Splanchnic and vagal denervation attenuate central Fos but not AVP responses to intragastric salt in rats

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Carlson, Scott H., and John W. Osborn. Splanchnic and vagal denervation attenuate central Fos but not AVP responses to intragastric salt in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1243–R1252, 1998.—We have recently reported that an acute intragastric hypertonic saline load increases plasma arginine vasopressin (\(P_{AVP}\)) and Fos immunoreactivity in several central nuclei, including the supraoptic nucleus (SON), paraventricular nucleus (PVN), nucleus of the solitary tract (NTS), area postrema (AP), and lateral parabrachial nucleus (LPBN). We hypothesized that these responses are mediated by stimulation of peripheral osmoreceptors with splanchnic and/or vagal afferent projections. To test this hypothesis, we examined the effect of bilateral subdiaphragmatic vagotomy and bilateral splanchnic denervation on the \(P_{AVP}\) and Fos immunoreactivity responses to intragastric hypertonic saline infusion in awake rats. Compared with responses in sham rats, Fos immunoreactivity responses were significantly reduced in vagotomized rats in the AP, SON, and PVN, whereas normal Fos levels were observed in the LPBN. However, vagotomized rats exhibited a normal increase in \(P_{AVP}\). Splanchnic-denervated rats also exhibited similar changes in \(P_{AVP}\) in response to intragastric hypertonic saline compared with sham-denervated rats, and no differences were observed in Fos immunoreactivity in the LPBN, SON, and PVN compared with sham rats. However, splanchnic-denervated rats were observed to have significantly lower Fos staining in the NTS and AP compared with sham rats. The inability of splanchnic or vagal denervation alone to block the \(P_{AVP}\) response to intragastric hypertonic saline suggests that either peripheral osmoreceptors project via both splanchnic and vagal afferents to mediate AVP release or that the observed response of \(P_{AVP}\) is due to the activation of central osmoreceptors in the absence of measurable changes in plasma osmolality.

C-Fos; arginine vasopressin; vagal afferents; hepatic nerves

THERE IS GROWING EVIDENCE supporting the view that peripheral osmoreceptors with central projections exist in the rat (1, 4, 17). We recently reported that an acute intragastric hypertonic saline load increases portal venous osmolality and stimulate the release of plasma arginine vasopressin (\(P_{AVP}\)) in conscious rats (3). We also observed that a number of central nuclei are activated in response to this stimulus, indicated by Fos immunoreactivity, including the nucleus of the solitary tract (NTS), area postrema (AP), lateral parabrachial nucleus (LPBN), supraoptic nucleus (SON), and paraventricular nucleus (PVN). These responses were independent of changes in plasma osmolality (\(P_{osmol}\)), indicating that central osmoreceptor activation did not contribute to these responses.

It remains unclear, however, whether peripheral osmoreceptor control of AVP release is mediated by vagal or splanchnic afferent projections. The inconsistency of previous reports is due, in part, to the methods used to stimulate peripheral osmoreceptors and the physiological responses measured. Studies supporting a vagal pathway include demonstrations that portal venous infusions of hypertonic saline modulate the firing rate of both the vagal afferent nerves (1) and NTS neurons (13, 14). Additional studies examining drinking behavior (17), salt intake (5), and ileal water absorption (20) also support a vagal afferent pathway. Alternatively, a splanchnic pathway is supported by studies measuring \(P_{AVP}\) (4), sympathetic nerve activity (10), and electrical activity of lateral hypothalamic neurons (28) in response to peripheral osmoreceptor stimulation. Finally, other studies implicate both vagal and splanchnic afferents within the hepatic nerve plexi (22–24, 26).

The present study was designed to clarify the role of splanchnic and vagal afferent pathways in mediating the central Fos immunoreactivity response patterns to peripheral osmoreceptor activation. We hypothesized that selective denervation of either abdominal vagal or splanchnic nerves would abolish both the \(P_{AVP}\) and Fos immunoreactivity responses to peripheral osmoreceptor stimulation. To test this hypothesis, the response of \(P_{AVP}\) and Fos immunoreactivity to intragastric hypertonic saline was measured in intact rats and rats with subdiaphragmatic vagotomy or bilateral splanchnic denervation.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in all experiments. Rats were housed in individual stainless steel cages in an isolated room under a 12:12-h light-dark cycle and allowed ad libitum access to rat chow and water. All studies were conducted in accordance with institutional and National Institutes of Health guidelines.

