Portal Osmopressor Mechanism Linked to Transient Receptor Potential Vaniloid 4 and Blood Pressure Control

Julia McHugh, Nancy R. Keller, Martin Appalsamy, Steven A. Thomas, Satish R. Raj, André Diedrich, Italo Biaggioni, Jens Jordan, David Robertson

Abstract—Human subjects with impaired baroreflex function cannot buffer rises or falls in blood pressure (BP), thus allowing BP effects of endogenous or environmental stimuli that previously escaped detection to emerge dramatically. Studies in these patients led us to discover that water ingestion induced a robust increase in BP and vascular resistance. Here, using a mouse model of baroreflex impairment, we show that the increase in blood pressure after water ingestion is mediated through sympathetic nervous system activation and that the osmosensitive transient receptor potential vaniloid 4 channel (Trpv4) is an essential component of the response. Although portal osmolality decreases after water ingestion in both wild-type and Trpv4−/− mice, only the wild-type animals show a pressor response. The same volume of physiological saline does not elicit an increase in BP, suggesting osmolality as the stimulus. The osmopressor response to water, and Trpv4 thus represent new factors now implicated in the physiology of BP regulation. (Hypertension. 2010;55:1438-1443.)

Key Words: Trpv4 ■ blood pressure ■ osmopressor ■ sympathetic nervous system ■ baroreflex

Studies in patients with baroreflex impairment led us to discover that water ingestion increases blood pressure (BP) and vascular resistance. We found that ingestion of 16 oz (473 mL) of water induces a profound increase in systolic BP, averaging 40 mm Hg, with occasional increases >75 mm Hg. The effect appears within 10 minutes, is maximal at 25 to 40 minutes, and largely dissipates by 90 minutes after ingestion. Although the effect of water was greatest in individuals with impaired baroreflex buffering, it was also present in healthy persons. In healthy young subjects with intact baroreflexes, water elicits an increase in peripheral vascular resistance without an increase in BP because of a compensatory reduction in cardiac output. Importantly, water ingestion raises plasma norepinephrine but not renin or vasopressin, supporting a sympathetic nervous system mechanism. Furthermore, induction of reversible autonomic blockade with the autonomic ganglionic blocking drug trimethaphan abolishes the pressor action of water.

Although these studies in human subjects suggest a sympathetic nervous system mechanism for the pressor action of water, we sought to discover the physiological and molecular basis of the response. There has been increasing interest in the location and nature of the pressor response to water, as well as potential molecular mediators of the response.

Materials and Methods

All of the protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 80-23).

Mice

Wild-type C57BL/6j mice (n=53; Jackson Laboratories) were used in all of the experiments unless otherwise noted. Dopamine
β-hydroxylase knockout mice (Dbh−/−; n=5) were developed by standard gene-targeting methods, as described by Thomas et al.4 Trpv4−/− mice (n=21) were developed and provided by Wolfgang Liedtke (Duke University).13 Animals were 3 to 12 months of age at the time of the experiment.

Continuous BP Recordings
Mice were anesthetized with 4% isoflurane and maintained on 1% isoflurane in oxygen delivered from a precision vaporizer. Body temperature was maintained at 36°C to 37°C with an isolothermal pad (Braintree Scientific, Inc). Drugs were administered through a venous catheter in the left jugular vein, and BP was measured through a catheter in the right femoral artery (Micro-Renathane, Braintree Scientific Inc) connected to a pressure transducer (DTX Plus-4812, Becton-Dickinson) and carrier amplifier (Gould Instruments). BP signals were recorded using a WINDAQ data acquisition system (DATAQ). Data were analyzed by PVwave (Visual Numerics).

Gastric/Duodenal Cannulation
An upper abdominal midline incision was made to expose the stomach for gastric and duodenal cannulation. The fundus was punctured at the greater curvature with blunt forceps. A PE-50 catheter was inserted into the gastric lumen or passed just beyond the pyloric sphincter into the duodenum. Sutures placed around the blunted and flanged end of the catheter secured the catheter in place and prevented reflux of GI fluids. Normal saline or water was infused into the stomach or duodenum at a volume of 25 μL/g of body weight over a 6-minute period.

Subdiaphragmatic Vagotomy
The esophagus was isolated and secured at 2 points with silk sutures; one suture was placed where the thoracic esophagus emerges through the diaphragm and the other placed closer to the gastroesophageal junction. The sutures acted as retractors to separate the esophagus from other structures in the area, as well as to prevent leakage of GI contents into the peritoneum. The esophagus was severed just below the diaphragm, along with the dorsal and ventral vagus nerves.

