Effect of Alcohol, Neurohypophysectomy, and Vasopressin Antagonists on Hemorrhage-Induced Bradycardia in the Rat

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JOHN H. MILLER,[1] DAVID R. GRATTAN,[2] AND ROBERT L. W. AVERILL
Animal Physiology Research Unit, School of Biological Sciences, Victoria University, Wellington, New Zealand

Abstract. **During initial stages of hemorrhage in the rat, cardiovascular compensation leads to a tachycardia (mean ± SE, 5.2 ± 0.7%; $n = 23$) that helps prevent a large fall in blood pressure. This compensatory phase is followed by a decompensatory phase in which mean arterial pressure and heart rate fall. A rise in arginine vasopressin (AVP) levels has been postulated as the cause of this hemorrhage-induced bradycardia (HIB). The object of the present study was to determine whether interference with AVP release by alcohol anesthesia or neurohypophysectomy or by blockade of AVP receptors in the plasma or cerebral spinal fluid could attenuate HIB. Male Wistar rats were anesthetized with pentobarbital, surgically prepared, and bled to maintain a blood pressure of 40–50 mm Hg. After hemorrhage, heart rate decreased 15 ± 2% ($n = 6$) with alcohol anesthesia compared with 32 ± 3% ($n = 7$) with pentobarbital. After neurohypophysectomy, however, HIB remained unchanged (−15 ± 2%; $n = 5$) compared with sham-operated controls (−19 ± 3%; $n = 6$). Peripheral administration of two nonselective V_1/V_2 antagonists and one V_2 antagonist had no effect on HIB, whereas a V_1 antagonist significantly attenuated the heart rate decrease (−15 ± 4%; $n = 6$) compared with controls (−32 ± 3%; $n = 7$). None of the AVP antagonists tested at one tenth the peripheral dose had any effect on HIB when administered into the lateral ventricle of the brain, although a mixed serotonin, dopamine, and catecholamine antagonist, spiperone, potentiated the response. It was concluded that although peripheral release of AVP may be partially involved in the heart rate response to hemorrhage, central AVP release and central AVP receptors were not involved in HIB.** [P.S.E.B.M. 1993, Vol 202]

During hemorrhage, arterial baroreceptors detect a fall in blood pressure and send afferent information to the cardiovascular control center in the medulla, causing a reflex increase in sympathetic activity to the heart and vessels and a decrease in parasympathetic activity to the heart. This compensatory response results in tachycardia and an increase in peripheral resistance that helps to maintain arterial blood pressure at close to prehemorrhage values (1). In severe hemorrhage (>20% of blood volume), a decompensatory phase occurs in which blood pressure (BP) and heart rate (HR) fall due in part to an abrupt decrease in sympathetic nerve activity (1–6). Although the mechanism of the decompensatory response is not known, intact vagus nerves are required since bilateral vagotomy abolishes 50% or more of the cardiovascular effects (2, 4, 5, 7). Roles for opioids and serotonin (4, 8, 9) and for arginine vasopressin (AVP) (5, 10, 11) have also been suggested. A recent study by Peuler *et al.* (5) reported that Brattleboro rats with congenital diabetes insipidus failed to show hemorrhage-induced bradycardia (HIB), nor did they show a decompensatory fall in renal sympathetic nerve activity (RSNA) unless AVP replacement therapy was given during hemorrhage. Earlier studies, however, have reported typical HIB responses in Brattleboro rats compared with Long-

[1] To whom requests for reprints should be addressed at School of Biological Sciences, Victoria University, P.O. Box 600, Wellington, New Zealand.

[2] Current address: Department of Physiology, School of Medicine, University of Maryland at Baltimore, 655 West Baltimore Street, Baltimore, MD 21201-1559.

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Evans (11) and Sprague-Dawley (12) rats. Although AVP may have some limited pressor activity at basal levels in certain vascular beds and may also alter baroreceptor sensitivity at low levels (6, 13–16), the main effect of AVP at low concentrations is to cause antidiuresis. In severe hemorrhage, however, AVP levels in the blood increase markedly to values sufficiently high enough to cause general peripheral vasoconstriction (11, 16–21). Attempts to block the action of AVP on heart rate changes or baroreceptor sensitivity during hemorrhage using specific antagonists have met with little success. V_1 receptor antagonism failed to prevent HIB in anesthetized rats (12) or in conscious rats (21–23) and rabbits (8) and did not prevent an increase in baroreceptor sensitivity caused by AVP in nonhemorrhaged rats (13). AVP antagonism, however, increased the extent of fall in blood pressure during hemorrhage in conscious rats (23) and rabbits (24). In a different study, V_1 antagonism had no effect on blood pressure responses to hemorrhage in conscious male rats, but impaired blood pressure compensation in female rats (21, 25). Although cardiovascular receptors for vasopressin are generally considered to be of the V_1 subtype, some evidence exists that V_2 receptors may be involved in heart rate changes and in baroreceptor sensitivity control (13, 23).