Surgical Procedures

Subdiaphragmatic vagotomy. Male Sprague-Dawley rats (275–325 g, \(n = 6\)) were given atropine (0.4 mg/kg ip) and anesthetized with pentobarbital sodium (65 mg/kg ip). Rats were then chronically instrumented with Silastic catheters into the abdominal aorta and the inferior vena cava via the left femoral vessels. The stomach was retracted through a midline abdominal incision, and the anterior and posterior subdiaphragmatic vagi were isolated along the esophagus close to the diaphragm. Proximal and distal sutures were tied around both vagi, and the nerves were sectioned between the sutures with the use of cautery. A Silastic catheter was then implanted in the greater curvature of the stomach. All catheters were tunneled subcutaneously to the dorsal surface of the skull where they were exteriorized, secured with stainless steel screws and dental acrylic, and threaded through

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a lightweight spring attached to a swivel. Rats were given a single dose of an antibiotic (gentamicin sulfate, 2 mg im) and an analgesic (butorphanol tartrate, 0.075 mg sc) and, on recovery from anesthesia, were housed in individual stainless steel cages in an isolated room. Rats were maintained under a 12:12-h light-dark cycle and allowed ad libitum access to rat chow and water. Rats were allowed a recovery period of at least 1 wk before experimentation, during which time the arterial and venous catheters were flushed daily with heparinized saline (1,000 U/ml, arterial; 50 U/ml, venous). The procedure for the sham abdominal vagotomy (n = 6) was identical to that described above, with the exception that the vagi were left intact.

Because vagotomized rats exhibit hypophagia, hypodipsia, and weight loss (16), a dietary supplement (Clinicare, Pet-Ag, Hampshire, IL; diluted 1:1 with water) or vitamin fortification (Vitamin mix AIN-76A, Research Diets, New Brunswick, NJ, mixed with a ratio of sweetened condensed milk and water; 1:28:9) was administered daily through the gastric catheter (1.0 ml) and flushed with 1.0 ml 0.9% NaCl saline. Additionally, rats were allowed ad libitum access to the suprarenal and celiac ganglia were also sectioned. The incisions were closed, and rats were given a single dose of atropine (0.4 mg/kg ip) and anesthetized with pentobarbital sodium (65 mg/kg ip), and the adrenal glands were isolated through lateral flank incisions. The nerves innervating the adrenal glands were traced back to the sympathetic chain to the suprarenal ganglia were identified and cut rostral to the ganglia (11). All nerves between the suprarenal and celiac ganglia were also sectioned. The incisions were closed, and rats were given a single dose of antibiotic (gentamicin sulfate, 2 mg im) and analgesic (butorphanol tartrate, 0.075 mg ip) and were returned to their home cage. Sham surgery for splanchnic denervation (n = 6) was identical to that described above, with the exception that the splanchnic nerves were not sectioned.

After a recovery period of at least 1 wk, rats were instrumented with femoral and gastric catheters as described above, returned to their home cage, and allowed to recover for 3 days before experimentation. The arterial and venous catheters were flushed daily with heparinized saline (1,000 U/ml, arterial; 50 U/ml, venous), and the gastric catheters were flushed daily with saline.

Experimental Protocol

Rats were studied in their home cages, and water was removed at the beginning of the experiment. The protocol was identical to that used in our recent study that characterized central Fos immunoreactivity and plasma vasopressin responses to intragastric saline infusion (3). Briefly, mean arterial pressure (MAP), heart rate (HR), plasma vasopressin (P_{AVP}), plasma osmolality (P_{osmol}), plasma protein concentration (P_{Prot}), and hematocrit (Hct) responses to intragastric infusion of hypertonic saline were measured in conscious rats. MAP was measured by connecting the arterial catheter to a pressure transducer that was connected to a polygraph (Grass Instruments, model 7D, Quincy, MA). A perfusion blood sample (1.4 ml) was drawn into a chilled microcentrifuge tube containing EDTA (0.038 mg in 75 µl) and immediately replaced by an equal volume of blood from a conscious, chronically catheterized donor rat. We have shown that this sampling protocol does not alter basal levels of MAP, HR, P_{osmol}, or P_{AVP} (3). After an equilibration period of at least 30 min, changes in MAP, HR, P_{AVP}, P_{osmol}, P_{Prot}, and Hct were measured in response to an intragastric infusion of 0.59 ml/min over 5 min of hypertonic (600 mosmol/l) saline. This stimulus increases portal venous osmolality ∼10 mosmol/l above control levels, but does not alter systemic P_{osmol} (3). Ten minutes after termination of the intragastric infusion, a second blood sample (1.4 ml) was drawn and replaced with an equal volume of donor blood.

