Role of Spinal V1a Receptors in Regulation of Arterial Pressure during Acute and Chronic Osmotic Stress

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Abstract

Vasopressinergic neurons in the paraventricular nucleus (PVN) project to areas in the spinal cord from which sympathetic nerves originate. This pathway is hypothesized to be involved in the regulation of mean arterial pressure (MAP), particularly under various conditions of osmotic stress. Several studies measuring sympathetic nerve activity support this hypothesis. However, the evidence that spinal vasopressin influences MAP under physiological or pathophysiological conditions in conscious animals is limited. The purpose of this study was to investigate, in conscious rats, if the increases in MAP during acute or chronic osmotic stimuli are due to activation of spinal vasopressin (V1a) receptors. Three conditions of osmotic stress were examined: acute intravenous hypertonic saline, 24- and 48-hour water deprivation, and 4 weeks of DOCA-salt treatment. Rats were chronically instrumented with an indwelling catheter for intrathecal (i.t.) injections and a radiotelemeter to measure MAP. In normotensive rats, i.t. vasopressin and V1a agonist increased MAP, heart rate, and motor activity; these responses were blocked by pretreatment with an i.t. V1a receptor antagonist. However, when the i.t. V1a antagonist was given during the three conditions of osmotic stress to investigate the role of endogenous vasopressin, the antagonist had no effect on MAP, heart rate, or motor activity. Contrary to the hypothesis suggested by previous studies, these findings indicate that spinal V1a receptors are not required for elevations of MAP under conditions of acute or chronic osmotic stress in conscious rats.

Key words: paraventricular nucleus, vasopressin, osmolality, sympathetic nerve activity, intrathecal
Introduction

A number of reports implicate arginine vasopressin (AVP) as a neurotransmitter involved in the regulation of spinal sympathetic preganglionic neurons (SPNs) (10, 39). Forty percent of the spinally-projecting neurons in the paraventricular nucleus (PVN) of the hypothalamus, a key sympathoregulatory site (16, 52, 56), contain AVP mRNA (17); and PVN stimulation increases the amount of AVP in spinal fluid (36). Intrathecal administration of AVP causes a dose-dependent increase in arterial pressure (38), and this increase is prevented by pretreatment with a V1-specific antagonist (29). Likewise, a V1-specific antagonist is able to completely block increases in renal sympathetic nerve activity (SNA) and arterial pressure due to chemical stimulation of the PVN (28). Anatomically, V1a receptors have been identified in all lamina of the gray matter along the length of the spinal cord (57), including the intermediolateral cell column (IML) neurons (49); and fibers from the PVN have been found to terminate near SPNs in the IML (9, 32, 41, 50). Electrophysiological studies have shown that V1a receptors in the spinal cord depolarize neurons when activated, and this is blocked with a V1a receptor antagonist (27, 40, 47). Although these studies suggest that spinally released AVP influences SNA and arterial pressure at the level of the spinal cord, the physiological conditions which activate spinally projecting vasopressinergic pathways have not been established.

Osmotic stress is associated with increased SNA, and previous studies suggest that either spinal vasopressin or glutamate is responsible for the elevated SNA under conditions of increased osmolality (2, 15, 52). Osmoreceptors in the circumventricular organs (CVOs) detect small changes in plasma osmolality (6) and change the firing patterns of neurons that project to the PVN (52). PVN activation results in hormone release from the pituitary gland and affects SNA through direct spinal projections or via the rostral ventrolateral medulla (RVLM), which sends glutamatergic projections to the spinal cord (3, 52, 56). Several studies have found evidence for activation of PVN-spinal vasopressinergic neurons during various types of osmotic stress. Recent data from our laboratory suggest that vasopressinergic PVN neurons are activated in DOCA-salt hypertensive rats (1), which are known to have elevated plasma osmolality (33, 34). Increased osmolality during water deprivation is thought to influence arterial pressure in part through activation of descending brain pathways (7). Also, vasopressin mRNA within the PVN is enhanced during dehydration (12), and spinally-projecting PVN neurons show increased Fos labeling in water-deprived rats (51). Finally, in an in situ rat preparation, acute intravenous infusion of hypertonic saline is accompanied by an increase in lumbar SNA which is blocked by pretreatment with intrathecal V1a antagonist (2).
Taken together, the above studies are consistent with the hypothesis that increased plasma osmolality stimulates PVN vasopressinergic neurons to act on spinal V1a receptors on SPNs and elevate arterial pressure. However, this hypothesis has not been tested under conditions of osmotic stress in conscious animals. The present study tested this hypothesis by measuring the response of arterial pressure to intrathecal administration of a V1a antagonist in conscious rats under conditions of acute (intravenous hypertonic saline), semi-chronic (24- and 48-hour water deprivation), and chronic osmotic stress (4 weeks DOCA-salt treatment). Contrary to the hypothesis supported by earlier studies, we found that V1a receptors are not required for the pressor responses to osmotic stress.
Methods

Animals

Male Sprague Dawley rats were purchased from Charles River Laboratory (Wilmington, MA) and housed in a temperature-controlled animal room with a 12 hour light/dark cycle. Unless otherwise noted, animals ate normal rat chow (Lab Diet 5012) and drank distilled water ad libitum. All surgical procedures in this study were approved by the Institutional Animal Care and Use Committee.

