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The role of the subfornical organ in AngII-salt hypertension in the rat

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Abstract

Hypertension caused by chronic infusion of angiotensin II (AngII) in experimental animals is dependent, in part, on increased activity of the sympathetic nervous system. This chronic sympathoexcitatory response is amplified by a high salt diet suggesting an interaction of circulating AngII and dietary salt on sympathetic regulatory pathways in the brain. The present study tested the hypothesis that the subfornical organ (SFO), a forebrain circumventricular organ known to be activated by circulating AngII, is crucial to the pathogenesis of hypertension induced by chronic AngII administration in rats on a high salt diet (AngII-salt model). Rats were randomly selected to undergo either subfornical organ lesion (SFOx) or sham surgery (SHAM) and then placed on a high salt (2% NaCl) diet. One week later rats were instrumented for radiotelemetric measurement of mean arterial pressure (MAP) and heart rate (HR) and placed in metabolic cages to measure sodium and water balance. Baseline MAP was slightly (but not statistically) lower in SFOx compared to SHAM rats during the 5 day control period. During the subsequent 10 days of AngII administration, MAP was statistically lower in SFOx rats. However, when MAP responses to AngII were analyzed by comparing the change from the 5 day baseline period, only on the 5th day of AngII was MAP significantly different between groups. There were no differences between groups for water or sodium balance throughout the protocol. We conclude that, although the SFO is required for the complete expression of AngII-salt hypertension in the rat, other brain sites are also involved.

Keywords

sympathetic; telemetry; blood pressure; angiotensin; brain; subfornical organ

Introduction

Hypertension caused by chronic administration of angiotensin II (AngII) in experimental animals is mediated, in part, by activation of the sympathetic nervous system (Brody *et al.*, 1978; Osborn & Fink, 2010; Osborn *et al.*, 2011). Recent studies suggest that the magnitude of the neurogenically mediated AngII-induced hypertension is amplified by a high salt diet (Osborn & Fink, 2010; Osborn *et al.*, 2011). This concept is supported by the observation

that, in addition to the AngII-induced increase in arterial pressure, “whole body” sympathetic activity is also greater in AngII-infused animals on a high salt diet than those on a normal salt diet (King & Fink, 2008; Osborn & Fink, 2010). The increase in sympathetic activity observed in the “AngII-salt” model is delayed in onset, not appearing until approximately 5 days of AngII administration (Osborn & Fink, 2010; Osborn *et al.*, 2011).

The mechanisms by which circulating AngII modulates SNA are not clear but likely involve forebrain circumventricular organs (CVO) which, due to their weak blood brain barrier, act as the key sensory sites for central autonomic pathways (Johnson & Loewy, 1990). The subfornical organ (SFO) is a CVO that mediates many of the physiological responses to AngII including regulation of salt appetite (Thunhorst *et al.*, 1990; Weisinger *et al.*, 1990), thirst (Smith *et al.*, 1995), and the central pressor and dipsogenic effects of this hormone (Simpson, 1981; Ferguson & Wall, 1992; Ferguson & Bains, 1997). Direct application of AngII to the SFO elicits a pressor response that is blocked by an AngII receptor antagonist (Mangiapane & Simpson, 1980b) and the acute pressor response to intravenous AngII is blocked by lesion of the SFO (Mangiapane & Simpson, 1980a). The organum vasculosum of the lamina terminalis (OVLT) is another forebrain CVO that may contribute to the salt-sensitive component of AngII-salt hypertension. The OVLT has been proposed to act as the “central osmoreceptor” and is a key site in mediating the sympathoexcitatory responses to acute increases in plasma sodium concentration (Toney *et al.*, 2003; Toney & Stocker, 2010). Moreover, chronic increases in salt intake have been reported to markedly amplify sympathoexcitatory responses in general (Ito *et al.*, 1999; Adams *et al.*, 2007; Stocker *et al.*, 2008), not just to AngII, and this effect is modulated by the OVLT (Adams *et al.*, 2009).

We have hypothesized that circulating AngII and dietary salt converge on sympathoexcitatory pathways in the brain by signaling in the SFO and OVLT, respectively, and thereby activate downstream pathways in the brain (Osborn *et al.*, 2007). Both the SFO and OVLT send projections to the median preoptic nucleus (MnPO), a site which is necessary for AngII mediated hypertension (Ployngam & Collister, 2007). The MnPO is an established integration station with excitatory projections to the paraventricular nucleus (PVN), a brain site which drives SNA by at least two pathways: excitatory projections to sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) and direct projections to preganglionic neurons in the spinal cord (Toney *et al.*, 2003; Toney & Stocker, 2010).