Two hours after the intragastric infusion, rats were deeply anesthetized with pentobarbital sodium (65 mg/kg iv) and transcardially perfused, first with heparinized saline (20 U/ml; 150 ml) followed by a 4% paraformaldehyde-PBS solution. Brains were removed and stored overnight in 4% paraformaldehyde-PBS and then transferred to a 30% sucrose-PBS solution for several days. Sections (40 µm) were taken serially through the medulla and alternately through the hypothalamus, and the sections were processed for immunocytochemistry using a primary Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA) complexed with avidin-biotin (ABC Kit, Vector Laboratories, Burlingame, CA) and subsequently incubated in diaminobenzidine (DAB; Polysciences, Warrington, PA) to produce a visibly dark reaction product within the nucleus of the Fos-positive neurons. Sections were then mounted on microscope slides, and Fos immunoreactivity was quantitated using a commercially available imaging software program (Image-1, Universal Imaging, Westchester, PA). Nuclei displaying differences in Fos expression between the sham and denervated groups were identified, and the Fos-labeled nuclear profile (Fos-positive neurons per nucleus per section) was counted along the rostrocaudal extent of the nucleus of interest. Because each animal contained a varying number of sections per nucleus, the total cell counts were averaged per number of sections.

This protocol allowed us to examine the relationship between P_{AVP} and Fos immunoreactivity in the splanchnic denervation study. However, technical problems with the AVP assay prevented us from measuring both plasma AVP and Fos immunoreactivity in the vagotomy study. Therefore, the experiment was repeated in a second group of vagotomized and sham-vagotomized rats for measurement of P_{AVP}. Fos immunocytochemistry was not performed in these experiments.

Analysis of Plasma Samples

Blood samples were centrifuged at 4°C, and the plasma was saved for subsequent analysis of P_{AVP}, P_{osmol}, and P_{Prot} concentration. P_{Prot} was measured using a Reichert refractometer (Cambridge Instruments, Buffalo, NY). P_{osmol} was analyzed with a vapor pressure osmometer (Wescor, model 5500, Logan, UT). Hct was measured by the microcapillary technique before centrifugation of the samples. P_{AVP} was quantitated by radioimmunoassay at the Core Assay Laboratory in the Department of Physiology at the Medical College of Wisconsin (6).

Statistical Analysis

Statistical analysis was performed using commercially available software (StatView 4.01 and SuperANOVA 1.11, Abacus Concepts, Berkeley, CA). Changes in HR, P_{AVP}, P_{osmol}, P_{Prot}, and Hct between control and postinfusion periods were compared within groups using a paired t-test. Differences in Fos expression between sham and denervated groups were compared using an unpaired t-test. Intergroup comparisons and changes in MAP were performed using a one-way ANOVA followed by a means comparison linear contrast test to
identify points of significance for within-group and between-

group comparison. In all cases, a value of \( P < 0.05 \) was 

considered statistically significant. Results are expressed as 

means \( \pm \) SE.

**RESULTS**

**PAVP, P_{osmol}, and Cardiovascular Responses**

The effect of hypertonic saline on P_{AVP} concentration in splanchnic-denervated (n = 6) and sham-denervated (n = 6) rats is illustrated in Fig. 1. Intragastric saline significantly elevated P_{AVP} in the sham group, and the P_{AVP} response occurred in the absence of any changes in either P_{osmol} or vascular volume, as indicated by Hct and P_{Prot}. Moreover, the P_{AVP} response to intragastric hypertonic saline was not affected by splanchnic denervation. Intragastric hypertonic saline also significantly increased P_{AVP} in sham-vagotomized (n = 7) rats (Fig. 2), again without measurable changes in either P_{osmol} or volume. Furthermore, when the experiment was repeated in vagotomized (n = 8) rats, vagotomy also failed to block the observed increase in P_{AVP} (Fig. 2).