Surgical Procedures

To continuously measure mean arterial pressure (MAP) and heart rate (HR), a telemetry transmitter (model TA11PA-C40, Data Sciences International, St. Paul, MN) was implanted into the descending aorta. At the same time, for some experiments, an intravenous (i.v.) catheter (silastic tubing, Dow Corning 508-002) was implanted for drug delivery. Rats were anesthetized with 2% isoflurane (after a brief 4% induction), given atropine sulfate (0.2 mg/kg i.p., Baxter) and gentamicin sulfate (2.0 mg i.m., Hospira), and the left femoral artery and vein were exposed. The vein was cut, and the tip of the catheter was advanced 6 cm into the inferior vena cava and tied securely into place. The catheter was tunneled under the skin to exit between the scapulae. For the telemetry device, a midline abdominal incision was made, and the body of the telemetric transmitter was placed in the abdominal cavity and sutured to the abdomen wall. The fluid-filled catheter of the transmitter was then tunneled through the abdominal wall, inserted into the femoral artery, advanced 4cm until the tip lay in the abdominal aorta caudal to the renal arteries, and tied securely into place. The femoral incision was sutured close, and the abdominal incision was closed with 9-mm surgical wound clips.

At the time of telemetry transmitter and i.v. catheter implantation, an intrathecal catheter was also implanted. A 3cm incision was made near the midline over the lumbar vertebrae, and the rat was placed in the prone position over a 150 ml beaker to slightly separate the vertebrae. A 32-gauge intrathecal catheter (CR3212 Cth RSR 32G 12 w/stylet; ReCathCo, LLC; Allison Park, PA) was threaded into a 23 gauge needle, which was inserted between L6 and S1 vertebrae until a tail flick indicated penetration of the dura. The needle was angled along the spinal column, and the catheter was advanced slightly to check for resistance. If no resistance was felt, the catheter was advanced 7cm cranially, so the tip was positioned within spinal segments T11-T13. This position was chosen based on preliminary experiments with Evans blue dye that suggested the injectate would travel rostrally and cover the length of the thoracic cord. The needle and stylet were removed, and cyanoacrylate adhesive was applied to point of
exit. A loop was made in the catheter tubing and sutured in several places to secure. The tubing was then glued to 34cm of PE10 tubing (Intramedic TM; BD, Sparks, MD) attached to 1cm of PE50 to complete the catheter. The catheter was tunneled under the skin to exit between the scapulae along with the venous catheter. The two catheters were threaded through a spring and attached to a swivel that allowed the rat to move freely. Each rat was caged individually. For all surgeries performed, amoxicillin (1mg/ml in drinking water, Westward Pharmaceutical Co.) and the analgesic buprenorphine (0.3mg/ml in 0.02ml, PharmaForce) were administered post-operatively.

On the final day of recovery, catheter placement was checked by injecting 20μl of lidocaine (10mg/ml, Hospira) into the intrathecal space. Immediate hindlimb paralysis indicated intrathecal placement; animals showing no paralysis were eliminated from the study.

**General Protocol**

Each cage was placed on a receiver (model RPC1) that was connected to a computer via a Data Exchange Matrix (DSI; St. Paul, MN). Data was acquired and analyzed with Dataquest A.R.T. 4.0 software (DSI; St. Paul, MN). MAP and HR data were collected at 500Hz over 10s every 1 min, except during the 10 min before and after injection in which the sampling rate was continuous at 500Hz. Also, an index of motor activity was monitored by the DSI system by counting the number of times the signal strength fluctuated due to changes in the animal’s position or orientation. The counts were summed and reported in counts per time.

Animals were allowed at least 5 days to recover from surgery before beginning the experimental protocols. All experiments were conducted in conscious rats in their home cages, and the experiments consisted of intrathecal (i.t.) and/or intravenous (i.v.) injections. At least ten minutes of a stable MAP baseline was collected before each injection, and MAP and HR were recorded for 1 hour following the injection. When more than one compound was injected (i.e. antagonist pretreatment before agonist injection), five minutes were allowed between the two injections. At least one day was given for recovery between different injection treatments.

