Effects of acute hypotensive stimuli on arginine vasopressin gene transcription in the rat hypothalamus

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Effects of acute hypotensive stimuli on arginine vasopressin (AVP) gene transcription in the rat hypothalamus. Am J Physiol Endocrinol Metab 279: E886–E892, 2000.—We investigated the baroregulation of arginine vasopressin (AVP) gene transcription in the supraoptic (SON) and paraventricular nuclei (PVN) in conscious rats by use of intronic in situ hybridization. Hemorrhage of 16 ml/kg body wt decreased mean arterial pressure (MAP) by 57% and increased both plasma AVP (control, 1.2 ± 0.3 pg/ml; 16 ml/kg body wt, 38.9 ± 3.2 pg/ml) at 10 min and AVP heteronuclear (hn)RNA levels (SON, 150%; PVN, 140% of control values) at 20 min. On the other hand, hemorrhage of 7 ml/kg body wt had no significant effect on MAP, plasma AVP, or the AVP hnRNA levels. To better understand the baroregulation, we also examined the effects of sodium nitroprusside (SNP), which induces hypotension without a change in blood volume. The subcutaneous injection of 2 mg/kg body wt SNP, which decreased the MAP by 60%, increased both plasma AVP (control, 1.6 ± 0.4 pg/ml; 2 mg/kg body wt, 8.1 ± 0.4 pg/ml) at 10 min and AVP hnRNA levels (SON, 150%; PVN, 140% of control values) at 30 min. The injection of 0.1 mg/kg body wt SNP, which reduced the MAP by 10%, failed to increase either the plasma AVP or AVP hnRNA levels. These results indicate that AVP gene transcription increases rapidly after both hypotensive hemorrhage and normovolemic hypotension. In addition, it is suggested that the set point for AVP synthesis in the baroregulation is similar to that for AVP release.

arginine vasopressin (AVP), an antidiuretic hormone, is synthesized in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus, transported axonally, and stored in the posterior pituitary until it is released into the peripheral circulation. The release of AVP is regulated physiologically by plasma osmolality and plasma volume or blood pressure (30), and the osmoregulation of AVP release is so precise that a change in plasma osmolality of as little as 1% can affect plasma AVP levels. On the other hand, changes in blood pressure and blood volume have little effect on plasma AVP until the reductions reach 10%; beyond that point, plasma AVP levels increase exponentially in relation to the degree of hypotension or hypovolemia (31).

Changes in blood pressure and blood volume are detected at pressure-sensitive receptors located in the large arteries and cardiac atria (33). The signals are then transferred through the vagal and glossopharyngeal nerves to the nucleus solitarius in the brain stem, from which postsynaptic pathways project to the magnocellular neurons in the SON and PVN (4, 33). Although it has been shown that the plasma AVP response to changes in blood volume is similar to the response to changes in blood pressure (30), the relative importance of these two variables under physiological conditions has not been well established. In rats, volume depletion by hemorrhage produces little or no elevation in plasma AVP levels until changes in blood volume are sufficient to affect arterial blood pressure (33), suggesting that arterial blood pressure is more important than blood volume in the regulation of AVP release in hemorrhage (33).

There is evidence that AVP mRNA is increased after chronic stimuli such as water deprivation and salt loading (24, 35). However, these conditions induce not only an increase in plasma osmolality but also a change in plasma volume, thus making it difficult to evaluate the relative importance of these variables in AVP gene regulation. In addition, the abundance of AVP mRNA levels in the SON and PVN could mask a change after acute stimuli such as intraperitoneal injection of hypertonic saline (18), hypovolemia (8), or hemorrhage (11). On the other hand, intronic in situ hybridization, which measures heteronuclear (hn)RNA levels, allows one to assess rapid and small changes in gene transcription. Recently, we used intronic in situ hybridization to show that AVP synthesis in the SON and PVN is regulated precisely by plasma osmolality (1).