The responses of MAP and HR to intragastric hypertonic saline in sham and denervated rats are illustrated in Fig. 3. There were no statistically significant changes in MAP during the infusion, with the exception of the sham splanchnic-denervated rats, which increased to significant levels at minutes 3 and 5. A modest and statistically significant tachycardia was observed in both sham splanchnic-denervated and splanchnic-denervated groups.

**Fos Immunoreactivity**

Figures 4–10 illustrate the response of Fos immunoreactivity in the four groups of rats studied: vagotomy (n = 6), sham vagotomy (n = 6), splanchnic denervation (n = 6), and sham splanchnic denervation (n = 6). As previously reported by our laboratory (3), intragastric hypertonic saline infusion in intact rats resulted in marked Fos immunoreactivity in the SON (Fig. 4 and 6) and PVN (Figs. 5 and 6). The Fos response in both the SON and PVN was significantly reduced by abdominal vagotomy (Figs. 4–6). In contrast, Fos immunoreactivity was not significantly altered in these nuclei by splanchnic denervation (Figs. 4–6).

Fos immunoreactivity was also observed in the NTS in the sham groups (Figs. 7 and 8). Moreover, NTS Fos immunoreactivity was significantly lower in splanchnic-denervated compared with sham-denervated rats (Figs. 7 and 8). In contrast, Fos immunoreactivity in the NTS was not significantly different when comparing vagotomy to sham-vagotomized rats (Figs. 7 and 8). However, it should be noted that a significant shift in the NTS subnuclei staining pattern was observed. Whereas Fos immunoreactivity was confined primarily to the medial subnucleus of the NTS in sham-vagotomized rats, it was abolished in the medial subnucleus of vagotomized rats but significantly increased in the central subnucleus, such that overall NTS staining was not significantly different between the two groups (data not shown).

A third nucleus where Fos immunoreactivity was observed was the AP (Figs. 7 and 8). Both vagotomy and splanchnic denervation significantly lowered Fos immunoreactivity in this medullary nucleus (Figs. 7 and 8). Finally, Fos immunoreactivity was observed in the LPBN in both sham groups. Although Fos immunoreactivity was reduced by 60% in vagotomized compared with sham-vagotomized rats (Figs. 9 and 10), this was not statistically significant (\( P = 0.05 \)). Similarly, there
was no statistically significant difference between the sham-denervated and splanchnic-denervated groups (Figs. 9 and 10).

**DISCUSSION**

Previous studies from our laboratory have demonstrated that intragastric hypertonic saline loads stimulate the release of P_{AVP} and activate several central nuclei, including the NTS, AP, LPBN, SON, and PVN (3). These responses were independent of changes in either $P_{osmol}$ or volume, supporting the idea that peripheral osmoreceptors modulate the release of AVP in response to sodium ingestion. However, it remains unclear whether the responses were mediated by vagal or splanchnic afferent projections. The present study therefore examined the effect of splanchnic denervation and abdominal vagotomy on the response of plasma AVP and central Fos immunoreactivity to intragastric saline loads.

**Mechanisms Mediating the AVP Response to Intragastric Hypertonic Saline**

Although there is an increasing body of evidence supporting peripheral osmotic control of AVP release, the afferent pathway from the gastrointestinal tract (or its circulation) to the central nervous system remains unclear. Although a number of reports indicate a vagal pathway (1, 5, 13, 20), there are studies suggesting that the information travels via splanchnic afferent pathways (4, 10, 28). To date, Choi-Kwon and Baertschi (4) have provided the only direct comparison of the effects of vagotomy and splanchnic denervation on the AVP response to intragastric saline loads. They reported that bilateral splanchnic denervation attenuated the AVP response by $60\%$, whereas bilateral vagotomy had no effect, and thereby concluded that the response was mediated by splanchnic afferents. However, inability to completely abolish the AVP response has left open the possibility that either additional neural pathways...
are involved or the AVP response can be attributed, at least in part, to classical controllers of AVP release.

In the present study we were also unable to abolish the AVP response to intragastric saline using either vagotomy or splanchnic denervation. Moreover, whereas Choi-Kwon and Baertschi (4) reported a 60% reduction of the AVP response after splanchnic denervation, we did not observe such an attenuation. Failure of either splanchnic denervation or abdominal vagotomy to affect the AVP response to intragastric hypertonic saline leads to several possible conclusions. First, the response may indeed be mediated by peripheral osmoreceptors whose afferent projections include both vagal and splanchnic afferent fibers. This would be consistent with the observations of Morita et al. (22–24, 26), who have extensively examined hepatic control of renal nerve activity in response to intraportal sodium. Thus neither the vagal nor splanchnic denervation technique employed in this study would be individually sufficient to block the observed AVP response, as the remaining afferent pathway could modulate plasma AVP. In support of this, we have previously observed that intragastric hypertonic saline increases portal venous osmolality but not systemic P_{osmol}, thereby creating a localized osmotic signal within the portal circulation that would be capable of stimulating such hepatic receptors (3). However, the only way to test such a hypothesis would be to repeat the experiment in rats in which both vagal and splanchnic afferents were sectioned.