All intrathecal injections were administered with a 50μl Hamilton syringe in a volume of 10μl and a rate of ~0.5μl/sec. Each intrathecal injection was followed by a 25μl flush with vehicle solution in order to empty the 23μl dead space of the catheter. Intravenous injections were weight-dependent and were followed by a flush of 0.2ml of 0.9% saline. The following drugs were administered intrathecally or intravenously: artificial cerebrospinal fluid (aCSF; Harvard Apparatus, i.t.); V1a agonist (Phe2,Ile3,Orn8-Vasopressin, American Peptide Company, 10ng i.t.); V1a antagonist [(B-mercapto-B, B-cyclopentamethylene-propionyl]1, O-Me-
Tyr 2, Arg 8)-vasopressin, Sigma-Aldrich, 100ng i.t., 100μg/kg i.v.]; [Arg8]-vasopressin (Sigma, 0.1ng i.t., 10μg/kg i.v.).

When the experiments were complete, the rats were euthanized with isoflurane and the spinal cord was dissected to determine the precise location of the intrathecal catheter tip. Approximately 1/3 of the rats in the study were also given an intrathecal injection of 10μl saturated Evans blue dye to analyze injectate spread.

Specific Experimental Protocols

**Effect of an Intrathecal V1a Antagonist on the Pressor Responses to V1a Agonists**

The purpose of this study was to determine the MAP and HR responses to intrathecal V1a agonists and confirm that these responses could be blocked by pretreatment with the selected dose (100ng) of the V1a antagonist. Normotensive rats (225-325g) with ad libitum water and normal rat chow were instrumented as described above. After at least 5 days recovery, the responses to the following were measured on separate days: 1) artificial cerebrospinal fluid (aCSF); 2) V1a agonist; and 3) the V1a agonist injected 5 minutes after pretreatment with the V1a antagonist. In a separate group of rats, intrathecal [Arg8]-vasopressin was administered with and without pretreatment 5 minutes prior with the V1a antagonist. A third group of rats was used to verify that the intrathecal V1a antagonist effectively blocked V1a receptors at a site distant from the tip of the catheter. Rats in these experiments were implanted with a second intrathecal catheter via the atlanto-occipital membrane, and the catheter was advanced caudally until the tip lay at segment T1. These rats received the V1a antagonist (or vehicle) via the lumbar catheter, followed 5 minutes later by the V1a agonist via the atlanto-occipital catheter.

**Effect of Blockade of Spinal V1a Receptors on the Acute Pressor Response to Intravenous Hypertonic Saline**

The purpose of this study was to determine if spinal V1a receptors mediate the acute pressor response to i.v. hypertonic saline (HS). Rats (250-400g) were instrumented with transmitters and catheters as described above and recovered for five days before injections began. Intravenous HS was administered by injection of 3M saline in a volume of 0.14ml/100g over a 40 second period. The same volume of isotonic saline was administered as a control.

Since cardiovascular responses to i.v. HS may be mediated by spinally released AVP or increases in plasma AVP, the following treatment groups were studied: 1) i.v. HS alone; 2) i.v.
isotonic saline alone; 3) i.v. HS 5 minutes after intrathecal administration of V1a antagonist; 4) i.v. HS 5 minutes after intravenously administered V1a antagonist. This i.v. dose of the V1a antagonist was shown in a pilot study to completely block the pressor response (50 ± 5 mmHg) to i.v. injection of 100μg/kg AVP.

The response of plasma osmolality to HS or isotonic saline was also measured in a separate study. Briefly, blood was collected with heparinized syringes at the following times: 10 and 5 minutes before the i.v. hypertonic or isotonic injection; every minute for 5 minutes after the injection; and at 30, 60, 90, 120, and 180 minutes. The volume of plasma collected (200μl/sample) was replaced with isotonic saline at each sample point. Samples were centrifuged at 4°C at 5000 RPM for 10 minutes, and the plasma osmolality was determined using a freezing-point micro-osmometer (Advanced Instruments, model 3320).

Effect of Blockade of Spinal V1a Receptors on Arterial Pressure in Water Deprived Rats

The purpose of this study was to determine if spinal V1a receptors are responsible for the sustained pressor response during water deprivation. Rats (250-275g) were instrumented with transmitters and catheters as described above. Six days later, their water bottles were removed for 48 hours. Intrathecal injections of the V1a antagonist were given at 24 hours and 48 hours of water deprivation; plasma osmolality was measured at those times in a separate group of rats. Water bottles were returned approximately one hour after the injection at the 48-hour time point. Rats were given 3 days to recover from water deprivation and then received the following injections a day apart to measure the response to i.t. V1a antagonist in rehydrated rats and confirm effectiveness of the antagonist: 1) i.t V1a antagonist; 2) i.t. V1a antagonist 5 minutes before i.t. V1a agonist; 3) and i.t V1a agonist alone.