A role for the SFO in AngII-induced models of hypertension in animals consuming a *normal* salt diet is supported by recent studies. We have reported that SFO lesion abolishes the arterial pressure response to 10 days of intravenous AngII in rats (Hendel & Collister, 2005). Studies of AngII-induced hypertension in mice indicate that reactive oxygen species (ROS) are increased in the SFO and interference with this signaling pathway in the SFO ameliorates the neurogenic phase of AngII-induced hypertension (Zimmerman *et al.*, 2004). However, the role of the SFO in models induced by the combination of AngII and a *high* salt diet are not as clear. Although AngII-induced hypertension in the mouse is salt-dependent (Cholewa *et al.*, 2005), the contribution of the SFO to AngII-salt hypertension has not been investigated in this species. In an earlier study we reported that lesion of the SFO exacerbated, rather than attenuated, AngII induced hypertension in rats on a high salt intake (Bruner *et al.*, 1985). However, in that study daily sodium intake was increased by intravenous infusion of isotonic saline, and AngII was infused intravenously for only 5 days. In light of our more recent work demonstrating that the neurogenic phase of AngII-salt hypertension does not begin until approximately the 5th day of AngII administration (Osborn *et al.*, 2011), investigation of the SFO’s role during longer periods of AngII administration in animals consuming a high salt diet are required.

Therefore, the present study was designed to test the hypothesis that signaling in the SFO is a major contributor to AngII-salt hypertension in the rat. We tested this hypothesis by measuring the arterial pressure and fluid balance responses to 10 days of AngII administration in SFO lesioned and sham-operated rats consuming a high salt diet. Previous studies of AngII-salt hypertension have been conducted in tethered (King & Fink, 2006; King & Fink, 2008; Yoshimoto *et al.*, 2010) and non-tethered (Osborn & Fink, 2010) rats. It is often presumed that tethering causes at least low-level stress in experimental animals. Since increased salt intake has been reported to augment the response of the sympathetic nervous system to acute stress (Falkner *et al.*, 1981; Scrogin *et al.*, 1991), studies were conducted in tethered and non-tethered animals.

Methods

General Procedures

All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with institutional and National Institutes of Health guidelines. 275-300 g male Sprague-Dawley rats (Charles River Laboratory; Wilmington, MA) were housed in a 12:12-hr light-dark animal housing facility and provided fresh distilled water and high-salt diet (2.0% NaCl) rat pellets (Research Diets; New Brunswick, NJ) ad libitum until the beginning of the protocol. Following the SFO lesion or SHAM surgeries, rats were housed in metabolic cages (Nalgene, Nalge Nunc International; Rochester, NY), and provided distilled water and powdered high-salt diet (2.0% NaCl (Research Diets; New Brunswick, NJ) ad libitum.

Surgical Procedures

Rats were randomly selected for either electrolytic lesion of the subfornical organ (SFOx; n = 23) or sham (SHAM; n = 20) operation. Rats were preanesthetized with pentobarbital (32.5 mg/kg, ip) and atropine (0.2 mg/kg, ip). Surgical anesthesia was achieved with a second intramuscular injection containing a cocktail of anesthetic agents (acetylpromazine, 0.2 mg/kg; butorphanol tartrate, 0.2 mg/kg; ketamine, 25 mg/kg). Rats were then placed in a stereotaxic apparatus and the SFO was lesioned as previously described (Collister & Hendel, 2003). Briefly, a dorsal midline incision was made through the skin of the skull. Bregma and lambda landmarks were exposed and a 3 mm hole was made in the skull 0.8 mm posterior to bregma. A Teflon-insulated monopolar tungsten electrode was lowered to four predetermined coordinates, and a current of 1 mA was passed for 8 seconds. Sham operations were identical to lesions with the exception that ventral coordinates were 1.5 mm less so as to not damage the SFO, and no current was passed. The hole in the skull was repaired with bone wax and the skin was closed with 3-0 silk suture. After surgery, all rats were given an intramuscular antibiotic injection of 2.5 mg gentamycin and a subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes.