Baroreflex Impairment Model Preparation
Our method is similar to previously described methods of baroreflex deafferentation.15,16 Briefly, a ventral midline incision was made in the neck, exposing the carotid bifurcation and allowing isolation of afferent components of the baroreflex. The superior cervical ganglion and superior laryngeal nerves were isolated and removed, along with the carotid sinus nerve. In addition, the adventitia and associated connective tissue were stripped from the carotid sinus regions. Baroreflex impairment was confirmed by comparing Δheart rate (HR)/ΔBP on phenylephrine challenge before and after deafferentation. (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org)

Conscious BP Measurements for Restraint Stress Test
Mouse BP radiotelemetry (Data Sciences International) has been described previously.17 Briefly, the left carotid artery was isolated, a vessel clamp was placed 8 to 10 mm below the bifurcation to occlude blood flow, and the lumen was cut to allow insertion of the transmitter catheter to the point of the bifurcation. The transmitter body was placed in a lateral subcutaneous pocket. The overlying muscles and skin were secured via sutures.

Restraint Stress Test
A universal mouse restrainer (Braintree Scientific, Inc) was used to induce restraint stress in telemetered mice. Mice were placed in the restrainer for 2 minutes. Beat-to-beat BP and HR measurements were recorded using the DSI ART Gold software (Data Sciences International). Data were analyzed by PVwave (Visual Numerics).

Drugs
Prazosin hydrochloride (Sigma) was dissolved in distilled water with the application of heat. Final dilutions were made with saline and given IP at 0.5 mg/kg in 100 μL saline. This dose was chosen because it sufficiently blocked the α-1 pressor effect of phenylephrine. Water was infused intraduodenally 20 minutes after IP prazosin. Phenylephrine hydrochloride (Sigma) was dissolved in saline and given intravenously.

Osmolality Measurement
Blood was drawn from the portal vein and carotid artery 10 minutes after duodenal infusion of water or saline and centrifuged right after collection (600 g for 10 minutes) to reduce cell lysis. Plasma osmolality was measured using the Vapro Vapor Pressure Osmometer 5520 (Wescor Inc).

Statistics
The data in this study consist of multiple BP measurements before and after the intervention. We have used a response-feature approach to account for the repeated-measures aspects of our data while avoiding complex longitudinal models.18 Average BPs were derived for each mouse during the baseline and post-treatment intervals (BPib and BPiv, respectively). The average change from baseline for the ith mouse was BPi=BPi−BPib. BPs were recorded continuously throughout the experiment. The baseline interval was from 10 minutes before the onset of treatment until the start of infusion. Infusion lasted 6 minutes. The posttreatment interval was from the end of infusion and lasted 34 minutes. Tests of the change in BP in response to treatment within each treatment group were assessed by comparing BPiv with BPib using the Wilcoxon signed-rank test. The difference in BP response to infusion between separate treatment or genetic groups was assessed by comparing ΔBPi in the 2 groups using a Mann-Whitney U test. Data are presented as the mean±SD. SPSS was used for all of the statistical calculations. All probability values were derived with respect to 2-sided alternative hypotheses.

Results
Baroreflex Deafferentation Unmasks Pressor Response
To unmask and better characterize the response in mice, we used a model of baroreflex failure (baroreflex deafferentation). Unless otherwise noted, BP data shown here are from animals after bilateral baroreflex deafferentation. Effective baroreflex deafferentation was documented in each animal by the absence of opposing changes in HR in response to alterations in BP (Figure S1, available in the online Data Supplement). Our mouse model responded to water with an increase in BP similar in magnitude and time course to that observed in human subjects with baroreflex impairment (Figure S2).

Location of the Action of Water
Neurons in the pharyngeal and esophageal areas are sensitive to both mechanical and chemical stimuli (including water) and can elicit cardiovascular reflexes when activated.19,20 To investigate pharyngeal and esophageal involvement in the pressor effect of water, water (25 μL/g in 6 minutes) was infused directly into the stomach via an intragastric tube. BP began to increase near the end of water infusion, but maximal pressure was not reached until 20 minutes later (10.0±13.2 mm Hg; P<0.05). The experiment was repeated with direct duodenal infusion of water (catheter advanced beyond the pyloric sphincter). Intraduodenal infusion of water resulted in robust BP elevation (14.9±7.4; P<0.005) of similar magnitude to intragastric infusion (P=0.2 between groups; Figure 1).
Role of Plasma Volume in Pressor Response
To address the possibility that water increases BP by increasing plasma volume, saline (150 μL, ≈10% of circulating blood volume) was infused intravenously in mice. Intravenous saline caused a small, transient spike in BP, with values returning to baseline within 2 minutes (Figure S3). This intravenous saline did not elicit the persistent pressor effect seen with water.