Effect of Blockade of Spinal V1a Receptors on Arterial Pressure in DOCA-salt Hypertensive Rats

The purpose of this study was to determine if spinal V1a receptors are involved in the chronic elevation of pressure in DOCA-salt hypertensive rats. Rats (200-225g) were anesthetized as described above and received a left nephrectomy and subcutaneous implantation of 50 mg of deoxycorticosterone acetate (DOCA) pellets as previously described (35). After the surgery, the animals were housed individually and were given 0.1% sodium chloride food and 0.9% saline to drink. Sham animals received a left nephrectomy and 0.1% sodium food, but their implanted pellets contained no DOCA and they were given ad libitum access to distilled drinking water.
Three weeks later, the rats were instrumented with transmitters and intrathecal catheters as described above. The experimental protocols began the following week, so all rats received 4 weeks of DOCA-salt treatment at the time of the experimental injections. The following injections were given on separate days: 1) i.t. V1a antagonist; 2) i.t. V1a antagonist 5 minutes prior to i.t. agonist; and 3) i.t. aCSF 5 minutes prior to i.t. agonist. Plasma osmolality was also assessed after 4 weeks of DOCA-salt or Sham treatments.

**Data Analysis and Statistics**

MAP and HR data were plotted as 2 minute averages, and activity was plotted in 5 minute averages. All data are shown as means ± standard errors. Two-way repeated measures analysis of variance (ANOVA) was used to determine differences between and within treatments. The Bonferonni’s post hoc test of Multiple Comparisons versus Control was used to identify differences between and within groups. The aCSF data were used as the control between groups, and measurements 6 minutes prior to drug treatments (t = -6 min) was used as the control within groups. A p value of <0.05 was defined as statistically significant.
Results

Effect of an Intrathecal V1a Antagonist on the Pressor Responses to V1a Agonists

The MAP, HR, and activity responses to 10μl intrathecal injections are shown in Figure 1. MAP, HR, and motor activity were unaffected by vehicle (aCSF) injection (Fig 1a). Intrathecal injection of the V1a agonist increased MAP (23 ± 5 mmHg), HR (105 ± 18 bpm), and motor activity (Fig 1b). MAP and HR both remained elevated 60 minutes following injection. Intrathecal pretreatment with 100ng of a V1a antagonist blocked all of these responses (Figure 1b), but the antagonist had no effect on its own. Therefore, this dose of V1a antagonist was selected for the remainder of the experiments.

Intrathecal injection of 0.1ng of AVP increased MAP and HR by approximately the same amount as the V1a agonist, although all three measured variables returned to control levels within approximately 30 minutes (Fig 1c). Pretreatment with 100ng of intrathecal V1a antagonist also blocked the increases in MAP, HR, and motor activity in response to this dose of intrathecal vasopressin.

In another group (n=3), we investigated whether administration of the V1a antagonist to the lower thoracic cord was effective in blocking responses to administration of the V1a agonist administered to the upper thoracic cord. Rats were instrumented with two intrathecal catheters: one advanced from the lumbar region with the tip at T11 – T13; one advanced from the atlanto-occipital region with the tip near T1. ACSF was delivered via the lumbar catheter and 5 minutes later, administration of the V1a agonist via the atlanto-occipital catheter increased MAP (16±6 mmHg at 10 min) and HR (107 ± 21 bpm at 10 min). The next day, pretreatment with the V1a antagonist via the lumbar catheter abolished the MAP (1 ± 2 mmHg at 10 min) and HR (9 ± 10 bpm at 10 min) responses to the V1a agonist delivered via the atlanto-occipital catheter.

Effect of Blockade of Spinal V1a Receptors on the Acute Pressor Response to Intravenous Hypertonic Saline

Plasma osmolality was increased to 315 ± 4 mOsm/kg after intravenous hypertonic saline but was unaffected by intravenous isotonic saline (Fig 2). Intravenous isotonic saline also had no effect on MAP, HR, or motor activity (data not shown). Intravenous hypertonic saline increased MAP by 20 ± 3 mmHg (Fig 3) but caused no change in HR or motor activity (data not shown). This pressor response was attenuated, but not abolished, by intravenous V1a antagonist (Fig 3a). Pretreatment with an intrathecal V1a antagonist had no effect on the pressor response to i.v. hypertonic saline (Figure 3b).
Effect of Blockade of Spinal V1a Receptors on Arterial Pressure in Water Deprived Rats

Rats deprived of water for 24 - 48 hours showed elevated daytime arterial pressure (Table 1). When these animals were given intrathecal injections of the V1a antagonist at 24 hours (Fig 4a) and 48 hours (Fig 4b) of water deprivation, there was no change in arterial pressure or heart rate. At the end of the experiment, the effectiveness of the antagonist used was verified with a challenge injection of the V1a agonist (data not shown). In a separate group of rats (n=6), plasma osmolality was significantly elevated after 48 hours of water deprivation (308 ± 1 mOsm/kg) compared to euhydrated levels (298 ± 1).