In this study, to investigate the physiological regulation of AVP gene transcription by blood pressure and/or volume, we employed intronic in situ hybridization and examined the changes in AVP hnRNA levels...
in the SON and PVN after acute hemorrhage. We also used the vasodilatory agent sodium nitroprusside (SNP) to decrease arterial pressure in an attempt to compare the effects of hypotensive hemorrhage to pharmacologically induced hypotension without blood loss.

**MATERIALS AND METHODS**

*Animals and maintenance conditions.* The experiments were carried out with the use of conscious unrestrained male Sprague-Dawley rats (body wt 250–270 g: Chubu Science Materials, Nagoya, Japan). One hundred seventy rats were housed in individual cages in a room with a controlled environment (lights on 0900–2100, 23.0 ± 0.5°C) and had free access to food and water until the time of the experiments. All experiments were performed in a room with a temperature of 23.0°C and 55% humidity. The rats were deprived of food and water during the experiments. The procedures were in accordance with institutional guidelines for animal care at Nagoya University School of Medicine.

*Surgical procedures.* The day before the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg body wt) by intraperitoneal injection, and a PE-50 polyethylene catheter, pulled to a fine tip, was implanted into the right carotid artery. The catheter was filled with heparinized (100 U/ml) 0.9% saline and was sealed and exteriorized at the nape of the neck. Each rat was housed in an individual cage in which it was allowed to move freely. To ensure normal fluid balance after surgery, the rats were given access to a 20-ml 5% dextrose solution in addition to the regular drinking water (6, 7). Although most rats drank almost all the dextrose, some drank smaller amounts of the solution. One hundred fifty-six rats used for the subsequent experiments drank at least 18 ml of the solution.

**Experiment 1a: effects of hemorrhage on arterial blood pressure and heart rate.** Eighteen rats were divided into 3 groups in which 7, 8, or 16 ml/kg body wt of total blood volume were withdrawn. It has been estimated that 8 ml/kg body wt is ~10% of the total blood volume (6, 7, 10). Hemorrhage was accomplished via blood removal over a 3-min period from the right carotid catheter. Arterial blood pressure and heart rate (HR) were continually recorded before and after experimental manipulation by connecting the arterial line to a blood pressure transducer (Gould, Oxnard, CA), via an amplifier connected to a hot wire recorder, to measure pulsatile blood pressure. Mean arterial pressure (MAP) was via an amplifier connected to a hot wire recorder, to measure and after experimental manipulation by connecting the arterial pressure and heart rate (HR) were continually recorded before hemorrhage was accomplished via blood removal over a 3-min period from the right carotid artery.

**Experiment 1b: effects of hemorrhage on plasma AVP and hypothalamic AVP hnRNA levels.** Three independent experiments were performed to determine the effects of 7, 8, and 16 ml/kg body wt hemorrhage on plasma AVP and AVP hnRNA levels. In each experiment, rats were decapitated 10, 20, or 30 min after completion of the hemorrhage. The control rats, which were also implanted with catheters the day before the experiment, were decapitated without hemorrhage.

**Experiment 2a: effects of SNP injection on arterial blood pressure and HR.** To determine the effect of SNP on arterial blood pressure and HR, 18 rats were divided into 3 groups that received SNP [0.1% (wt/vol) in isostonic saline] subcutaneously at doses of 0.1, 1, or 2 mg/kg body wt. Arterial blood pressure and HR were continually recorded with a blood pressure transducer connecting the arterial line implanted into the right carotid artery.

**Experiment 2b: dose-response effects of SNP injection on plasma AVP and hypothalamic AVP hnRNA levels.** Rats were decapitated 30 min after the subcutaneous injection of isotonic saline or SNP at doses of 0.1, 1, or 2 mg/kg body wt.

**Experiment 2c: time-course effects of SNP injection on plasma AVP and hypothalamic AVP hnRNA levels.** Rats were decapitated 10, 30, or 60 min after the subcutaneous injection of 2 mg/kg body wt SNP. The control rats were decapitated 30 min after the injection of isotonic saline.

