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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 (ΔBP_{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.

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

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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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 Niijima, 1982; Adachi et al., 1976). TRPV4 is a non-selective Ca^{2+}-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; Schreihofer 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.