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Pressor doses of vasopressin result in only transient elevations in plasma peptide levels

Gina L.C. Yosten and Willis K. Samson

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, Saint Louis, Missouri, 63104

Abstract

We recently reported that neuronostatin, a novel neuropeptide, biphasically increased mean arterial pressure, first through the activation of the sympathetic nervous system followed by the release of vasopressin. In those experiments, we found that centrally administered neuronostatin increased plasma vasopressin levels only 2–3 times greater than levels observed in saline-treated controls, and that the increase in mean arterial pressure (approximately 15 mm Hg) could be blocked by pretreatment with a V1-vasopressin antagonist. Here we report the relationship between two to three fold elevations in plasma vasopressin levels and concomitant changes in mean arterial pressure in conscious, unrestrained male rats. We injected increasing doses of vasopressin (5, 20, and 100 ng/kg, intra-arterially) and measured both changes in plasma vasopressin levels and the elevation in mean arterial pressure achieved. At five minutes post injection, plasma levels of vasopressin and mean arterial pressures were similar to those observed following central neuronostatin administration in our earlier study. Thus we conclude that small increases in circulating vasopressin levels can result in significant elevations in mean arterial pressure at least in the conscious rat.

Keywords

Vasopressin; Blood Pressure

1.1 Introduction

Vasopressin was first identified in 1895 by Oliver and Schafer based on the ability of pituitary extracts to induce a pressor effect in isolated vascular beds [6]. Almost twenty years later, pituitary extracts were shown to exert antidiuretic effects as well, but it was not until the 1950s, when the amino acid sequence of vasopressin was determined, that vasopressin was discovered to possess both pressor and antidiuretic activities [4,10]. It is now known that arginine vasopressin (AVP) signals through G protein coupled receptors to regulate blood pressure by acting in both the kidney and directly in the vasculature [1,4]. In the vasculature, vasopressin binds to V1 receptors on vascular smooth muscle cells,

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Corresponding Author: G.L.C. Yosten, Ph.D., Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, MO 63104, Tel: (314) 977-6354, Fax: (314) 977-8670, gyosten@slu.edu.

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ultimately leading to calcium mobilization and vasoconstriction [4]. V1 receptor signaling in the vasculature appears to be an important mechanism by which AVP maintains blood pressure during acute hemorrhage [8,9] and septic shock [5].

We recently have shown that neuronostatin, a peptide derived from the somatostatin preprohormone, when administered into the cerebroventricle biphasically increased mean arterial pressure (MAP) and led to a significant increase in AVP release in a time course that was consistent with the second phase of MAP increase. Furthermore, the second phase was abrogated by pretreatment with a V1 antagonist [11], indicating that this elevation in MAP was due to an increase in AVP secretion. Despite numerous studies evaluating the role of vasopressin as a pressor hormone [1,4,5,8,9], it is not clear what absolute levels of vasopressin must be achieved to elicit vasoconstriction in the rat and whether those plasma levels can be attained under physiological circumstances. In these studies, we sought to determine what doses of intra-arterially administered vasopressin were necessary to elicit a pressor response in conscious, unrestrained male rats, and what plasma levels of vasopressin were attained following those doses.

2.1 Materials and Methods

2.1.1 Animals

All procedures and protocols were approved by the Saint Louis University Animal Use and Care Committee. Adult, male rats (Sprague-Dawley, Harlan Laboratories, Indianapolis, IN, 230–250g) were housed under controlled conditions (23–25 degrees C, lights on 0600–1800) with unrestricted access to food and water. Rats were anesthetized with a mixture of ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA)/xylazine (TranquiVed, Vedco Inc. St. Joseph, MO) anesthesia (60mg/8mg mixture/ ml, 0.1 ml/100 gram body weight, intraperitoneal injection), and a polyethylene catheter (PE50, Intramedic, Clay Adams) was implanted into the left carotid artery as previously described [12]. The catheter was filled with heparinized saline (200 U/mL in sterile saline) to ensure patency. Following surgery, rats received a 10mL subcutaneous infusion of sterile saline (0.9% NaCl) to compensate for anticipated fluid loss.