Effect of Blockade of Spinal V1a Receptors on Arterial Pressure in DOCA-salt Hypertensive Rats

Rats that received 4 weeks of DOCA-salt treatment developed sustained hypertension (arterial pressure daytime average: 129 ± 10 mmHg; heart rate daytime average: 392 ± 8 bpm) A separate group of rats showed elevated plasma osmolalities (299 ± 1 mOsm/kg) compared to Sham rats (294 ± 1 mOsm/kg). After 4 weeks of DOCA-salt treatment, intrathecal injections of the V1a antagonist resulted in no change in arterial pressure or heart rate (Figure 5). At the end of the experiment, the effectiveness of the antagonist used was verified with a challenge injection of the V1a agonist (data not shown).
Discussion

The PVN is a key brain region involved in regulating the central nervous system’s response to osmotic stress (46, 49). It is also the site of origin of vasopressinergic neurons that terminate near SPNs (10, 39, 41, 49), and it plays a role in regulating SNA (16, 28). Therefore, we hypothesized that the pressor responses to osmotic stress are due to the activation of a PVN-to-spinal cord vasopressinergic pathway. To our knowledge, this is the first study to investigate spinal control of MAP under conditions of both acute and chronic osmotic stress in conscious intact animals. We found that spinal V1a receptors are not required for the pressor responses during intravenous hypertonic saline, 24- and 48-hour water deprivation, or DOCA-salt hypertension. Our findings do not support the hypothesis that spinal V1a receptors are involved in the regulation of MAP under conditions of acute or chronic osmotic stress in conscious rats.

Cardiovascular and Motor Responses to Intrathecal Injections of V1a Agonists in Normal Rats

Before injecting the intrathecal V1a antagonist under conditions of osmotic stress, it was necessary to verify that the dose of antagonist was sufficient to prevent increases in MAP in response to activation of spinal V1a receptors. In normotensive rats, pretreatment with the V1a antagonist blocked the pressor response to intrathecal administration of both the V1a agonist and AVP. This demonstrated that the antagonist dose was sufficient to block the pressor response and, since AVP can act on both V1 and V2 receptors, that the response to AVP is mediated exclusively by V1a receptors. This is in agreement with the findings of Porter and Brody, who first demonstrated that intrathecal vasopressin increases MAP in conscious rats and that the response relies on V1 receptors (37).

One novel aspect of these control experiments was the discovery that the responses to the intrathecal V1a agonist followed a different time course than AVP. The AVP responses lasted approximately 20 minutes, while the responses to the V1a agonist lasted more than 50 minutes. A possible explanation may be that AVP and the V1a agonist are metabolized differently. Both are peptides, but they differ in three amino acids, including the site of action of an aminopeptidase primarily responsible for breaking down AVP. The rate-limiting step of AVP metabolism in the brain involves cleaving the Cys-Tyr bond (8), which is replaced with Cys-Phe in the V1a agonist.

Another result that requires speculation is the fact that heart rate increased along with MAP. Typically, a baroreflex-mediated bradycardia accompanies pressor responses. Instead,
a profound tachycardia (ΔHR=105±18 bpm) occurred, although it was slightly delayed in comparison to the MAP response (MAP peaked within four minutes while HR took at least ten minutes to reach its maximum). Previous studies have reported variable effects on HR in response to i.t. AVP in both anesthetized (44, 53) and conscious rats (29, 37). Activation of cardiac sympathetic nerves, either directly or via activation of ascending neurons or interneurons, is one possible explanation of the tachycardia shown here. The delay in HR response could be due to diffusion time for the injectate to reach the cardiac SPNs; however, it could also be due to an initial offsetting by the baroreflex before it is overridden by sympathetic activation. Another possibility is that the tachycardia is due to activation of adrenal nerves that cause epinephrine release into the circulation. However, Riphagen et al. found no change in systemic epinephrine levels in response to intrathecal AVP (42).

Finally, intrathecal V1a agonist and AVP cause an increase in motor activity, which was prevented by V1a antagonist pretreatment. It was previously shown that i.t. V1 antagonist can block the scratching behavior to i.t. AVP (54), but the current study extends the finding to show that MAP and HR responses follow the same time course as motor activity. The injections seem to cause some change in somatosensation - the behavioral response consists primarily of scratching, biting, or licking at the hind limbs. However, it is unclear whether the change in somatosensation involves pain or other sensory circuits. AVP is thought to be antinociceptive at the spinal cord (54, 58), the antinociception and scratching behavior are thought to involve separate mechanisms (55), and AVP-induced scratching behavior continues after morphine pretreatment (54). Intrathecal applications of other compounds, such as morphine, are known to produce pruritus (22), but this remains to be investigated for intrathecal AVP.