Role of Sympathetic Nervous System Efferents in Pressor Response to Water
To test the hypothesis that sympathetic activity underlay the pressor effect of water, mice were given the α1 adrenoceptor antagonist prazosin before water infusion. Pretreatment with prazosin resulted in loss of the pressor response to water (3.8±2.7 versus 14.9±7.4 mm Hg; P=0.01; Figure 2A). In addition, Dbh knockout mice with no detectable norepinephrine in blood, urine, or tissue did not produce an increase in BP in response to water infusion (1.3±1.2 mm Hg; P=0.36; Figure 2B).

Vagal Involvement in Pressor Response to Water
To investigate vagal afferent involvement in the response to water, surgically baroreflex-impaired mice underwent bilateral subdiaphragmatic vagotomy before water infusion. The vagotomized mice responded to water infusion with a similar increase in BP as intact animals (10.5±4.8 versus 14.9±7.4 mm Hg; P=0.2; Figure 3).

Effect of Gastric Luminal Stretch and Osmolality on BP
To differentiate between luminal stretch and osmolality as triggers of the water response, mice were given equivalent doses (25 μL/g) of saline or water. In contrast to water, intraduodenal saline infusion did not elicit an increase in BP (2.3±8.1 mm Hg; P=0.6; Figure 4A).

Figure 1. Location of water’s action. Change in BP after intragastric or intraduodenal infusion of water (25 μL/g). Infusions began at t=10 minutes and ended at t=16 minutes. Both gastric (orange; n=7) and duodenal (blue; n=11) infusion of water resulted in a robust increase in BP.

Figure 2. Water’s pressor response is mediated by sympathetic nervous system activation. Change in BP after duodenal infusion of water (25 μL/g). A, The pressor response to water was greatly attenuated with prazosin (0.5 mg/kg IP) before water infusion (green; n=5). B, Dbh−/− mice display no response to intraduodenal water (red; n=5).
Portal Versus Systemic Osmolality After Water or Saline Infusion

To determine the effect of intraduodenal water or saline infusion on osmolality in mice, both portal and systemic osmolality were measured 10 minutes after the infusion. Water infusion lowered portal osmolality relative to systemic osmolality (292.7±4.7 and 304.4±6.9 milliosmol/kg, respectively; P<0.002), whereas saline infusion did not lower portal osmolality relative to systemic osmolality (307.1±4.4 and 307.4±1.7 milliosmol/kg, respectively; P=0.88; Figure 4B).

Response of Trpv4−/− Mice to Water Infusion

We used Trpv4 knockout animals to investigate the potential role of these channels in our osmopressor response. The pressor response to intraduodenal infusion of water was virtually abolished in mice lacking the Trpv4 channel compared with wild-type controls (1.6±4.3 mm Hg, P=0.3; Figure 5). To verify that the absence of the pressor response in Trpv4−/− animals was not because of a general abnormality in neural pathways or efferent sympathetic nerve function, mice were placed under restraint, which increases sympathetic output from the central nervous system. Both wild-type and Trpv4−/− mice showed similar BP and HR changes when placed under restraint, whereas Dbh−/− mice (absent sympathetic adrenergic tone) under restraint did not show increases in BP or HR (Figure S4).

Portal Versus Systemic Osmolality in Trpv4−/− After Water Infusion

Portal and systemic osmolalities were measured 10 minutes after duodenal infusion of water in Trpv4−/− animals. Portal and systemic osmolalities in these mice were found to be 301.0±6.0 and 310.2±6.1 milliosmol/kg, respectively (P=0.003; Figure 6).

Figure 3. Vagal afferents are not essential for water’s pressor effect. Change in BP after duodenal infusion of water (25 μL/g) in vagotomized (green; n=6) and intact (blue; n=11) mice.

Figure 4. Effect of osmolality on BP. Change in BP after intraduodenal infusion of water (blue; n=11) or saline (pink; n=6; 25 μL/g). A, Attenuation of the pressor response during saline infusion implicates hypo-osmolality as the stimulus. B, Systemic (●) and portal (○) osmolality 10 minutes after intraduodenal infusion of water (●, ○; n=9) or saline (●, □; n=9). The decrease in portal osmolality after water but not after saline infusion is consistent with portal/hepatic osmosensor involvement in the pressor response to water.
Figure 5. Trpv4 is an essential mediator in the osmopressor response. Change in BP after duodenal infusion of water (25 μL/g) in Trpv4<sup>−/−</sup> mice (red; n=11) vs wild-type (blue; n=11).