Fig. 4. Photomicrographs of Fos immunoreactivity in the supraoptic nucleus (SON) in sham-Vx (A), Vx (B), sham-Sx (C), and Sx (D) rats receiving an intragastric infusion of hypertonic saline.

Fig. 5. Photomicrographs of Fos immunoreactivity in the paraventricular nucleus (PVN) in sham-Vx (A), Vx (B), sham-Sx (C), and Sx (D) rats receiving an intragastric infusion of hypertonic saline.
Second, the failure of either vagal or splanchnic denervation to abolish the AVP response may also be attributed to baroreceptor modulation of AVP release independent of a peripheral osmoreceptor signal. It is possible that a fluid shift from the vascular compartment to the gastric lumen occurred in response to a high osmolality in the stomach. Such a shift would elicit AVP release via baroreceptor pathways secondary to vascular volume depletion. However, if water were osmotically drawn into the gastric lumen, increases in both $P_{Prot}$ and Hct should be observed as a result of hemoconcentration due to volume contraction. In contrast, we have observed, in this and our recent study (3), that $P_{Prot}$ decreases or remains unchanged, which is

![Fig. 6. Effect of Vx (n = 6), sham Vx (n = 6), Sx (n = 6), and sham Sx (n = 6) on Fos immunoreactivity in the SON (A) and PVN (B) in response to intragastric infusions of hypertonic saline. Nuclear profiles are displayed as average Fos-positive nuclear profile per nucleus per section. *P < 0.05 vs. sham levels.](image)

![Fig. 8. Effect of Vx (n = 6), sham Vx (n = 6), Sx (n = 6), and sham Sx (n = 6) on Fos immunoreactivity in the NTS (A) and AP (B) in response to intragastric infusions of hypertonic saline. Nuclear profiles are displayed as average Fos-positive nuclear profile per nucleus per section. *P < 0.05 vs. sham levels.](image)

![Fig. 7. Photomicrographs of Fos immunoreactivity in the nucleus of the solitary tract (NTS) and area postrema (AP) in sham-Vx (A), Vx (B), sham-Sx (C), and Sx (D) rats receiving an intragastric infusion of hypertonic saline.](image)
consistent with either no volume shift or volume expansion. Moreover, we have observed moderate increases, rather than decreases, in arterial pressure during the intragastric infusion. This response, coupled with the observation of volume expansion, would serve to inhibit AVP release via arterial and cardiopulmonary baroreceptors.

A third explanation would be that central osmoreceptor activation accounts for the observed AVP response but that the change in plasma osmolality was below the limits of detection of our osmometer. Intravenous (rather than intragastric) infusion of 2.95 ml of a 600 mosmol/kg saline solution (total of 1.77 mosmol) into a 300-g rat would increase $P_\text{osmol}$ a maximum of 5 mosmol/kg after equilibration between the extracellular and intracellular compartments (assuming total body water = 66% body wt). Hence, the $P_\text{osmol}$ response to an intragastric infusion of the same solution would be between 0 and 5 mosmol/kg, depending on how much of the sodium chloride was absorbed across the intestinal epithelium. In theory, this fluid shift may have occurred by the time the plasma sample was taken (10 min postinfusion). Although this represents <2% maximum increase in $P_\text{osmol}$, it is enough to cause a modest increase in plasma AVP in the dog (32). This explanation seems unlikely, however, based on our recent study in which we simultaneously measured the response of portal venous and systemic arterial osmolality before, during, and after intragastric infusion of 600 mosmol/kg saline (3). Repeated measurements of portal venous osmolality at 2-min intervals revealed statistically significant increases of 5 mosmol/kg and greater during the 10-min postinfusion period. This change from preinfusion portal venous osmolality (294 mosmol/kg) was a 1.7% increase. In contrast, systemic $P_\text{osmol}$ remained constant in the same animal during that period. This observation suggests that we are able to detect <2% increase in $P_\text{osmol}$ and that portal venous osmolality increases when systemic $P_\text{osmol}$ does not. Although this supports a role for a localized stimulus to peripheral osmoreceptors, we cannot exclude a contribution of central osmoreceptors entirely.