One week later rats were instrumented with radiotelemetric pressure transducers (Model #TA11PA-C40, Data Sciences International, Inc., St. Paul, MN) for the continuous sampling of mean arterial pressure (MAP) and heart rate (HR). A 2 cm incision was made in the groin area and any visible connective tissue was teased apart to visualize the left femoral artery and vein. Then, a 3 cm midline abdominal incision was made to expose the abdominal cavity and the transmitter catheter was passed through the abdominal muscles with a 15 gauge hypodermic needle. The body of the transmitter was secured into the abdominal cavity by suturing it to the abdominal muscles with 3-0 silk suture thread, and the skin was closed with surgical staples. The femoral artery was isolated, a small incision was made, and the catheter was advanced proximally into the femoral artery so the tip was placed just distal to the renal arteries. The catheter was tied to the vessel with 3-0 silk suture, and a drop of

medical adhesive (Vetbond TM; 3M, St. Paul, MN) was applied to secure the catheter in place. The wound was then closed with 3-0 silk suture.

24 of the 43 animals in this study were tethered. These rats were fitted with a custom made rodent vest which had a 15 inch flexible spring that was connected to a freely rotating swivel placed above the cage. The other 16 rats were placed in the cage without a vest or tether. Rats were then given a one week recovery period before beginning the experimental protocol.

Experimental Protocol

MAP and HR were sampled using Dataquest V software (Data Sciences International, St. Paul, MN) every 4 minutes for 10 seconds throughout the protocol. 24 hour food and water intake and urine output were measured gravimetrically. Sodium intake was calculated as the product of the food intake and the sodium content of the food (2.0% NaCl, 0.35 mmol/g). Urinary sodium concentration was measured with an ion-specific electrode (NOVA-5⁺, Biomedical; Waltham, MA). 24 hour urinary sodium excretion was calculated as the product of the urine flow rate and urinary sodium concentration. 24 hour sodium and water balances were calculated as the difference between intake and urinary excretion of sodium and water, respectively.

The experimental protocol ran for 15 days and consisted of 5 days of baseline measurements followed by 10 days of continuous AngII (Sigma Chemical Corp., St. Louis, MO) treatment. AngII was administered subcutaneously at a rate of 150 ng/kg/min by osmotic minipump (Model 2ML2, Durect Corp, CA). The minipump implantation was done under isoflurane anesthesia immediately following measurements on day 5 of the protocol. This dose and route of AngII administration was chosen for comparison of the findings with our previous studies (Osborn & Fink, 2010; Osborn *et al.*, 2011. Although we haven't measured plasma AngII under these conditions, other have reported that this protocol results in a small increase of plasma AngII that is well within the physiological range (Huang *et al.*, 2010).

Histological Verification of Subfornical Organ Lesion

Upon completion of the protocol, rats were anesthetized as described above and perfused intracardially with 4% paraformaldehyde. Whole brains were dissected and soaked in 4% paraformaldehyde overnight and then transferred to a 30% sucrose solution for a minimum of 2 days. Frozen serial sagittal sections (40 μ m) were made at the lateral edge of the third ventricle, mounted on slides and stained for Nissl substance with cresyl violet stain. Confirmation of complete SFO lesion or intact SFO (SHAM) was made under light microscopy.

Data Analysis and Statistics

All values are presented as 24 hour means $\pm$ standard errors. Analysis of variance (ANOVA) with repeated measures was used to determine significant differences between and within groups. Holm-Sidak's post-hoc test was used to determine between group differences at determined sampling intervals using Sigmapstat software (SPSS, Science, Chicago, IL). Statistical significance was defined as $P < 0.05$.

Results

Rats were considered lesioned if $\geq 80\%$ of the SFO was ablated by the lesion including the rostrocaudal efferent fibers of the SFO. Shown in Figure 1 are representative examples of a SHAM rat (left panel) and SFOx rat (right panel). All SFOx rats included in the final

analysis of the data were confirmed to have complete lesions of the SFO. 6 rats out of 23 were excluded due to incomplete SFO lesions.

Effect of tethering on the response of SHAM and SFOx rats to AngII administration

Shown in Figure 2 are the mean arterial pressure (MAP; top panel) and heart rate (HR; bottom panel) responses to AngII in the 4 groups; non-tethered SHAM (n = 9), non-tethered SFOx (n = 7), tethered SHAM-T (n = 11) and tethered SFOx-T (n = 10). AngII increased MAP in all 4 groups. There was no statistical effect of tethering within either SHAM or SFOx groups for MAP or HR throughout the protocol. Therefore, tethered and non-tethered animals were combined for subsequent analysis and are presented as two groups, SFOx (n = 17) and SHAM (n = 20).