*Plasma AVP assay.* Immediately after decapitation, trunk blood was collected into an EDTA-containing tube for the determination of plasma AVP. After immediate separation, plasma AVP was extracted through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) and was measured with a radioimmunoassay kit (provided by Mitsubishi Chemical, Tokyo, Japan). The sensitivity of the assay for AVP was 0.063 pg/tube, with <0.01% crossreactivity with oxytocin (28). AVP concentrations in plasma were corrected for recovery, which averaged 85.2–91.3%.

*In situ hybridization.* In situ hybridization was performed as described previously (2). Brains were rapidly removed, frozen on dry ice, and stored at −80°C until they were sectioned (12 μm) in a cryostat. Sections were thaw mounted onto gelatin/chrome alum-coated slides and stored at −80°C until they were processed for in situ hybridization. Slides were thawed, fixed with 4% formaldehyde in PBS for 5 min, washed twice in PBS, acetylated for 10 min in 0.9% NaCl containing 0.25% acetic anhydride and 0.1 M triethanolamine, and then dehydrated in 70 (1 min), 80 (1 min), 95 (2 min), and 100% ethanol (1 min) and delipidated in 100% chloroform (5 min) and 100 (1 min) and 95% ethanol (1 min) before air drying.

Riboprobe templates were made by PCR with rat genomic DNA as a template. The primers used were synthesized on the basis of sequences 946—965 upstream and 1661—1677 downstream from the coding region of intron 1 of the rat AVP gene, and the SP6 promoter sequences were added to the 5’ end of the latter primer. PCR products were run on 0.9% agarose gel, and gel slices containing the resulting fragments were purified with a QIA quick gel extraction kit (QUIAGEN, Chatsworth, CA) according to the manufacturer's instructions. The sequence of the PCR product was ascertained by restriction analysis. A 35S-labeled cRNA probe was produced with the use of an in vitro transcription kit (Ambion, Austin, TX). The reaction contained 0.1 μg PCR product, 1× SP6 transcription buffer, 250 μCi [α-35S]thio-UTP (>1,000 μCi/mmol; NEN), 500 μM ATP, 500 μM CTP, 500 μM GTP, 10 μM UTP, and 1 U/μl SP6 RNA polymerase. The reaction mixture was incubated for 60 min at 37°C, labeled probes were extracted with phenol-chloroform, and then ethanol was precipitated. The specificity of the probe was described previously (18). 35S-labeled cRNA was applied to each section (1×106 cpm), and hybridization proceeded under paraffin coverslips in a 55°C humidity chamber for 16–24 h. The sections were then washed in 2× standard sodium citrate (SSC; 0.15 M NaCl and 0.015 M sodium citrate (pH 7.4)-50% formamide for 2×15 min at 55°C and treated with RNase A (100 μg/ml) at 37°C for 30 min. The sections were then washed in 2× SSC-50% formamide for 3×15 min at 55°C and in 2× SSC twice for 5 min at room temperature. Sections were dehydrated through 70% ethanol, allowed to dry, and exposed to hyperfilm MP (Amersham International, Amersham, UK) together with the 14C-labeled standards of known radioactivity (American Radiochemical, St. Louis, MO) for 1 wk.

The AVP exonic probe was a 48-base oligonucleotide, complementary to part of the exonic mRNA sequence coding the...
last 16 amino acids of the AVP glycopeptide (19). The hybrid-
ization for the AVP exonic probe was performed as described
previously (2).

The relationship between the signal intensities and the
exposure time was examined by exposing sections to the
X-ray films for various periods, from which were determined
the exposure times yielding appropriate signal intensities
within the linear range of the detection system. The autora-
diographic images were quantified by computerized image
analysis software (NIH Image 1.41). The data representing
the hybridization signals were expressed as percentages of
the control level.