The possibility that the failure of either vagal or splanchnic denervation to abolish the AVP response may also be attributed to incomplete denervations must be considered. Although we did not employ techniques to verify the completeness of lesions, we find such a possibility unlikely due to both the uniformity of the AVP response among the rats as well as the ease of accessing and sectioning both the splanchnic and vagal nerves. Additionally, the splanchnic nerves were sectioned in two separate places to prevent either incomplete lesions or possibility of regrowth. Furthermore, in all rats vagotomy was followed by a time of marked hypodipsia and hypophagia that served as an indicator of successful denervation. Moreover, the fact that both denervations had marked affects on the Fos immunoreactivity staining pattern demonstrates that afferent...
projections were indeed interrupted. Therefore, failure to observe even modest effects on AVP responses cannot be explained by insufficient denervation procedures.

Finally, our results raise the possibility that peripheral osmoreceptors may stimulate AVP release independent of vagal or splanchnic afferent nerves. This would include alternate neural afferent projections or possibly nonneural hormonal signals. For example, sodium chloride ingestion may stimulate the release of gastrointestinal hormones (e.g., secretin, cholecystokinin), which then circulate to circumventricular organs (e.g., AP) to modulate AVP release. These possibilities remain open for investigation.

Central Fos Immunoreactivity Responses to Intragastric Hypertonic Saline

As we previously reported, Fos immunocytochemistry indicated that intragastric hypertonic saline activated several nuclei, including the NTS, AP, and LPBN in addition to the SON and PVN (3). The expression of Fos in each of these nuclei was affected by both vagotomy and splanchnic denervation. In the vagotomized group, Fos immunoreactivity was significantly lower in the SON and PVN as well as the AP and medial NTS. In addition, Fos staining was reduced in the LPBN in vagotomized rats by 60% compared with sham-vagotomized rats, but this did not quite reach statistical significance (P = 0.05). Although splanchnic denervation did not affect Fos immunoreactivity in the SON, PVN, or LPBN, expression in the AP and NTS was significantly reduced.

The observations that vagotomy markedly reduced Fos immunoreactivity in both the SON and PVN, but had no effect on the AVP response to hypertonic saline, suggest that not all of the Fos staining observed was in AVP magnocellular neurons. It is important to note that Fos immunoreactivity in the SON of vagotomized rats receiving hypertonic saline (∼50/section) was elevated compared with that observed in intact rats receiving isosotonic saline (∼15/section) (3). In other words, it appears that vagotomy markedly reduced Fos staining in SON, but we cannot conclude that it was abolished. Whether the remaining Fos-positive cells were indeed AVP cells can only be ascertained using double-labeling techniques.

This interpretation necessarily implies that intragastric hypertonic saline activated SON and PVN cells that mediate responses other than AVP release. For example, it is possible that oxytocin cells in the SON and PVN are also activated by this stimulus. It has been shown that a hyperosmotic challenge administered either intraperitoneally (2) or systemically (19) stimulates the release of oxytocin from the hypothalamus. Moreover, intraperitoneal injection of hypertonic saline also activates oxytocinergic neurons in the SON, as indicated by Fos expression (33). Based on the involvement of oxytocin in maintaining sodium homeostasis (30), the idea that peripheral osmoreceptors modulate the release of oxytocin presents an interesting hypothesis. It is known that cholecystokinin (CCK) activates vagal afferent fibers within the gastrointestinal system to stimulate oxytocin release, and this response is abolished by abdominal vagotomy (31).

Another possibility is that peripheral osmoreceptor activation of splanchnic and vagal afferents is involved in control of sympathetic activity rather than the release of AVP. As described above, the work of Morita and colleagues (9, 22–24, 27) suggests that osmotic information travels via both vagal and splanchnic afferents to the NTS to modulate renal sympathetic nerve activity. In an elegant series of experiments in conscious dogs, it was shown that a high-NaCl meal inhibited renal sympathetic nerve activity and the response abolished by hepatic denervation (23). Moreover, this stimulus did not stimulate AVP release in these dogs. Although we did not measure renal sympathetic nerve activity in this experiment, it is possible that the lower levels of Fos immunoreactivity observed in the AP, NTS, and the PVN in both denervated groups correlates with removal of afferents involved in the control of sympathetic activity rather than AVP release. Taken together, these observations support the view that activation of peripheral osmoreceptors may result in a number of neurohumoral responses.