Effect of SFO Lesion on AngII-salt Hypertension

Shown in the left panels of Figure 3 are the MAP (top-left) and HR (bottom-left) responses of SHAM and SFOx rats to AngII administration. ANOVA revealed a significant difference between groups for MAP ($F=10.84$, $P=0.002$) but not HR ($F=0.613$, $P=0.439$). During the 5 day control period MAP in SFOx was slightly lower but was not statistically different from SHAM rats and HR was similar in SFOx and SHAM groups. However, beginning on the first day of AngII treatment, MAP was significantly higher in SHAM compared to SFOx rats and remained so for the duration of the AngII period. HR decreased in both groups in response to AngII (bottom left panel) and there were no statistical differences between groups.

Since SFOx rats had slightly lower basal levels of MAP compared to the SHAM control group, the cardiovascular responses to AngII were re-plotted as the change from the 5 day control period (Figure 3 right panels). The hypertensive response to AngII was modestly less in SFOx rats ($F=4.731$, $P=0.041$) reaching statistical significance only on day 5 of AngII treatment. Moreover, the bradycardic response to AngII was significantly less in SFOx rats ($F=9.616$, $P=0.004$) reaching statistical significance on days 6-8 of AngII administration.

Effect of SFO lesion on Sodium and Water Balance Responses to AngII Administration

Animals were studied in metabolic cages to measure sodium and water balance. These measurements are somewhat imprecise in that, although water and sodium intake are measured accurately, urinary water and sodium excretion are consistently less than intakes due to extrarenal losses and the technical challenges of recovering 100% of the 24h urine sample. Nonetheless, they do provide a essential information related to salt and water loading and relative changes between groups.

As shown in Figure 4, there were no differences between groups for water intake ($F=0.607$, $P=0.441$), urine output ($F=1.480$, $P=0.232$) or water balance ($F=0.464$, $P=0.506$). AngII had no significant effect on water intake, urine output or water balance in either group. There were also no differences between groups for cumulative water balance over the duration of the study (data not shown).

The effects of AngII on sodium balance are shown in Figure 5. Similar to water balance, there were no differences between groups for sodium intake ($F=1.433$, $P=0.240$), sodium excretion ($F=3.129$, $P=0.086$) or sodium balance ($F=1.292$, $P=0.264$). There were also no differences between groups for cumulative sodium balance over the duration of the study (data not shown).

Discussion

There were two major findings from this study. First, in contrast to our hypothesis, hypertension in AngII-salt rats was only modestly affected by lesion of the SFO. Although SFO lesioned rats had statistically lower levels of arterial pressure during the entire AngII administration period compared to SHAM rats, this was mainly due to the fact that the basal level of pressure was also lower in lesioned rats. When we specifically compared the response of arterial pressure to AngII by plotting the change from control levels, there was no statistical effect of SFO lesion with the exception of day 5 of the 10 day AngII period. Second, the hypertensive response to AngII was identical in tethered and non-tethered rats suggesting that this form of minor restraint does not alter the development of hypertension of this model. We conclude that although the SFO contributes to the hypertensive response to AngII administration in rats consuming a high salt diet, other brain sites are required for full expression of AngII-salt hypertension.

Interpretation of Results Compared to Previous Studies

A key difference between our study in which lesion of the SFO had a minor effect on AngII-induced hypertension, and previous studies in which interventions that targeted the SFO abolished AngII-induced hypertension (Zimmerman *et al.*, 2004; Hendel & Collister, 2005), is the duration of AngII administration and the prevailing level of salt intake. To our knowledge there is only one other investigation of the effect of SFO lesion on AngII-induced hypertension in animals on a high salt intake. Surprisingly SFO lesioned rats showed an greater response to AngII administration compared to intact rats (Bruner *et al.*, 1985). However, there are two key differences between this previous report and the present study that explains these disparate findings. First, although daily sodium intake was similar (~ 6-8 mEq/day), our earlier investigation achieved this by intravenous infusion of 40 ml/day of isotonic saline, in contrast to the current study in which rats ingested a high salt diet and water *ad libitum*. Infusion of isotonic saline would not be expected to increase plasma osmolality and activate osmosensitive pathways as does a high salt diet, which we believe is a key component in the activation of sympathetic activity in the AngII-salt model (see below). Moreover, sodium administered by intravenous infusion also bypasses hepato-portal osmoreceptors which may play an important role in central responses to salt ingestion (Morita *et al.*, 1993; Carlson *et al.*, 1997; Carlson & Osborn, 1998). Another key difference between these studies is the duration of AngII administration, which was 5 days in the previous report (Bruner *et al.*, 1985) and 10 days in the present study. We have previously reported that activation of the sympathetic nervous system in AngII-salt rats is delayed in onset, not occurring until 5 days of AngII administration (King & Fink, 2006; King & Fink, 2008). This suggests that the hypertension during the initial 5 days of AngII is mediated by non-neural mechanisms and therefore interventions which alter the sympathetic response to AngII, such as SFO lesion, will not attenuate the arterial pressure response during this non-neurogenic phase. Taken together both studies are consistent with the hypothesis that signaling in the SFO is not required for the initial phase of AngII-salt hypertension.