2.1.2 Analysis of Mean Arterial Pressure

The day after implantation of the carotid catheter, rats were habituated to a quiet recording room for at least 2 hours. The carotid catheter was connected to a pressure transducer (Digi-Med Blood Pressure Analyzer, Micro-Med, Louisville, KY) and flushed with heparinized saline. Baseline mean arterial pressure (MAP) and heart rate (HR) were recorded at one-minute intervals for at least 30 minutes. Rats then were injected with either vehicle alone (sterile saline) or vehicle containing 5, 20, or 100 ng/kg arginine vasopressin (Phoenix Pharmaceuticals, Burlingame, CA) intra-arterially. MAP and HR were recorded at one-minute intervals for at least 30 minutes. Data are represented as change from pre-injection baseline (average of MAP or HR for 5 minutes prior to injection of AVP).

2.1.3 Determination of Plasma AVP Levels

Rats bearing carotid cannulae were habituated to a quiet room for at least 2 hours. Following habituation, rats were injected with either saline vehicle alone, or vehicle containing 5, 20, or 100 ng/kg AVP intra-arterially. Rats were decapitated and trunk bloods collected at 1, 5, or 10 minutes following injection of AVP. Plasma levels of AVP were determined by radioimmunoassay, as previously described [7]. Plasmas were acidified (0.5 ml 1 N HCl in 1.0 ml plasma) and centrifuged (4 min, 6 C, 6000 g). Supernatants were then applied to a C-18 column (Sep Pak, Fisher) that had been activated by elution first of 4 ml absolute methanol and then washed with 10 ml distilled water. After entry of the acidified plasma

into the gel bed, the column was washed (gravity flow) with 10 ml, 4% acetic acid. Vasopressin was then eluted from the gel bed with 4 ml mixture of acetonitrile: 4% acetic acid (3:1 mixture). Eluates were dried in a rotary evaporator (Speed Vac, Savant Instruments) and reconstituted in radioimmunoassay buffer (0.1% gelatin in 0.05 M phospho-buffered NaCl, pH 7.0) before inclusion in the assay. A common plasma pool collected from donor rats (male) was employed to calculate recovery efficiency (historically > 90%). Aliquots of the plasma pool were extracted similarly to unknowns as blanks (measurement of endogenous peptide levels) or with the addition 5, 20 or 50 pg synthetic vasopressin (Phoenix Pharmaceuticals). Measured levels of vasopressin in the unknowns were corrected for percent recovery. Intra- and inter-assay coefficients of variability have remained less than 8% using this polyclonal vasopressin antibody (code: 728-4) raised by our lab in rabbits [7].

2.1.4 Statistics

Blood pressure and HR data were analyzed using a Mann-Whitney U test, and radioimmunoassay data were analyzed using ANOVA with Scheffe's multiple comparison. A non-parametric statistic (Mann-Whitney U) was used to analyze MAP and HR data because data were transformed to reflect change from pre-injection baseline to account for the natural differences in resting MAP and HR between animals.

3.1 Results

To determine the relationship between the dose of vasopressin administered and the pressor response elicited by a given dose, we began by injecting rats intra-arterially with a published pressor dose of AVP (20ng/kg) [11] and tested both a lower (5ng/kg) and higher (100ng/kg) dose of AVP. All three doses of AVP significantly increased MAP (Figure 1A), with 100ng/kg AVP yielding the greatest peak change in MAP (approximately 35 mmHg) at 1 minute post-injection. The peak changes in MAP in animals treated with 5ng/kg and 20ng/kg were approximately 15 and 25 mmHg, respectively, at 1 minute post-injection.