Brattleboro rats with congenital diabetes insipidus fail to demonstrate HIB in some studies (5), but may also have other problems associated with their genetic defect; therefore, the present study was undertaken to determine whether HIB can be blocked by treatments that are known to reduce peripheral AVP levels in otherwise normal rats, including alcohol anesthesia and neurohypophysectomy. Preliminary studies on the effects of alcohol, hydration, and hypophysectomy on HIB have already been reported (10). In addition, it was planned to investigate the effect of peripheral and central addition of AVP antagonists on the HR response to hypotensive hemorrhage. Although studies on peripheral AVP antagonist addition have been reported (8, 12, 13, 22, 23), no results from central administration are available.

Materials and Methods

Animals. Male Wistar rats were bred and maintained in the animal facility at Victoria University. The rats were kept in a 12:12-hr light:dark cycle and were given water and food *ad libitum*. Diet 86 rat pellets were obtained from C. F. Mills (Carterton, New Zealand). Rats to be treated with alcohol were fasted overnight prior to anesthesia and surgery.

Surgical Procedures. *Anesthesia.* As standard procedure, rats were anesthetized prior to hemorrhage by intraperitoneal injection of 50 mg/kg of pentobarbitone sodium (Sagatal, 60 mg/ml; May & Baker NZ, Ltd, Lower Hutt, New Zealand). Additional doses of pentobarbital were given as required either intraperitoneally or intravenously.

For alcohol anesthesia, 50 ml/kg of 12% (v/v) alcohol were administered by stomach tube under light ether anesthesia. Additional 12% alcohol was given as required.

For removal of the posterior lobe of the pituitary gland and for placement of a stainless steel cannula into the lateral ventricle of the brain, animals were anesthetized with a mixture of ketamine and xylazine by intramuscular or intraperitoneal injection at doses of 30 mg/kg and 6 mg/kg, respectively. Additional ketamine and xylazine were given as required. Ketamine (2-[2-chlorophenyl]-2-[methylamino]cyclohexanone HCl) was used at 100 mg/ml (Sigma Chemical Co., St. Louis, MO) and xylazine (2-[2,6-dimethylphenylamino]-4*H*-5,6-dihydro-1,3-thiazine HCl) at 20 mg/ml (Rompun, Bayer, NZ Ltd, Wellington, New Zealand).

Neurohypophysectomy. During ketamine/xylazine anesthesia, the posterior lobe of the pituitary gland was exposed by a parapharyngeal approach and removed by controlled suction, as described previously (26). The animals were selected at approximately 200 g body wt to facilitate surgical procedures. Penstrep LA (0.2 ml of a 200,000 unit/ml penicillin and 250-mg/ml streptomycin suspension, Rosco, Veterinary Ethicals Ltd., Auckland, New Zealand) was injected subcutaneously immediately after surgery to prevent postoperative infection. Sham-operated animals were subjected to the same operation without removal of the neurohypophysis. Water consumption was monitored for 3 days before surgery and for 3 to 4 days after neurohypophysectomy, when hemorrhage tests were carried out. Only animals having no visible posterior lobe on autopsy and showing a daily water consumption increase of greater than 50% were considered lobectomized. Animals with either residual posterior lobe material present on autopsy or no posterior lobe but with water consumption increases of less than 50% were considered partially lobectomized.

Lateral ventricle cannulation. Under ketamine/xylazine anesthesia, a stainless steel cannula guide (21 gauge) was inserted through a hole drilled through the skull so that its ventral tip lay 2 mm above the lateral ventricle. The correct position of the cannula was determined from micrometer measurements using a stereotaxic instrument. The guide tube was held in place by dental cement. Rats were given penicillin-streptomycin subcutaneously and left to recover a minimum of 4 days before carrying out hemorrhage tests.