Recent studies suggesting an important role for the SFO in AngII-induced hypertension have been conducted in animals on a normal salt diet, rather than a high salt diet. We previously observed that SFO lesion abolished the arterial pressure response to 10 days of AngII administration (Hendel & Collister, 2005). However, rats in that study consumed a normal salt diet and the magnitude of the hypertensive response was less (~10 mmHg) than the present study in which rats consumed a high salt diet (~35 mmHg). It is interesting to note that the magnitude of the effect of SFO lesion on the AngII-induced increase in arterial pressure was similar in both studies, on the order of 5-10 mmHg. This is consistent with the idea that AngII acting in the SFO is required for the hypertensive response, but the amplification of this response by a high salt diet involves other brain sites. In another study,

AngII-induced hypertension in mice on a normal salt diet was abolished by adenoviral delivery of superoxide dismutase (SOD) to the brain by intracerebroventricular administration (Zimmerman *et al.*, 2004). Based on the robust expression of the virus in the SFO, it was suggested that AngII induced hypertension was secondary to increased production of superoxide in the SFO resulting in increased sympathetic activity and that overexpression of SOD in the SFO prevented this response (Zimmerman *et al.*, 2004). However, it is important to note that in that study, as well as another from the same group investigating the contribution of AngII to sympathetic activity in heart failure, the virus was also expressed in other brain regions such as the OVLT and choroid plexus of the 3rd ventricle (Lindley *et al.*, 2004; Zimmerman *et al.*, 2004).

Role of osmo- or Na⁺- sensitive sites in amplification of AngII-induced hypertension

The hypothesis that increased plasma osmolality, secondary to a high salt intake, interacts with plasma AngII to increase sympathetic activity has been proposed (Brooks *et al.*, 2001; Brooks *et al.*, 2005; Osborn *et al.*, 2007; Toney & Stocker, 2010). Indeed, the contribution of the sympathetic nervous system to AngII-induced hypertension is enhanced in animals consuming a high salt diet compared to animals on a normal or low salt diet (King & Fink, 2008). The results of the present study suggest that the neurogenic component of hypertension induced by the combination of AngII and a high salt diet is not mediated exclusively by signaling in the SFO, suggesting that osmosensitive neurons in other CVOs are also likely to play a role. It is important to note that measurable increases in plasma osmolality in the AngII-salt model have not been reported. This may be due to the fact that such responses are small in magnitude and transient due to osmotically driven fluid transfer from intracellular to extracellular compartments. Nonetheless, decreases in cell volume could still act as an “osmosensor” in key brain structures.

One brain site where increased salt intake may act to amplify the sympathetic response to AngII, and other stimuli, is the OVLT (Toney & Stocker, 2010). Several studies have shown that rats on a chronic high salt diet exhibit exaggerated sympathoexcitatory responses (Scrogin *et al.*, 1991; Ito *et al.*, 1999; Adams *et al.*, 2007). It was recently reported that the enhanced sympathetic nerve activity and arterial pressure responses to activation of sympathetic premotor neurons in the RVLM in rats on a high salt diet was abolished by lesion of the OVLT (Adams *et al.*, 2009). Since chronic AngII administration is likely to increase sympathetic activity by stimulation of the RVLM, this observation supports the hypothesis that a high salt diet amplifies the neurogenic actions of AngII secondary to stimulation of osmosensitive neurons in the OVLT which activate an OVLT-PVN-RVLM pathway (Toney & Stocker, 2010). This hypothesis remains to be tested by further experiments.