Another possibility is that both denervations remove mechanoreceptive input from the stomach, thereby accounting for some of the observed decreases in Fos immunoreactivity. Fraser et al. (8) demonstrated that gastric distension increased Fos expression in the dorsal motor nucleus of the vagus nerve (DMNV) as well as medial subnucleus of the NTS (mNTS). We do not feel our results are due to activation of gastric mechanoreceptors, however. In this and our previous report (3), we did not observe Fos immunoreactivity in the DMNV, indicating that volume of saline infused in these studies (∼2.9 ml) was probably of insufficient magnitude to cause gastric distension and activate mechanoreceptors. Additionally, we have observed that isosotonic saline infused intragastrically at the same rate (0.59 ml/min) for 15 min (total volume = 8.9 ml) resulted in significantly less Fos expression in the LPBN, a site known to receive gastric mechanoreceptive input (unpublished results). This argues against gastric distension being the primary stimulus for changes in Fos immunoreactivity in the LPBN.

We have reported that intragastric hypertonic saline increases Fos immunoreactivity in the LPBN (3). In the present study it is interesting to note that neither vagotomy nor splanchnic denervation altered Fos staining in this nucleus. It is important to point out, however, that, despite the failure to show a statistically significant effect, Fos staining was ∼60% lower in vagotomized rats and ∼30% lower in splanchnic-dener-
vated rats compared with sham controls. These results are suggestive of dual inputs to the LPBN from both splanchnic and vagal afferents. The LPBN receives extensive afferent input from the NTS and AP, and the fact that staining was maintained in light of significant decreases in both the NTS and AP (in vagotomized and splanchnic-denervated rats) is difficult to explain. It is possible that both the vagus and splanchnic nerves contain afferents that project to AP and NTS neurons, which then project to the LPBN, that one input is able to compensate for loss of the other, thereby accounting for Fos staining in the LPBN. This could add support to peripheral osmoreceptor control of sympathetic activity, as the LPBN is a site of sympathetic regulation (7, 12, 21). However, to fully test this hypothesis would require experiments in rats with combined abdominal splanchnic and vagal denervation.

Technical Considerations Concerning Denervation Techniques

The results of the present study are not consistent with those of Choi-Kwon and Baertschi (4) in which a bilateral splanchnic denervation reduced the AVP response to intragastric hypertonic saline by 60%. Because we used the identical osmotic stimulus, the reasons for this conflict are unclear. The most likely cause is differences in the denervation procedures. With the use of the midline technique for splanchnic denervation described in their report, we were unable to access the right splanchnic nerves above the level of the celiac ganglia. Instead, we used the lateral approach described by Jasper and Engeland (11) to transect both splanchnic nerves above the suprarenal ganglia. It is possible that the splanchnic side-branch lesion procedure they used removed mixed hepatic innervation, thus sectioning both splanchnic and vagal afferents, which may account for the attenuation of the AVP response in their report (4). However, because definitive verification of afferent nerve section was not performed in either study, the reason for the discrepancy between studies remains unclear.

Perspectives

An increasing number of studies support a role for peripheral osmoreceptors in regulation of body fluid homeostasis. One mechanism that has been proposed is through the stimulation of AVP release. However, the present study fails to support the concept of peripheral control of vasopressin release in rats, because neither abdominal vagotomy nor splanchnic denervation altered the response of plasma AVP to intragastric hypertonic saline. Although this does not entirely exclude the possibility that peripheral osmoreceptors modulate the release of AVP in response to sodium ingestion, it leaves open the possibility that the central osmoreceptors are responsible for the observed increase in AVP, a position supported by other reports (18, 29). However, the Fos immunocytochemistry results support the hypothesis that peripheral osmoreceptors modulate functions other than AVP release, including oxytocin release, drinking behavior (5, 15, 17), sympathetic nerve activity (9, 23, 24), and jejunal (25, 26) and ileal (20) sodium and water reabsorption. Therefore, the effects of peripheral osmoreceptors may be multifaceted, affecting sodium and water reabsorption and sympathetic activity, and behavior.

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