Rats that were treated with intra-arterially administered AVP also demonstrated dose-related decreases in HR (Figure 1B), with the highest dose of AVP yielding the greatest decrease in HR (peak decrease in HR of 99 beats per minute). The peak decreases in HR for the 5 ng/kg and 20 ng/kg doses of AVP were 16 and 56 beats per minute, respectively.

To compare the pressor response of AVP to the plasma levels of AVP achieved at these doses, rats were injected with 5, 20, or 100 ng/kg AVP, and animals were decapitated and trunk bloods collected 1, 5, or 10 minutes later. Animals that were injected with the highest dose of AVP (100 ng/kg) exhibited the highest levels of plasma AVP at 1 minute-post injection (1512 pg/ml) (Table 1). Plasma levels of AVP achieved after injection of 5 and 20 ng/kg were 7.2 and 59.4 pg/ml, respectively. While plasma levels of AVP did not differ significantly at 1 minute between controls and animals administered 5 ng/kg AVP when the group data were analyzed by ANOVA, this is an artifact of the wide spread in plasma AVP levels among the three treatment groups. If on the other hand an independent t test was used to analyze just the control and 5 ng/kg values, the difference did attain significance ($p < 0.05$). The same can be said at 1 minute sampling for the AVP levels in the animals that received 20 ng/kg i.a. compared to controls. When analyzed by independent t test the difference between plasma AVP levels in controls versus animals in this group was highly significant ($p < 0.001$).

At 5 minutes post-injection, animals that received 100 ng/kg AVP had an approximately 99% reduction in plasma AVP levels compared to 1 minute post-injection (1512 vs. 21.8 pg/ml). However, rats that were infused with 20 ng/kg AVP exhibited only an approximately

70% reduction in plasma AVP levels at 5 minutes (18.34 pg/ml) compared to 1 minute-post injection, and plasma levels of rats that received the lowest dose of AVP were reduced by only 14% at 5 minutes post-injection (6.25 pg/ml) compared to the 1 minute time point. By 10 minutes post-injection, all AVP-injected rats had levels of AVP in their blood that were comparable to that of saline-injected controls.

4.1 Discussion

All three doses of intra-arterially injected AVP led to significantly elevated MAP, but only two of the doses (20 and 100 ng/kg) led to a significant increase in plasma AVP levels in conscious, male rats. Interestingly, the lowest dose of AVP tested (5 ng/kg) elevated MAP by approximately 15 mmHg, despite yielding only small changes in plasma AVP levels, indicating that in vivo, plasma AVP levels must only double or triple to cause significant changes in MAP. We can only speculate that the continued elevation in mean arterial pressure at 10 minutes post injection is a reflection of the lasting effect of AVP once bound to its vascular receptors and therefore cleared from the circulating plasma. We have in several published reports been able to accurately detect changes in plasma AVP levels that range within the physiologic range with this extraction and assay technique [7, 11], and do not think, therefore, that the failure to detect a continued elevation in plasma AVP levels is a problem of assay sensitivity.

We recently have shown that central injection of neuronostatin, a novel neuropeptide, led to a significant increase in plasma AVP levels at 30 minutes post-injection that was approximately 2–3 times larger than saline-injected controls [11]. This rise in plasma AVP levels induced a significant increase in MAP of approximately 10–15 mmHg [11], which is comparable to the changes observed in this study. Proof that this small a rise in plasma AVP levels could elicit a significant change in MAP came in those studies when it was observed that pretreatment with a V1 vasopressin antagonist could significantly block the MAP rise to neuronostatin [11]. While these studies were motivated by our earlier observation [11] that neuronostatin acted within the brain to biphasically increase mean arterial pressure in conscious male rats due to an initial, transient increase in sympathetic activity, followed by a longer lasting increase in plasma AVP levels, it is difficult to compare the results from the current study with those of the earlier work with regards to effects on heart rate. However, following i.a. administration there was a clear decrease in heart rate possibly reflecting activation of the baroreflex following bolus administration of AVP. When neuronostatin was administered i.c.v. there was a more delayed increase in plasma AVP levels as opposed to the abrupt increase with i.a. administration.