Theoretically, since the magnitude of the neurogenic response to chronic AngII may be dependent on sodium sensing neurons in the OVLT, it is possible that the arterial pressure response to AngII in animals on a normal salt diet may be attenuated by interventions which block sodium sensing pathways in the brain. If this true then any intervention that abolishes the ability of the “sodium sensor” to modulate AngII responses would block the neurogenic phase of AngII-induced hypertension. This is supported by several findings. First, the magnitude of the arterial pressure response to AngII increases in direct proportion to dietary salt intake (Osborn & Fink, 2010). Second, similar to other models of neurogenic salt-sensitive hypertension (Huang & Leenen, 2002; Abrams *et al.*, 2010), chronic intracerebroventricular administration of the sodium channel blocker, benzamil, abolishes the neurogenic phase of AngII-induced hypertension in animals on a high salt diet but has no effect in animals on a low salt diet (Hirsch *et al.*, 2010; Toney & Stocker, 2010). Although the exact type of sodium channel blocked by benzamil is not known, or its location in the brain, this finding nonetheless supports the hypothesis that a sodium sensing mechanism in

the brain is an essential component of AngII-induced hypertension. Third, we have recently reported that lesion of the OVLT abolishes AngII-induced hypertension in rats on a normal salt diet and this is correlated with a reduction in sympathetic activity (Vieira *et al.*, 2010). Finally, recent evidence suggests that, similar to AngII (Zimmerman *et al.*, 2004), increased salt intake alone activates ROS in sympathetic regulatory pathways in the brain (Ando & Fujita, 2011; Hirooka, 2011). If this is true, then viral delivery of SOD by ICV delivery may attenuate AngII-induced hypertension in the mouse by knocking out the sodium sensitive component of this model, similar to the effect of blocking sodium channels with ICV benzamil in the rat (Abrams & Osborn, 2008). This hypothesis remains to be tested by further studies.

Perspectives

Although it is well established that AngII and dietary salt are both known factors in the pathogenesis of hypertension and that they act synergistically to chronically increase arterial pressure, the mechanism of this interaction is still poorly understood. The present study tested the hypothesis that a single brain site, the SFO, was key in mediating AngII-salt hypertension. However, similar to findings in another model of salt-sensitive neurogenic hypertension caused by co-administration of a salt retaining hormone and a high salt intake, the DOCA-salt model, in which lesion of the SFO has minimal effects on arterial pressure (Osborn *et al.*, 2006), the results of the present study suggest that other brain sites are essential to the pathogenesis of AngII-salt hypertension. Future studies are needed to elucidate the role of these other sites with the goal of developing new therapies to treat salt-sensitive forms of neurogenic hypertension.

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REFERENCES

- Abrams JM, Engeland WC, Osborn JW. Effect of intracerebroventricular benzamil on cardiovascular and central autonomic responses to DOCA-salt treatment. *Am. J. Physiol.* 2010; 299:R000–R000.
- Abrams JM, Osborn JW. A role for benzamil-sensitive proteins of the central nervous system in the pathogenesis of salt-dependent hypertension. *Clin Exp Pharmacol Physiol.* 2008; 35:687–694. [PubMed: 18387084]
- Adams JM, Bardgett ME, Stocker SD. Ventral lamina terminalis mediates enhanced cardiovascular responses of rostral ventrolateral medulla neurons during increased dietary salt. *Hypertension.* 2009; 54:308–314. [PubMed: 19506102]
- Adams JM, Madden CJ, Sved AF, Stocker SD. Increased dietary salt enhances sympathoexcitatory and sympathoinhibitory responses from the rostral ventrolateral medulla. *Hypertension.* 2007; 50:354–359. [PubMed: 17592069]
- Ando K, Fujita M. Reactive oxygen species and central nervous system in salt-sensitive hypertension: Possible relationship to obesity induced hypertension. *Clin Exp Pharm and Phys.* 2011 “Accepted Article”.
- Brody MJ, Fink GD, Buggy J, Haywood JR, Gordon FJ, Johnson AK. The role of the Anteroventral Third Ventricle (AV3V) region in experimental hypertension. *Circulation Research.* 1978; 43((1) Supp. 1):I-2–I-13.
- Brooks VL, Haywood JR, Johnson AK. Translation of salt retention to central activation of the sympathetic nervous system in hypertension. [Review] [95 refs]. *Clinical & Experimental Pharmacology & Physiology.* 2005; 32:426–432. [PubMed: 15854153]
- Brooks VL, Scrogin KE, McKeogh DF. The interaction of angiotensin II and osmolality in the generation of sympathetic tone during changes in dietary salt intake. An hypothesis. *Ann N Y Acad Sci.* 2001; 940:380–394.