Surgical preparation of animal for HIB measurements. When surgical anesthesia was complete (Sagatal or alcohol anesthesia), the trachea was cannulated with polyethylene tubing (PE-205-240 Clay Adams, Parsippany, NJ). For peripheral drug administration tests, alcohol anesthesia, and neurohypophysectomy tests,

the right jugular vein was cannulated with soft medical grade Silastic tubing (o.d. 1 mm; Dow Corning, Midland, MI) for drug administration and for blood removal or replacement. The left or right common carotid artery was cannulated with PE tubing (PE-50) for BP measurement. For central drug administration tests, the right femoral artery and ventral tail artery were cannulated for BP measurements and for blood removal and replacement, respectively. This alteration to the procedure was made after completion of the earlier studies in order to minimize interference with vagal and carotid sinus activity in the cervical region. No qualitative differences were found between rats cannulated in the carotid artery and those cannulated in the femoral artery-tail artery; however, there was a change in the blood volume needed to be removed to induce the hypotensive state (Table I) and the extent of the bradycardia was altered (compare Fig. 1A and Fig. 3). All vascular cannulae were filled with heparinized saline (16 units/ml) to prevent blood clot formation. Needle electrodes were inserted under the skin of both forepaws and one hindpaw for electrocardiograph monitoring of heart rate. Body temperature was monitored using a rectal probe connected to a Scanning Tele-Thermometer (model 47; Yellow Springs Instrument Co., Yellow Springs, OH), and body temperature was maintained between 35°C and 37°C on a temperature-controlled operating table.

Blood Pressure and Heart Rate Monitoring. BP and HR were recorded on a Gould RS 3200 two-channel, pressurized ink or thermal pen recorder (Gould, Inc., Cleveland, OH) fitted with Universal amplifiers (Gould model 13-4615-58). A Gould P23XL pressure transducer was used for BP measurement. Heart rate was calculated from an electrocardiograph recording using needle electrodes connected to the recorder via a Gould defib protector and isolated preamp.

Hemorrhage Procedure. One hour after completion of surgery, blood was removed from either the jugular vein (alcohol anesthesia, neurohypophysectomy, or peripheral drug tests) or the tail artery (central drug tests) using a heparinized 5-ml syringe until mean arterial pressure (MAP), calculated as diastolic pressure plus one third of pulse pressure, fell to between 40 and 50 mm Hg. The time required to remove sufficient blood to lower MAP to the set value varied between experiments from 30 to 90 sec but averaged about 45 sec. HR and BP were then monitored for 4 to 8 min after reaching the desired pressure. Compensatory rises in BP during this posthemorrhage period were prevented by withdrawing additional blood as required to maintain hypotension. At the end of the hypotensive period, the blood was replaced. In some cases, repeat hemorrhages in the same animal gave significantly attenuated responses after only a 30-min recovery; hence, data were collected from first hemorrhages only in the alcohol, neurohypophysectomy, and peripheral drug addition tests. In the central drug addition tests, however, a 1-hr recovery period was allowed, and first and second hemorrhages in the same rat were found to give similar HR responses (Fig. 3). At the end of the experimental tests, the animals were sacrificed without regaining consciousness by intravenous injection of Euthatal 350 (May & Baker NZ).

Addition of Drugs. *Peripheral addition.* Drugs were administered either by bolus injection into the jugular vein 10 min before hemorrhage or by infusion using a Harvard infusion withdrawal pump (model 931; Harvard Apparatus, Millis, MA) set to deliver at a rate of 0.02 ml/min. The infusion was started 2–5 min before hemorrhage and continued throughout the posthemorrhage period before blood replacement. To assess the effects of the different AVP antagonists on the blood pressure response to vasopressin, AVP at a dose of 10 mU/min·kg was given by infusion into the jugular vein at 0.02 ml/min.

Central addition. A bolus of 5 μl of saline, with or without drug, was injected through the indwelling right lateral ventricle cannula (LVC) 5 min before hemorrhage. The injection was made using a Hamilton microliter syringe (model 710; Hamilton, Reno, NV) fitted to a 26-gauge needle with a stop that ensured that its tip lay in the lateral ventricle. Terminally, 5 μl of 1% Evan's blue dye in saline were injected and the dye distribution within the ventricles of the brain were examined on autopsy. In most cases, dye was clearly visible in the lateral ventricles (first and second ventricles), the median eminence (third ventricle), and in the cord (fourth ventricle).