Although this study was not designed specifically to assess the half-life of AVP in the plasma, our data infer some interesting details on the kinetics of AVP in vivo. While it is commonly accepted that the half-life of AVP is approximately 5–6 minutes [3,9], our data suggest that the half-life is actually much shorter, depending upon the dose administered. Rats that were injected with the highest dose of AVP (100 ng/kg, or ~25 ng total per rat) had average plasma AVP levels of 1512 pg/ml at one minute post injection. If we assume a half-life of 1 minute, then the levels of plasma AVP should be ~94 pg/ml at 5 minutes. However, we observed that at 5 minutes, these animals exhibited plasma AVP levels of 21.8 pg/ml—4 times less than that predicted by a 1 minute half-life. We did not assess the disappearance from plasma of exogenous AVP following the cessation of a constant infusion that would raise it to a stable, physiologic or supraphysiologic level. That study would be necessary if an accurate determination of plasma half-life is to be calculated using a sensitive method of measurement.

5.1 Conclusion

In conclusion, doses of AVP administered intra-arterially that in our hands range from approximately 1.25 to 25 nanograms per rat, significantly raised MAP and only transiently increased circulating AVP levels in plasma. While nanogram doses may appear high compared to circulating levels that are quite low (1–3 pg/ml under normal, unstimulated conditions), those doses are cleared very quickly from circulation (Table 1). Under pathophysiologic conditions (i.e. hemorrhage [2]), the neurohypophyseal content of AVP is depleted of microgram amounts, thus potentially resulting in high nanogram (or even microgram) quantities of the peptide entering the circulation. Therefore our doses, which were effective to raise MAP but only transiently elevate plasma levels, do not appear to us to be supraphysiologic.

Highlights

- Mean arterial pressure in conscious rats rises to i.a. vasopressin (AVP) injection.
- These injections result in small, but significant increases in plasma AVP levels.
- A two-three fold increase in plasma AVP levels can alter MAP in conscious rats.
- In vivo manipulations that double plasma AVP levels are capable of altering MAP.

Acknowledgments

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Figure 1.

Intra-arterially administered AVP increases mean arterial pressure. Rats bearing carotid cannulae were treated with either 5, 20, or 100 ng/kg body weight AVP, and changes in mean arterial pressure (A) and heart rate (B) were recorded for 20 minutes. Data are presented as change from pre-injection baseline (Mean, SEM). Data were analyzed using a Mann-Whitney U test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. Saline-Injected controls).

Table 1

Plasma levels of AVP attained with pressor doses of intra-arterial AVP

Treatment	1 minute	5 minutes	10 minutes
Saline	3.6 ± 0.6 (22)	2.8 ± 0.5 (24)	3.9 ± 0.6 (20)
5 ng/kg	7.2 ± 1.7 (14)	6.25 ± 1.7 (12)	3.1 ± 0.8 (7)
20 ng/kg	59.5 ± 5.7 (14)	18.4 ± 3.5 ** (16)	4.4 ± 0.6 (10)
100 ng/kg	1512.3 ± 66.2 *** (9)	21.8 ± 8.9 ** (10)	4.9 ± 1.2 (9)

Plasma AVP levels following intra-arterial administration of exogenous AVP. Rats were injected intra-arterially with either 5, 20, or 100 ng/kg body weight AVP, and sacrificed by rapid decapitation 1, 5, or 10 minutes later. Plasma AVP levels were measured using a radioimmunoassay, and are represented as picograms/ml plasma (Mean ± SEM, numbers in parenthesis indicate group size). Non-injected controls (n=13) exhibited plasma AVP levels of 2.0 ± 0.6 pg/ml. Data were analyzed using ANOVA with Scheffe's multiple comparison

*
p<0.05;

**
p<0.01;

p<0.001 vs. Saline-Injected controls.