- Bruner CA, Mangiapane ML, Fink GD. Subfornical organ. Does it protect against angiotensin II-induced hypertension in the rat? *Circ. Res.* 1985; 56:462–466. [PubMed: 3971518]
- Carlson SH, Beitz A, Osborn JW. Intragastric hypertonic saline increases vasopressin and central Fos immunoreactivity in conscious rats. *American Journal of Physiology.* 1997; 272:R750–R758. [PubMed: 9087636]
- Carlson SH, Osborn JW. Splanchnic and vagal denervation attenuate central Fos but not AVP responses to intragastric salt in rats. *American Journal of Physiology.* 1998; 274:R1243–R1252. [PubMed: 9644036]
- Cholewa BC, Meister CJ, Mattson DL. Importance of the renin-angiotensin system in the regulation of arterial pressure in conscious mice and rats. *Acta Physiol Scand.* 2005; 183:309–320. [PubMed: 15743391]
- Collister JP, Hendel MD. Role of the subfornical organ in the chronic hypotensive response to losartan in normal rats. *Hypertension.* 2003; 41:576–582. [PubMed: 12623962]
- Falkner B, Onesti G, Angelakos BH. Effect of salt loading on the cardiovascular response to stress in adolescents. *Hypertension.* 1981; 3:195–199. 1981.
- Ferguson AV, Bains JS. Actions of angiotensin in the subfornical organ and area postrema: implications for long-term control of autonomic output. *Clin Exp Pharmacol Physiol.* 1997; 24:96–101. [PubMed: 9043813]
- Ferguson AV, Wall KM. Central actions of angiotensin in cardiovascular control: Multiple roles for a single peptide. *Canadian Journal Physiology and Pharmacology.* 1992; 70:779–785.
- Hendel MD, Collister JP. Contribution of the subfornical organ to angiotensin II-induced hypertension. *Am J Physiol.* 2005; 288:H680–H685.
- Hirooka Y. Oxidative stress in the cardiovascular center has a pivotal role in the sympathetic activation in hypertension. *Hypertens Res.* 2011; 34:407–412. [PubMed: 21346766]
- Hirsch DM, Guzman P, Rodriguez M, Toney GM, Osborn JW. Effect of intracerebroventricular (ICV) and subcutaneous (SC) benzamil administration on AngII-salt hypertension. *Faseb J.* 2010; 24:982–989.
- Huang BS, Ahmadi S, Ahmad M, White RA, Leenen FH. Central neuronal activation and pressor responses induced by circulating AngII: role of the brain aldosterone-“ouabain” pathway. *Am. J. Phys.* 2010; 299:H422–H430.
- Huang BS, Leenen FH. Brain amiloride-sensitive Phe-Met-Arg-Phe-NH₂-gated Na channels and Na-induced sympathoexcitation and hypertension. *Hypertension.* 2002; 39(part 2):557–561. [PubMed: 11882607]
- Ito S, Gordon FJ, Sved AF. Dietary salt intake alters cardiovascular responses evoked from the rostral ventrolateral medulla. *Am. J. Physiol.* 1999; 276:R1600–R1607. [PubMed: 10362737]
- Johnson, AK.; Loewy, AD. Circumventricular organs and their role in visceral functions. In: Loewy, AD.; Spyer, KM., editors. *In Central regulation of autonomic functions.* Oxford University Press; New York Oxford: 1990. p. 247–267.
- King AJ, Fink GD. Chronic low-dose angiotensin II infusion increases venomotor tone by neurogenic mechanisms. *Hypertension.* 2006; 48:927–933. [PubMed: 17000931]
- King AJ, Fink GD. Whole body norepinephrine kinetics in AngII-salt hypertension in the rat. *Am.J.Physiol. (Reg.Int.Comp).* 2008; 294:R1262–R1267.
- Lindley TE, Doobay MF, Sharma RV, Davisson RL. Superoxide is involved in the central nervous system activation and sympathoexcitation of myocardial infarction-induced heart failure. *Circ. Res.* 2004; 94:402–409. [PubMed: 14684626]
- Mangiapane ML, Simpson JB. Subfornical organ lesions reduce the pressor effect of systemic angiotensin II. *Neuroendocrinology.* 1980a; 31:380–384. [PubMed: 7453950]
- Mangiapane ML, Simpson JB. Subfornical organ: forebrain site of pressor and dipsogenic action of angiotensin II. *Am.J.Physiol. (Reg.Int.Comp).* 1980b; 239:R382–R389.
- Morita H, Matsuda T, Furuya F, Khanchowdhury M, Hosomi H. Hepatorenal reflex plays an important role in natriuresis after high-NaCl food intake in conscious dogs. *Circulation Research.* 1993; 72:552–559. [PubMed: 8431983]
- Osborn JW, Fink GD. Region specific changes in sympathetic nerve activity in AngII-salt hypertension. *Exp Physiol.* 2010; 95:61–68. [PubMed: 19717492]