Statistical Analysis. All data are presented as the mean ± SE. Statistical significance of the data at the 95% confidence level was determined by the unpaired Student's t test or by repeated-measure analysis of variance (ANOVA-RM) using the Statview 512+ program (Brainpower Inc., Calabasas, CA) and an Apple Macintosh computer (Apple Computer, Inc., Cupertino, CA).

Materials. All chemicals used were of analytical grade. Although plasma samples were collected for measurement of AVP levels, a commercially available radioimmunoassay kit failed to perform to specification; therefore, no AVP values were obtained. Drugs and hormones were dissolved in saline (0.85% NaCl) and stored frozen at −20°C in appropriate stock solutions. Spiperone (8-[3-(*p*-fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspirol[4,5]decan-4-one) was dissolved in 0.4 *M* lactic acid. 1-Phenylbiguanide was purchased from Aldrich Chemical Co. (Milwaukee, WI). Spiperone, AVP, and the AVP antagonist (adamantaneacetyl^1,*O*-Et]-D-Tyr^2,Val^4, aminobutyryl^6,Arg^{8,9}]-vasopressin (Aaa-AVP) (27, 28) were from Sigma. Other AVP antagonists used in this

study were the generous gift of Professor Maurice Manning of the Medical College of Ohio. These included the mixed V_1/V_2 antagonist $d(CH_2)_5$D-Tyr(Et)^{2}Val^{4}AVP (27, 29), the V_1 antagonist $d(CH_2)_5$Tyr(Me)^{2}AVP (27, 30), and the V_2 antagonist $d(CH_2)_5$[D-Ile^2,Ile^4,Ala-NH_2^9]AVP (27, 31).

Results

Alcohol Effect on HIB. In seven control rats anesthetized with pentobarbital, HR decreased after hemorrhage 32.1 ± 2.9% after 8 min (Fig. 1A). After replacement of the blood, HR recovered to prehemorrhage values within 15 min, whereas BP immediately rose to above prehemorrhage values, gradually falling back to baseline over the next 15 min (data not presented). Approximately 3.5 ml of blood for a 270-g rat (1.28% of body wt) was removed to maintain MAP at ≤50 mm Hg (Table I). Assuming blood volume to be 5.4% of body wt (32), this represents a blood loss of about 24% of the total blood volume. Separation of the blood volume data into initial volume removed and subsequent volume removed during the 8-min period showed the same trends as that of total volume, and the two values were combined in the data of Table I.

Alcohol anesthesia significantly attenuated the bradycardia compared with control rats anesthetized with pentobarbital, since HR only decreased 15.2 ± 1.7% by 8 min (Fig. 1A) ($P < 0.0005$ by ANOVA-RM). Unfortunately, there is no adequate control available to determine whether the alcohol anesthesia has any non-AVP-related effects on HIB not present with pentobarbital anesthesia. Prehemorrhage HR and MAP in alcohol-anesthetized rats were not different from those of pentobarbital-anesthetized rats (Table I); however, the volume of blood required to reduce MAP to ≤50 mm Hg (1.95% of body wt) was significantly greater in alcohol-treated animals ($P < 0.001$, Student's t test).

Neurohypophysectomy Effect on Water Consumption and HIB. The effect of removal of the posterior lobe of the pituitary gland on water consumption is shown in Table II. Both complete and partial lobectomies increased water consumption over the 3- to 4-day period after lobectomy. Neither complete nor partial lobectomy, however, had any effect on HIB compared with sham-operated controls (Fig. 1B). The percentage of decrease in HR in sham-operated animals after 8 min (−18.5 ± 3.4%; $n = 6$) was not significantly different from either lobectomy (−14.5 ± 2.1%; $n = 5$) or partial lobectomy (−18.7 ± 3.5%; $n = 7$) animals. Sham-operated animals had much less of a bradycardia than unoperated controls (compare Fig. 1A and 1B), yet the blood volume depletion needed to generate the hypotensive state was much higher in sham-operated rats (1.69% of body wt) relative to unoperated rats (1.28% of body wt) (Table I).