**Role of Spinal V1a Receptors in Mediating the Cardiovascular Responses to Acute Administration of Hypertonic Saline**

Consistent with previous studies, acute intravenous injection of hypertonic saline increased plasma osmolality and MAP. Since the pressor response could be due to a combination of AVP release into the plasma and activation of SNA, we examined the effect of both intravenous and intrathecal V1a antagonist on this response. We found that systemic blockade of V1a receptors attenuated the pressor response but did not block it completely, suggesting that SNA might also contribute to the elevated MAP. However, i.t. V1a antagonist pretreatment had no effect on the pressor response. This does not support our hypothesis that spinal V1a receptors are involved in the pressor response to i.v. hypertonic saline.
Our results are supported by findings by Liu et al, who administered intracerebroventricular (i.c.v.) vasopressin receptor antagonists and found no effect on the pressor response to i.v. hypertonic saline (24). However, our findings differ from those of the recent study by Antunes and Paton (2). They reported that lumbar SNA was increased in response to either intravenous hypertonic saline or intrathecal V1a agonist, and this response was blocked with intrathecal V1a antagonist pretreatment or chemical inhibition of neurons in the PVN (2). These data imply that the PVN releases vasopressin in the spinal cord to mediate lumbar SNA responses to hypertonic saline. Our study differs from Antunes et al. in the preparation employed to test this hypothesis. The present study was conducted in conscious intact rats, in contrast to the study by Antunes et al. which used an in situ rat preparation that did not allow measurement of MAP. Another study in anesthetized rats also showed elevation of lumbar SNA in response to i.v. hypertonic saline, along with elevations in MAP (59). The reasons for the differences between these results and those of our study in conscious rats are not clear. Possibly, lumbar SNA is not elevated in response to i.v. hypertonic saline in conscious rats, or perhaps another neurotransmitter is responsible for its elevation. Another explanation could be that the lumbar SNA increases by the same amount (~30%) as the in situ or anesthetized rats (2, 59), but the increase in lumbar SNA does not cause the elevation in MAP. This possibility is supported by a discrepancy in timing between MAP and lumbar SNA during a 30-minute infusion of hypertonic saline in anesthetized, baroreceptor-intact rats (59). Weiss et al. found that MAP was significantly increased 5 minutes into the infusion, but lumbar SNA remained at baseline levels until 25 minutes of hypertonic saline infusion had occurred (59).

**Role of Spinal V1a Receptors in Mediating the Pressor Response to Water Deprivation**

Previous findings are consistent with the hypothesis that water deprivation increases SNA and this response may be due to activation of spinally projecting vasopressinergic neurons. Scroggin and co-workers measured lumbar SNA in water deprived rats and concluded that it was increased as a result of increased plasma osmolality (46). Others found that water deprivation increased Fos expression, an indicator of neuronal activity, in PVN neurons that contain vasopressin (12); and another report demonstrated increased Fos expression in spinally-projecting PVN neurons (48).

Although our findings show that MAP was increased in response to 24 and 48 hours of water deprivation, intrathecal administration of the V1a antagonist had no effect on MAP in these rats, suggesting that spinal vasopressin does not increase MAP during water deprivation.
How does this relate to previous studies? Although individual Fos studies support the idea of osmotic activation of a PVN-spinal vasopressinergic pathway, to our knowledge there is no report in which PVN neurons activated by water deprivation were both vasopressinergic and spinally-projecting, so it is possible that the spinally-projecting neurons activated by water deprivation use neurotransmitters other than vasopressin. Further studies are needed to answer this question.

Role of Spinal V1a Receptors in Mediating DOCA-salt Hypertension

Several studies suggest that DOCA-salt hypertension involves activation of both vasopressin and the sympathetic nervous system. For example, DOCA-salt hypertension does not develop in rats lacking vasopressin (4), and vasopressin neurons in the PVN show Fos expression with DOCA-salt hypertension (1). Also, acute, central injection of hypotonic saline in DOCA-salt rats causes a fall in MAP (34) and lumbar SNA (33), and this is blocked by a combination of ganglionic blockade and systemic V1a antagonist (34). Together, these data suggest that DOCA-salt hypertension is due to a combination of systemic vasopressin release and activation of the sympathetic nervous system. In the present study we tested the hypothesis that spinal vasopressin was partly responsible for increased MAP in DOCA-salt rats. However, our findings were inconsistent with our hypothesis in that intrathecal administration of a V1a antagonist had no effect on MAP in DOCA-salt hypertensive rats. We conclude that spinal V1a receptors are not required for sustained elevations in MAP in DOCA-salt hypertension.

Implications of Negative Findings

Most of the previous studies investigating spinally-released vasopressin have led to the conclusion that vasopressinergic neurons in the PVN activate sympathetic nerves in the spinal cord to increase MAP (28, 43, 45, 53, 60). However, the physiological conditions under which this pathway is activated have not been investigated. We tested the hypothesis that vasopressin acts on spinal V1a receptors to mediate pressor responses under conditions of acute and chronic osmotic stress – specifically, intravenous hypertonic saline, 24 and 48 hours of water deprivation, and 4 weeks of DOCA-salt treatment. Under all three conditions, intrathecal V1a antagonist had no effect on MAP. These findings do not support our hypothesis, suggesting that spinal V1a receptors do not support SNA control of MAP under these conditions.
An alternate explanation is that the intrathecally administered V1a antagonist did not reach the SPNs, where the endogenous vasopressin supposedly is released during osmotic stress. However, in control rats (Figure 1), the intrathecal V1a antagonist was able to block the response of exogenous intrathecal vasopressin or a V1a agonist. Additionally, the V1a antagonist delivered through the lumbar i.t. catheter was able to block the MAP and HR responses to the V1a agonist delivered through the atlanto-occipital catheter. This implies that the antagonist delivered via the lumbar i.t. catheter during hypertonic saline, water deprivation, and DOCA-salt hypertension likely blocks V1a receptors along the entire length of the thoracic spinal cord.