- Osborn JW, Fink GD, Kuroki MT. Neural mechanisms of angiotensin II-salt hypertension: Implications for therapies targeting neural control of the splanchnic circulation. *Curr Hypertens Rep.* 2011; 13:221–228. [PubMed: 21298369]
- Osborn JW, Fink GD, Sved AF, Toney GM, Raizada MK. Circulating angiotensin II and dietary salt: converging signals for neurogenic hypertension. *Curr Hypertens Rep.* 2007; 9:228–235. [PubMed: 17519130]
- Osborn JW, Jacob F, Hendel M, Collister JP, Clark L, Guzman PA. Effect of subfornical organ lesion on the development of mineralocorticoid-salt hypertension. *BRAIN RESEARCH.* 2006; 1:74–82. [PubMed: 16859651]
- Ployngam T, Collister JP. An intact median preoptic nucleus is necessary for chronic angiotensin II induced hypertension. *Brain Res.* 2007; 1162:69–75. [PubMed: 17618605]
- Scroggin KE, Hatton DC, McCarron DA. The interactive effects of dietary sodium chloride and calcium on cardiovascular stress responses. *American Journal of Physiology.* 1991; 261:R945–R949. [PubMed: 1928440]
- Simpson JB. The Circumventricular Organs and the Central Actions of Angiotensin. *NEUROENDOCRINOLOGY.* 1981; 32:248–256. [PubMed: 7012657]
- Smith PM, Beninger RJ, Ferguson AV. Subfornical organ stimulation elicits drinking. *Brain Res Bull.* 1995; 38:209–213. [PubMed: 7496814]
- Stocker SD, Osborn JL, Carmichael SP. Forebrain osmotic regulation of the sympathetic nervous system. *Clin Exp Pharmacol Physiol.* 2008; 35:695–700. [PubMed: 18067592]
- Thunhorst RL, Ehrlich KJ, Simpson JB. Subfornical organ participates in salt appetite. *Behavioral Neuroscience.* 1990; 104:637–642. [PubMed: 2206433]
- Toney GM, Chen QH, Cato MJ, Stocker SD. Central osmotic regulation of sympathetic nerve activity. *Acta Physiol. Scand.* 2003; 177(1):43–55. [PubMed: 12492778]
- Toney GM, Stocker SD. Hyperosmotic activation of CNS sympathetic drive: implications for cardiovascular disease. *J. Physiol.* 2010; 588:3375–3384. [PubMed: 20603334]
- Vieira AA, Nahey DB, Collister JP. Role of the organum vasculosum of the lamina terminalis for the chronic cardiovascular effects produced by endogenous and exogenous AngII in conscious rats. *Am.J.Physiol. (Reg.Int.Comp).* 2010; 299:R000–R000.
- Weisinger RS, Denton DA, Di Nicolantonio R, Hards DK, McKinley MJ, Oldfield BJ, Osborne PG. Subfornical organ lesion decreases sodium appetite in the sodium-depleted rat. *BRAIN RESEARCH.* 1990; 525:23–30. [PubMed: 2078815]
- Yoshimoto M, Miki K, Fink GD, King A, Osborn JW. Chronic angiotensin II infusion causes differential responses in regional sympathetic nerve activity in rats. *Hypertension.* 2010; 55:644–651. [PubMed: 20100996]
- Zimmerman M, Lazartigues E, Sharma RV, Davisson RL. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ. Res.* 2004; 95:210–216. [PubMed: 15192025]

Figure 1.
Typical photomicrographs of 40- μ m sagittal sections of SHAM (left) rat and SFOx (right) rat.

Figure 2.

Comparison of 24-hour average mean arterial pressure and heart rate between tethered SFOx (SFOx-T), non-tethered SFOx (SFOx), tethered shams (SHAM-T) and non-tethered shams (SHAM) rats. There were no statistical differences between tethered and non-tethered groups.

Figure 3.

Tether and non-tethered groups were combined for both SHAM and SFOx groups. Comparison of 24-hour absolute values (left two panels) and change from baseline (right two panels) for MAP and HR in SFOx and SHAM rats. * = $p < 0.05$ between SFOx and SHAM rats.

Figure 4.
Comparison of 24-hour water intake, urine output, water balance in SFOx vs. sham rats.

Figure 5. Comparison of 24-hour sodium intake, sodium excretion and sodium balance in SFOx vs. sham rats.