AVP Antagonist Effects on HIB: Peripheral Administration. The reported potencies and selectivities of the AVP antagonists are summarized in Table III. The effects of peripheral administration of the AVP antagonists on HIB are given in Figure 2A. The selective V_1 antagonist $d(CH_2)_5$Tyr(Me)^{2}AVP was administered either as a bolus dose of 10 nmol/kg 10 min before hemorrhage ($n = 3$) or by infusion at 2 nmol/min·kg beginning 2 min before hemorrhage ($n = 3$). There were no differences in the responses between the two different treatment regimes, and the data have, therefore, been grouped together in the results. This selective V_1 antagonist significantly reduced HIB (−15.3 ± 3.6%)

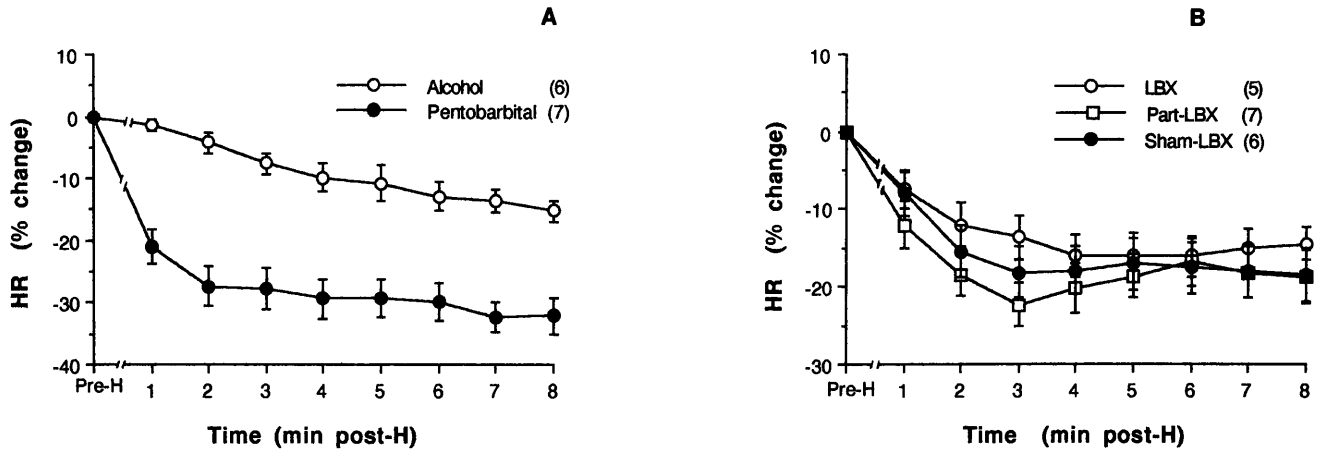

Figure 1. Effect of alcohol anesthesia and neurohypophysectomy on HIB. All hemorrhages were first hemorrhages (H1). HR is presented as the percentage of change from the prehemorrhage (pre-H) value measured immediately before blood removal. Time represents minutes after hemorrhage (min post-H) beginning from when MAP first reaches a value of ≤50 mm Hg during blood withdrawal. The number of rats (n) tested is given in parentheses. Data are presented as the mean ± SE. (A) Alcohol (12%) was administered by stomach tube to maintain anesthesia. Pentobarbital anesthesia was as described in the Methods. (B) Neurohypophysectomized rats, completely lobectomized (LBX) and partially lobectomized (Part-LBX), were compared with sham-operated controls (sham-LBX). Hemorrhage was performed under pentobarbital anesthesia 3–4 days after lobectomy.

Table I. Prehemorrhage Cardiovascular Parameters[a]