While the antagonist clearly reaches the site of action of exogenously administered agonist, it remains possible that the exogenous agonist activates V1a receptors somewhere other than on the SPNs. V1a receptors in the periphery, brain, dorsal horn, ventral horn, and spinal vasculature all must be evaluated as potential sites responsible for increased MAP in response to exogenous intrathecal vasopressin (or the V1a agonist).

We believe it is unlikely that the pressor response to intrathecal AVP is due to leakage of the agonist into the periphery causing direct peripheral vasoconstriction. Pressor responses to intrathecal AVP are twice as large as the same intravenous dose in conscious rats (29), and ganglionic blockade abolishes the response to intrathecal AVP (45). Additionally, i.v. administration of a V1a antagonist has no effect on the response to intrathecal vasopressin (44), and intrathecal injection of tritiated AVP shows that only very small amounts get into the plasma (45).

Another possibility is that intrathecal AVP could diffuse to the brain stem to activate descending pathways that affect SNA and elevate MAP. Indeed, faint traces of Evans blue dye injected at the end of our experiments appeared in the brainstem of all of the rats that were examined. However, a previous study that also showed MAP responses to i.t. AVP (in a volume of 3µl) concluded that, under these conditions, the spread of dye extended only to upper thoracic levels of the cord (37). Additionally, a study comparing responses of i.t. AVP with those of i.c.v. AVP demonstrated greater sensitivity to AVP in the spinal cord (53).

It is also possible that AVP acts in spinal regions other than the IML. V1a receptors have been localized in both dorsal and ventral horn neurons (25). If activation of V1a receptors in the ventral horn are responsible for the increased motor activity, perhaps MAP responds as an exercise pressor reflex (31). We believe this is unlikely because intrathecal vasopressin also increases MAP in anesthetized rats (29) that lack motor movement; however, further investigation is necessary to rule out the involvement of the exercise pressor reflex in conscious
rats. On the other hand, if dorsal horn neurons are activated, they could then activate SPNs directly or via activation of spinal interneurons or a spinal-bulbo-spinal pathway.

Indeed, the behavioral response to i.t. AVP seems to indicate a change in either somatosensation or motor neuron activation. Injection of i.v. phenylephrine to increase MAP the same amount as i.t. AVP does not cause scratching behavior (unpublished observations), suggesting that the motor response is due to activation of V1a receptors and not due to the increase in MAP. Intrathecal injection of substance P causes a similar scratching response to i.t. AVP, and this is not thought to be due to perception of pain (18). Instead, it appears that substance P induces convulsant-like behavior - likely through activation of motor neurons (5) - which can be attenuated with anti-convulsant drugs (14). This is also true for morphine, strychnine, and kainic acid (14). In contrast, anticonvulsants were shown to have no effect on the behavioral response to i.t. AVP (54). Finally, even if i.t. AVP causes changes in sensation through a morphine-insensitive mechanism as suggested by Thurston et al. (54), sensory and cardiovascular responses to i.t. injections may be due to separate mechanisms, as demonstrated in Khan et al.’s investigation of i.t. nicotinic agonists (20).

Finally, another possibility is that the pressor response to i.t. AVP is secondary to spinal ischemia caused by vasopressin acting on the spinal vasculature (45). V1a receptors have been identified on the blood vessels and capillaries of the spinal cord gray matter (48), and intrathecal vasopressin has been shown to cause reductions in blood flow in spinal vessels (26). However, this was seen with higher, paralysis-inducing doses of AVP; the dose used in this study showed no reduction in blood flow (26). Additionally, Riphagen et al. (45) discuss a variety of reasons to conclude that spinal ischemia is an unlikely cause of the responses to i.t. AVP: AVP applied to the outside of intact pial vessels had no effect (23); vasodilators do not reduce the pressor effects of i.t. AVP; and it is unlikely that spinal ischemia would affect SNA selectively, as has been demonstrated by i.t. AVP (42, 45).

While it remains possible that i.t. AVP acts in one or more of the above locations to ultimately increase MAP, the most likely site of action is in the IML. The spread of intrathecal AVP, specifically, has not been investigated; however, radiolabeled substance P - a peptide made of 11 amino acids - has been shown to spread far enough into the spinal cord to reach the IML one minute after injection (11). It is very likely that vasopressin, a smaller peptide, is able to reach the IML with a similar efficiency. Therefore, the most probable conclusion for this study is that intrathecal vasopressin and the V1a agonist acted on the SPNs to elevate MAP, and this was blocked by pretreatment with the V1a antagonist. This means that it is likely that the antagonist effectively blocked receptors on the SPNs during i.v. hypertonic saline, water
deprivation, and DOCA-salt hypertension, but the V1a receptor blockade did not reduce the elevated MAP during these conditions. Consequently, we can conclude that spinal V1a receptors are not required for the pressor responses under these acute and chronic conditions of osmotic stress.