For cellular localization of AVP hybrids, the slides were
subsequently dipped in nuclear emulsion (diluted 1:1 with
distilled water; NTB2, Eastman Kodak, Rochester, NY) and
exposed for 2 wk. The medial parvocellular AVP neurons in
the PVN were identified on the basis of location and the small
dark nuclei characteristic of parvocellular neurons (17).

Statistics. All results are expressed as means ± SE. Mul-
tiple comparisons were evaluated by one-way ANOVA fol-
lowed by Fisher’s protected least significant difference test.
Differences were considered statistically significant at
P < 0.05. The number of rats in each group was six in all exper-
iments.

RESULTS

Experiment 1a: effects of hemorrhage on arterial
blood pressure and HR. After the hemorrhage of 16
ml/kg body wt, MAP decreased from 113 ± 4 to 49 ± 10
mmHg at 3 min and remained lower than the initial
value at 30 min (Fig. 1). HR decreased from 411 ± 13
to 332 ± 18 beats/min at 10 min after the hemorrhage,
consistent with previous reports (5, 13). The hemor-
hage of 8 ml/kg body wt reduced the MAP from 110 ±
to 102 ± 5 mmHg (Fig. 1) and increased the HR from
420 ± 11 to 440 ± 14 beats/min at 10 min. On the other
hand, the 7-ml/kg body wt hemorrhage had no signifi-
cant effect on MAP (Fig. 1) or HR.

Experiment 1b: effects of hemorrhage on plasma AVP
and hypothalamic and AVP hnRNA levels. Plasma
AVP levels were significantly increased at 10 min after
the completion of hemorrhage of both 8 ml/kg body wt
(control, 1.7 ± 0.2 pg/ml; 8 ml/kg body wt, 11.4 ± 1.4
pg/ml, P < 0.01; Fig. 2A) and 16 ml/kg body wt (control,
1.2 ± 0.3 pg/ml; 16 ml/kg body wt, 38.9 ± 3.2 pg/ml, P
< 0.01; Fig. 2A). The AVP hnRNA levels in the SON
and PVN were increased significantly as early as 10
min and showed a peak at 20 min after the completion
of hemorrhage of both 8 ml/kg body wt (SON, 138.1 ±
7.5%; PVN, 124.5 ± 5.2% of control values; Fig. 2,
B and C) and 16 ml/kg body wt (SON, 153.7 ± 8.5%; PVN,
135.6 ± 6.1% of control values; Fig. 2, B and C). On the
other hand, the 7-ml/kg body wt hemorrhage had no
significant effect on plasma AVP (Fig. 2A) or AVP
hnRNA levels in the SON and PVN (Fig. 2, B and C).

Figure 3 shows representative dark-field photomi-
crographs of the AVP hnRNA transcripts in the SON
and PVN of a control and a 16-ml/kg body wt-hemor-
haged rat. Although AVP hnRNA transcripts were
seen in the posterior magnocellular PVN and ventral
SON, we did not find positive signals of AVP hnRNA in
the medial parvocellular neurons of the PVN in either
control or hemorrhaged groups. The levels of AVP
mRNA in the SON and PVN did not change signifi-
cantly after the hemorrhage of 16 ml/kg body wt at any
time point examined (data not shown).

Experiment 2a: effects of SNP injection on arterial
blood pressure and HR. After the injection of 2 mg/kg
body wt SNP, MAP decreased from 108 ± 5 to 42 ± 8

![Fig. 1. Effects of hemorrhage of 7, 8, or 16 ml/kg body wt on mean arterial pressure (MAP) at various points from 0 to 30 min. Results are expressed as means ± SE; n = 6 rats at each time point. *P < 0.05, **P < 0.01 compared with values at time 0.](image1)

