac ganglia display osmosensitive current, the nodose sensory neurons innervating the liver do not (Lechner et al., 2011). Our data showed that BP increased similarly in both CGX and sham mice after duodenal water infusion (Fig. 4B), indicating that the splanchnic nerve did not play an essential role in the initiation of the OPR. However, BP decreased to the baseline level faster in CGX mice than in the sham controls (Fig. 4C), suggesting that the splanchnic nerve was crucial in the maintenance of BP elevation, probably through increased splanchnic sympathetic activity, which is absent after CGX. Moreover, in human studies, it was shown that the maximal pressor response to water was observed around 20–30 min (Jordan et al., 2000; Jordan et al., 1999; Raj et al., 2006). In CGX mice, this response was profoundly attenuated at the time of expected maximal response. It is worth noting that in this experiment, the celiac ganglion that contained both splanchnic afferent and efferent nerves was severed. A study by Li et al. suggested that celiac ganglionectomy significantly reduced sympathetic innervation in splanchnic organs. The vasoconstrictor response of the mesenteric vessels to electrical stimulation (up to 10 Hz) was abolished in CGX animals (Li et al., 2010). As a result of the loss of vasoconstriction after CGX in the splanchnic region that holds ~25% of blood volume at rest (Tu et al., 2013), the baseline BP is reduced. The shortened duration of the OPR in CGX mice might be a result of the disruption of increased efferent sympathetic activity to the splanchnic organs by CGX surgery. Previous work by our lab showed that vagotomy did not abolish the OPR. It is possible that both vagal and splanchnic nerves play an important role in the OPR and when one is damaged, the other one could compensate to transfer the afferent signal. Research by Wright et al. showed that both the vagal and

splanchnic afferent nerves contribute to the reduction of meal size and prolongation of the intermeal interval induced by gastrin-releasing peptide-29 (Wright et al., 2012). To test whether denervation of both nerves is necessary to eliminate the OPR, both vagal and splanchnic nerves should be severed before hypo-osmotic fluid is infused directly into the portal vein. However, this experiment is better done pharmacologically with a compound that can denervate only the afferent arm of the two nerves. Surgically cutting both nerves denervates both afferent and efferent arms. A report by Brierley et al. indicates that TRPV4 is highly expressed on the mesenteric afferents and the signal is enhanced by a TRPV4 agonist (Brierley et al., 2008). Therefore, targeted deletion of TRPV4 by an antagonist on both nerves could be useful in determining the mechanism of the OPR in future experiments.

Patients with complete cervical spinal cord transaction display an OPR. In these patients, spinal sympathetic neurons are still intact although they are no longer connected to the brainstem (Tank et al., 2003). Multiple system atrophy patients with neuronal lesions in the brainstem also have a full response to water. Therefore, it is possible that the central nervous system is not needed for the OPR but that the OPR is the result of a spinal reflex, as long as some degree of spinal sympathetic activity still exists.

The enteric nervous system (ENS) is another important component that innervates the splanchnic area. It is composed of small ganglia that lie along the walls of the esophagus, stomach, small and large intestines, gallbladder and the nerves that innervate the muscle of the gut wall (Coruzzi et al., 2007; Ohlsson et al., 2007). Although the ENS is usually considered as an independent structure, it can also relay signals to the spinal cord by interacting with the vagal nerve and the pelvic nerve (Furness et al., 2014). A role of the ENS in the OPR cannot be eliminated, and the role of this nervous system in blood pressure regulation needs investigation.

Previous studies in our lab and the data presented in this paper indicate that the OPR likely involves multiple systems. The renal system has long been known to be key in BP and systemic osmolality regulation (Bernstein et al., 2014; Bichet, 2012a; Bichet, 2012b; Demerath et al., 2014; Tanimoto et al., 2014). The renal afferent nerves have cell bodies in the ipsilateral dorsal root ganglia and project into the spinal cord to convey sensory information to both spinal and supraspinal pathways (Johns, 2013). These neurons respond to hepatic portal vein infusion with hypertonic solution by reducing renal sympathetic nerve activity (Ishiki et al., 1991; Kopp et al., 2007; Lang et al., 1991). However, the role of the kidneys in the OPR had not been examined. Both human and mouse studies demonstrated that sympathetic nervous system activation is responsible for the rise of BP after water intake (Jordan et al., 2000; Lipp et al., 2005; Lu et al., 2003; McHugh, 2010; Schroeder et al., 2002; Shannon et al., 2002). Increased sympathetic signals can elevate renin levels via β_1 adrenergic receptor activation and subsequently angiotensin II increases (Osborn et al., 1981). Ang II increases BP through several mechanisms, including vasoconstriction and a further increase of sympathetic drive (Kim and Iwao, 2000; Peach, 1977; Ramchandra et al., 2013). Study in rats anesthetized with isoflurane showed that the renin-angiotensin system was potentiated but had minimal effect in blood pressure control (Ullman, 1999). Our data showed that blocking the Angiotensin I (AT1) receptor with AT1 antagonist Losartan had no effect on the OPR (Mai, 2014). Both human and mouse studies demonstrated that