**Perspectives**

Since the 1980's, a variety of neuropeptides and amino acids have been localized in descending fibers from supraspinal sites and proposed as likely putative neurotransmitters and/or neuromodulators of SPNs and therefore arterial pressure (9, 13, 19, 21, 30, 39). However, at the present time there are very few studies in which spinal neurotransmitter systems have been studied in conscious animals under physiological conditions. Since the spinal cord is the final point of integration in the CNS, it is a promising site for novel therapies aimed at modulation of sympathetic nervous system activity. Targeted treatment at the level of the spinal cord could eliminate side effects that are associated with drugs that target brain neurotransmitter systems. In this study, despite anatomical and neurophysiological studies supporting the central hypothesis, intrathecal blockade of spinal V1a receptors had no effect on pressor responses to the three tested conditions of osmotic stress. Further studies will be needed to investigate the role of other spinal neurotransmitters, particularly glutamate, in regulating MAP during osmotic stress in conscious animals.

**Grants**

This study was supported by NIH grant R01 HL64176 to J.W.O.
Table 1 – Twelve-hour daytime averages of MAP, HR, and motor activity for rats in water deprivation experiment. Data is reported as mean ± standard error. * = p < 0.05 vs. control.

Figure 1 – Effect of Intrathecal Injections in Normotensive Rats
A) MAP, HR, and motor activity responses to intrathecal injection of artificial cerebrospinal fluid (aCSF); B) MAP, HR, and motor activity effects of intrathecal injection of a V1a receptor agonist, with and without pretreatment (5 min prior) with intrathecal V1a receptor antagonist; C) MAP, HR, and motor activity effects of intrathecal injection of AVP, with and without pretreatment (5 min prior) with intrathecal V1a receptor antagonist. The dashed line indicates time=0, at which time the V1a agonist (or aCSF in Fig. 1a) was injected. The solid line indicates t= -5, at which time the V1a antagonist was injected. # = p < 0.05 within group for 0-60 min vs. t = -6 min. * = p < 0.05 V1a ago/AVP vs. antagonist-pretreated injection.

Figure 2 – Effect of Intravenous Hypertonic Saline on Plasma Osmolality
Plasma osmolality changes in response to intravenous injection of hypertonic saline or isotonic saline. # = p < 0.05 within group for 0-60 min vs. t = -5 min. * = p < 0.05 hypertonic saline vs. isotonic saline.

Figure 3 - Effect of Intrathecal V1a Antagonist in Rats Given Intravenous Hypertonic Saline
MAP and HR effects of intravenous hypertonic saline with and without pretreatment with an A) intravenous V1a receptor antagonist or B) intrathecal V1a receptor antagonist. Antagonist pretreatment occurred five minutes prior to hypertonic saline injection, indicated by the solid line. Hypertonic saline was administered at t = 0, indicated by the dashed line. # = p < 0.05 within group for 0-60 min vs. t = -6 min. * = p < 0.05 hypertonic saline vs. antagonist-pretreated injection.

Figure 4 – Effect of Intrathecal V1a Antagonist in Water Deprived Rats
MAP and HR effects of intrathecal injection of a V1a receptor antagonist in rats deprived of water for A) 24 and B) 48 hours. There were no differences within groups.

Figure 5 – Effect of Intrathecal V1a Antagonist in DOCA-salt Hypertensive Rats
MAP and HR effects of intrathecal injection of a V1a receptor antagonist in hypertensive rats after 4 weeks of DOCA-salt treatment. There were no differences within groups.
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<td>Control</td>
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<td>1.4 ± 0.3</td>
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</table>
Figure 1
Figure 2
Figure 3
A) 24-hour water deprivation

B) 48-hour water deprivation

Figure 4
Figure 5

- ΔMAP (mmHg)
- ΔHR (bpm)

n=8

IT V1a Ant (100ng)
References


41. **Ranson RN, Motawei K, Pyner S, Coote JH, Kamel M, Brooks VL, Qi Y, and O'Donaughy TL.** The paraventricular nucleus of the hypothalamus sends efferents to the spinal
cord of the rat that closely appose sympathetic preganglionic neurones projecting to the stellate ganglion.


Figure 1
Figure 2

Plasma Osmolality (mOsm/kg)

- • Isotonic saline (n=5)
- □ Hypertonic saline (n=5)

Time (min)
Figure 3
Figure 4

A) 24-hour water deprivation

B) 48-hour water deprivation

ΔMAP (mmHg)

ΔHR (bpm)

Time (min)

n=10
Figure 5

n=8

ΔMAP (mmHg)

ΔHR (bpm)

Time (min)
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