![Fig. 2. Effects of hemorrhage of 7, 8, or 16 ml/kg body wt on plasma arginine vasopressin (AVP) concentrations (A) and the AVP hetero-
nuclear (hn)RNA levels in supraoptic (SON, B) and paraventricular
(PVN, C) nuclei. AVP hnRNA levels are calculated as percentage of
control. Results are expressed as means ± SE; n = 6 rats at each
time point. *P < 0.05, **P < 0.01 compared with control.](image2)
mmHg at 3 min and returned to the control level at 60 min (Fig. 4). HR increased from 418 ± 10 to 490 ± 14 beats/min at 10 min as a result of baroreflex (22). After the injection of 1 mg/kg body wt SNP, MAP decreased from 105 ± 7 to 58 ± 4 mmHg at 3 min and returned to the control level at 30 min (Fig. 4), and HR increased from 417 ± 15 to 485 ± 18 beats/min at 10 min. The injection of 0.1 mg/kg body wt SNP reduced the MAP from 109 ± 3 to 98 ± 8 mmHg (Fig. 4) but did not affect HR significantly.

Experiment 2b: time-course effects of SNP injection on plasma AVP and hypothalamic AVP hnRNA levels. Plasma AVP levels were significantly increased from the control level at 10 min in the 2-mg/kg body wt SNP injection (control, 1.6 ± 0.4 pg/ml; 2 mg/kg body wt, 8.1 ± 0.4 pg/ml, p < 0.01; Fig. 5A). The AVP hnRNA levels were also increased significantly at 10 min and reached a peak at 30 min after the injection in both the SON (control, 100 ± 5.2%; 2 mg/kg body wt, 151.3 ± 9.8%, p < 0.05; Fig. 5B) and PVN (control, 100 ± 4.2%; 2 mg/kg body wt, 146.5 ± 10.5%, p < 0.05; Fig. 5C).

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Fig. 3. Effects of hemorrhage on AVP hnRNA expression in SON (A and B) and PVN (C and D). Brain slices obtained from control (A and C) and hemorrhaged rats (16 ml/kg body wt; B and D) were hybridized with AVP hnRNA probes. mp, Medial parvocellular region; pm, posterior magnocellular region.

Fig. 4. Effects of different doses of sodium nitroprusside (SNP) sc injection on MAP at various points from 0 to 60 min. Results are expressed as means ± SE; n = 6 rats at each time point. *P < 0.05, **P < 0.01 compared with values at time 0.

Fig. 5. Time-course effects of 2-mg/kg body wt SNP injection on plasma AVP concentrations (A) and AVP hnRNA levels in SON (B) and PVN (C). Rats were decapitated 10, 30 or 60 min after sc injection of 2 mg/kg body wt SNP. Control rats were decapitated 30 min after sc injection of equal volumes of isotonic saline. AVP hnRNA levels are calculated as percentage of control. Results are expressed as means ± SE; n = 6 rats at each time point. *P < 0.05; **P < 0.01 compared with control.
Experiment 2c: dose-response effects of SNP injection on plasma AVP and hypothalamic AVP hnRNA levels.

Consistent with experiment 2b, both plasma AVP and hnRNA levels in the SON and PVN were increased significantly after the injection of 2 mg/kg body wt SNP (Fig. 6). The AVP hnRNA levels were also increased after the injection of 1 mg/kg body wt SNP in both SON (control, 100 ± 15%; 1 mg/kg body wt, 120.3 ± 1.8%, P < 0.05; Fig. 6B) and PVN (control, 100 ± 2.3%; 1 mg/kg body wt, 137.2 ± 9.1%, P < 0.05; Fig. 6C). On the other hand, 0.1 mg/kg body wt SNP had no significant effect on the plasma AVP level (Fig. 6A) or the AVP hnRNA levels in the SON or PVN (Fig. 6, B and C).

**DISCUSSION**

In the present study, we examined the changes in AVP hnRNA levels induced by hypovolemia and/or hypotension. The AVP hnRNA levels in the SON and PVN significantly increased in response to hypotensive hemorrhage or normovolemic hypotension, accompanied by an increase in plasma AVP levels as well.

Although it has been shown that AVP hnRNA levels in the hypothalamus increase in response to osmotic stimulation (1, 18), this is the first direct report showing that AVP gene transcription is also regulated by blood pressure and/or volume.