sympathetic nervous system. Besides an effect on the renin-angiotensin system, the sympathetic nervous system also has a direct effect on BP via the renal nerves. Renal nerve denervation recently was acknowledged to reduce BP in some resistant hypertensive patients (Larsen et al., 2014). When patients with kidney transplant consumed water, their plasma norepinephrine increased 0.21 ± 0.07 nmol/l and systolic finger blood pressure increased 13 ± 2.3 mm Hg compared to 0.61 ± 0.29 nmol/l and ~ 35 mm Hg, respectively, in patients with multiple system atrophy and pure autonomic failure (May et al., 2011; Jordan et al., 1999). The kidney transplanted patients also had a smaller increase in BP compared to liver transplanted patients even though their plasma norepinephrine level showed a greater increase after drinking water (Jordan et al., 1999). It was possible that the lack of OPR in these patients and in renal denervated mice was due to the inability to increase renal sympathetic nerve activity after water infusion (Fig. 5D). Similar to patients with kidney transplanted patients, both afferent and efferent nerves were destroyed in mice underwent renal denervation with phenol in 70% EtOH (Consigny et al., 2014; Salman et al., 2010). We cannot distinguish from this experiment whether the afferent or efferent renal nerve contributes to the OPR.

These data suggest that the liver is the site of initiation of the OPR. The splanchnic nerve, although not needed to trigger the response, appeared to play an important role in the efferent arm to maintain the OPR. The renal nerves must be intact for the pressor effect of water. The diagram suggesting the neural flow in OPR is shown in Fig. 6. The involvement of the portal circulation and the renal system give new insights into the mechanisms underlying the OPR. Along with TRPV4, these two systems might represent sites of action for new drug targets in the treatment of hypotension and syncope.

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Abbreviations

OPR	Osmopressor Response
TRPV4	Transient Receptor Potential Vanilloid 4
BP	Blood Pressure
HR	Heart Rate
SAD	Sino-aortic Denervation
CGX	Celiac Ganglionectomy
RD	Renal Denervation

NE Norepinephrine

Epi Epinephrine

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Fig. 1. Hypo-osmotic fluid infused into the portal vein (Red, $n = 7$) of WT mice can initiate the osmopressor response. This response was absent when infusing normal saline (Blue, $n = 5$). Data was graphed as mean $\pm$ SE.

Fig. 2. Infusion of 0.45% NaCl solution directly into the jugular vein (Grey, $n = 7$) of WT mice failed to produce the pressor effect that was observed when infusing the same solution into the portal vein (Red, $n = 7$). Data was graphed as mean $\pm$ SE.

Fig. 3. Hypotonic fluid infusion (0.45% NaCl) into the portal vein of TRPV4^{-/-} mice (Green, n = 8) did not produce the OPR, in contrast to its effect when infused into WT mice (Red, n = 7). Data was graphed as mean $\pm$ SE.

Fig. 4.

A) Water infusion into the duodenum of CGX mice (Pink, $n = 11$) produced similar OPR to Sham mice (Blue, $n = 12$). B) The maximal change of BP in response to water was similar between the two groups. However, the duration of the response was significantly shorter (C). At $T = 20$ min after duodenal water infusion, BP stayed elevated in sham controls while it came back down to baseline in CGX mice. D) The AUC was significantly larger in the control groups, indicating a larger OPR. E) The recovery slope was significantly steeper in the CGX mice, showing a faster decrease in BP after the initiation of the OPR. F) Norepinephrine in the duodenum and spleen decreased significantly after CGX (Pink, $n = 12$) compared to WT mice (Blue, $n = 13$). Data was graphed as mean $\pm$ SE.

Fig. 5.

Bilateral renal denervation significantly attenuated the OPR in WT mice. A) Mice with bilateral renal denervation (Orange, $n = 5$) had significantly diminished OPR compared to sham controls (Blue, $n = 5$). B) Maximal change in BP was significantly higher in the sham controls, indicating a higher initiation of the OPR. C) AUC was significantly smaller in the renal denervated mice, showing a smaller response to water in these mice. D and E) Kidney norepinephrine and epinephrine were measured at the end of the experiment to confirm the renal nerve denervation (RD: Orange, $n = 5$ and Sham: Blue, $n = 5$). Data was graphed as mean $\pm$ SE.

Fig. 6.

Diagram suggesting the neural pathways in OPR: Hypotonicity is sensed in the portal vein/liver area and both vagal and splanchnic nerves transfer afferent signal to the spinal cord to increase efferent sympathetic activities.