Discussion

In this study, we sought to characterize the physiological and molecular mechanisms of the previously described pressor effect of water ingestion. The baroreflex deafferented mouse model allowed us to better observe the BP effects of water without buffering by an intact baroreflex system. Our results showing the presence of a robust pressor response after both intragastric and intraduodenal infusions place the location of water’s actions at or distal to the duodenum and show that water elicits its actions independent of pharyngeal and esophageal mechanisms. We also show that the stimulus eliciting the response is osmotic in nature, because only water, and not saline, produced a pressor response. Furthermore, the absence of a pressor response with saline infusion makes luminal stretch an unlikely explanation for the increase in BP, because both fluids would have induced equal duodenal stretch. However, luminal stretch may play a role during the period of infusion when the GI tract is experiencing the highest degree of stretch. The slight, transient dip in BP immediately after infusion when the GI tract is experiencing the highest degree of stretch might be explained by the acute luminal stretch experienced during infusion.

Hydration status may affect BP. However, we showed that acutely increasing plasma volume via saline infusion failed to elicit the pressor response seen with duodenal water administration in both magnitude and duration. Because intravenous administration would optimally increase plasma volume, these data effectively exclude an increase in plasma volume as the cause of the robust, sustained response observed after water ingestion.

Studies in humans provide indirect evidence that increased sympathetic nervous system activity underlie the pressor effect of water; plasma norepinephrine,<sup>2,3</sup> muscle sympathetic nerve activity,<sup>21</sup> and peripheral vascular resistance<sup>1</sup> all increase after water ingestion. In the present study, blockade of α<sub>1</sub> adrenoreceptors with prazosin attenuated the pressor response to water, implicating the sympathetic neurotransmitter norepinephrine in this response. Additional evidence strengthening the sympathetic nervous system hypothesis was obtained from Dbh<sup>−/−</sup> gene knockout mice. Although Dbh<sup>−/−</sup> mice display hypersensitivity to a variety of pressor and depressor stimuli, they were incapable of producing an increase in BP in response to water infusion (Figure 2B). Taken together, these data show that an intact efferent sympathetic nervous system is required to elicit the pressor response of water and, specifically, that sympathetic adrenergic mechanisms are critical to this response.

The neural pathways that form the loop of this pressor response to water might include brain stem centers, which receive splanchnic afferent input via the vagus nerve, or could be limited to spinal afferent nerves, which can directly influence sympathetic output at the level of the spinal cord.<sup>22</sup> However, the presence of a pressor response in bilaterally subdiaphragmatically vagotomized mice indicates that the vagus nerve is not essential for the osmoppressor response. Interestingly, patients with near-complete cervical spinal cord transection also have an intact pressor response to water ingestion.<sup>23</sup> Both of these findings suggest that the pressor response is most likely acting through a spinal reflex.

All of the contents absorbed by the GI tract enter the body by way of the portal blood vessels. The portal vein can exhibit a wider range of osmolality than the systemic circulation, and there is evidence of osmosensitive mechanisms in the portal system.<sup>24,25</sup> Our data show that water infusion lowered portal osmolality relative to systemic osmolality and produced a pressor response, whereas saline infusion did not alter portal/systemic osmolality or raise BP. These data suggest that water may be acting through osmosensors in the portal/hepatic circulation. For these reasons, we have termed this pressor response of water ingestion as the “osmopressor response.”

The location of action and the osmotic nature of the stimulus eliciting the pressor response made Trpv4 a top
candidate as a potential mediator. 


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**Portal Osmopressor Mechanism Linked to TRPV4 and Blood Pressure Control**

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S1. Blunted HR response to phenylephrine after baroreflex deafferentation. Representative BP and HR changes after i.v. phenylephrine (t = 0), pre and post baroreflex deafferentation. Phenylephrine challenge was used to validate successful baroreflex deafferentation in mice.
S2. BP profile during water consumption in humans and mice.
Mice with intact baroreflexes show little BP response to water infusion, since baroreflex buffering attenuates it. Surgically baroreflex-impaired mice, as well as patients with baroreflex impairment, show a robust increase in BP that is sustained well beyond the period of water ingestion. This enhanced pressor response facilitates mechanistic studies of water’s cardiovascular effects.
S3. **Role of plasma volume in pressor response.** 150 μL intravenous saline (*red*), given at 10 minutes elicited only a small, transient pressor response. Duodenal (*blue*) infusion of water is shown for comparison.
S4. *Trpv4*−/− have intact sympathetic efferents. A, Representative tracings of the change in BP and HR during restraint for wild-type (■), *Trpv4*−/− (○), and *Dbh*−/− (◊) mice. B, Average change in BP and HR during 2-minute restraint.