It has been firmly established that there are two major groups of receptors that can affect AVP release in response to changes in blood volume and arterial pressure, i.e., stretch receptors in the atria of the heart and the arterial baroreceptors (3, 16, 29, 32, 33). A question that has been of continuing interest concerns the relative roles of the atrial and arterial receptors in the blood volume-blood pressure control of AVP release. Although the relative contribution of each receptor type in mediating AVP release in response to blood withdrawal may be species dependent (33), it has been shown that plasma AVP does not increase during progressive hypovolemia until volume loss causes a frank fall in arterial pressure in rats (9, 12, 15). In this study, after the hemorrhage of 8 ml/kg body wt, which is estimated to be ~10% of the total blood volume (6, 7, 10), MAP significantly decreased, and plasma AVP and AVP hnRNA levels were increased significantly from the control level, whereas hemorrhage of 7 ml/kg body wt had no significant effect on MAP, plasma AVP, or AVP hnRNA levels. These results suggest that neither AVP synthesis nor AVP release increase during hemorrhage until there is a decrease in arterial blood pressure. We also showed that the decrease in MAP by SNP injections of 1 or 2 mg/kg, which does not cause a concomitant reduction in blood volume (5, 20, 21), increased AVP hnRNA levels in the SON and PVN. In addition, we showed that a 0.1-mg/kg body wt SNP injection did not cause any significant change in plasma AVP or AVP hnRNA levels in the SON and PVN. Although whether the signals sensed at cardiac and/or arterial receptors activate AVP release and synthesis through the same pathways remains to be clarified, our data indicate that the thresholds for the release and synthesis are similar in baroregulation.

Although the hemorrhage of 8 ml/kg body wt, which decreased MAP by 7%, increased both plasma AVP and the hnRNA levels, the 0.1-mg/kg body wt SNP injection, which decreased MAP by 10%, did not cause any significant change in plasma AVP or AVP hnRNA levels. One possible explanation for the discrepancy is that the threshold for AVP release and synthesis may be significantly affected by changes in blood volume. Alternatively, nitric oxide (NO) released by SNP (25) might affect AVP neurons. It has been reported that the inhibition of NO synthesis increases plasma AVP levels during hemorrhage in rats (14), although another study suggested that NO upregulates AVP hnRNA levels in the hypothalamus (23). It is therefore possible that these effects of NO on AVP neurons influenced our results.

Although Shoji et al. (34) reported that AVP mRNA in the hypothalamus increased at 6 h after acute hemorrhage, other studies failed to detect any significant increase in AVP mRNA levels after hemorrhage or...
hypovolemia (8, 11, and present study). Given that even a >300% increase in AVP hnRNA levels in the SON and PVN did not lead to any increase in the AVP mRNA levels after intraperitoneal injection of hypertonic saline (1, 26), the <60% increase in AVP hnRNA levels in the present study was unlikely to be enough to increase the mRNA levels. However, we cannot exclude the possibility that insufficient time elapsed in this study to allow sufficient accumulation of AVP mRNA.

Because hypovolemia is reported to activate the hypothalamic pituitary adrenal axis (11), it is possible that hemorrhage and hypotension might activate the AVP neurons in the parvocellular PVN. However, we did not detect AVP hnRNA expression in the parvocellular neurons in the PVN. Compared with corticotropin-releasing hormone (CRH) neurons, the response of AVP neurons to stress appears to be slow, because it was reported that AVP hnRNA did not increase significantly until 60 min, whereas CRH hnRNA increased as early as 5 min after intraperitoneal injection of hypertonic saline (26, 27). Because we observed for only ≤30 min after the stimuli, it is possible that the time course in the present study was not long enough to detect the changes in AVP hnRNA expression in the parvocellular neurons.

In conclusion, AVP gene transcriptional activity increases rapidly after hypotensive stimuli. It is also suggested that the set point for AVP synthesis in the SON and PVN induced by changes in blood pressure and/or volume is similar to that for AVP release.

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