Experiment	*n*	Body wt (g)	HR (beats/min)	MAP (mm Hg)	Hemorrhage (% body wt)
Peripheral tests					
Control H1	7	270 ± 10	405 ± 11	137 ± 6	1.28 ± 0.04
Alcohol	6	267 ± 13	433 ± 18	141 ± 8	1.95 ± 0.14[b]
Lobectomy	5	177 ± 15[b]	389 ± 14	108 ± 5[c]	1.28 ± 0.11[d]
Partial lobectomy	7	201 ± 17[b]	366 ± 19	111 ± 3[b]	1.49 ± 0.12
Sham lobectomy	6	201 ± 9[b]	384 ± 14	121 ± 4	1.69 ± 0.13[b]
V_1 Antagonist	6	258 ± 11	397 ± 21	118 ± 7	1.19 ± 0.09
V_2 Antagonist	6	270 ± 16	412 ± 15	133 ± 9	1.46 ± 0.08
V_1/V_2 Antagonist	6	270 ± 14	404 ± 46	109 ± 4[b]	1.03 ± 0.10[e]
Aaa-AVP	3	271 ± 13	422 ± 34	126 ± 10	1.20 ± 0.22
Central tests					
Control H1 (no LVC)	4	304 ± 13	406 ± 17	108 ± 1	0.96 ± 0.09[c]
Control H1 (LVC)	5	328 ± 12[b]	397 ± 10	123 ± 5	1.02 ± 0.13
V_1 Antagonist					
Control	4	356 ± 7	361 ± 15	104 ± 16	0.88 ± 0.01
Experimental	—	—	373 ± 25	97 ± 9	0.74 ± 0.02
V_1/V_2 Antagonist					
Control	4	298 ± 6	409 ± 16	113 ± 3	0.78 ± 0.19
Experimental	—	—	381 ± 20	112 ± 6	0.87 ± 0.09
Spiperone					
Control	6	338 ± 20	407 ± 14	114 ± 6	1.14 ± 0.15
Experimental	—	—	395 ± 28	103 ± 10	0.86 ± 0.10

[a] Hemorrhage, expressed as the percentage of body weight (v/w), refers to the total volume of blood removed to maintain MAP between 40 and 50 mm Hg. All data are presented as the mean ± SE, and statistical significance was determined by Student's *t* test.
[b] $P < 0.005$ (relative to peripheral control H1).
[c] $P < 0.01$ (relative to peripheral control H1).
[d] $P < 0.05$ (relative to sham lobectomy).
[e] $P < 0.05$ (relative to peripheral control H1).

Table II. Changes in Water Consumption after Neurohypophysectomy

Experiment[a]	Water consumption			Ratio (post/pre)
	n	Preoperation (ml/day·kg)	Postoperation (ml/day·kg)	
Lobectomy	5	123 ± 17	237 ± 26[b]	2.00 ± 0.23[b]
Partial lobectomy	7	112 ± 7	173 ± 13[c]	1.55 ± 0.09[d]
Sham lobectomy	6	112 ± 17	108 ± 16	0.99 ± 0.07

[a] Water consumption was monitored for 3 days before neurohypophysectomy and for 3 to 4 days after neurohypophysectomy. Lobectomy refers to animals that passed the two criteria given in the methods for complete lobectomy; partial lobectomy refers to animals that failed one or both criteria; sham lobectomy refers to animals that were operated on but on which no attempt was made to remove the posterior lobe. Data are presented as the mean ± SE.
[b] $P < 0.005$ (relative to sham-LBX).
[c] $P < 0.01$ (relative to sham-LBX).
[d] $P < 0.01$ (relative to sham-LBX).

relative to the control (−32.1 ± 2.9%) (Fig. 2A) ($P <$ 0.005 by ANOVA-RM). The selective V_2 antagonist $d(CH_2)_5$[D-Ile^2,Ile^4,Ala-NH_2^{9}]AVP given as a bolus dose at 10 nmol/kg 10 min before hemorrhage had no effect on HIB (−22.3 ± 2.7%). Neither of the two mixed receptor antagonists, the V_1/V_2 antagonist $d(CH_2)_5$D-Tyr(Et)^{2}Val^{4}AVP at a dose of 10 nmol/kg given 10 min before hemorrhage (−23.3 ± 2.6%) nor Aaa-AVP infused at a dose of 2 nmol/min·kg started 5 min before hemorrhage (−27.8 ± 8.3%), had any effect on HIB. Although prehemorrhage HR values were similar among the four groups, the V_1/V_2 antagonist caused a 12 ± 4% fall in MAP over the 10-min prehemorrhage period (Table I) ($P < 0.05$; Student's *t* test). This lower initial MAP may be the reason for a reduction in the amount of blood needed to be withdrawn to reduce MAP to ≤50 mm Hg (Table I). HR, however, was unaffected by this drug. None of the other three antagonists had any effect on either HR or MAP in the period preceding hemorrhage.

To demonstrate the antivasopressor (anti-V_1) effects of the different AVP antagonists, the antagonists were administered immediately before or during infusion of 10 mU/min·kg of AVP under nonhemorrhage

Table III. AVP Antagonist Potencies and Selectivities[a]

AVP antagonist	Anti-V_1 activity		Anti-V_2 activity		Ratio V_1:V_2
	Effective dose (nmol/kg)	pA_2	Effective dose (nmol/kg)	pA_2	
V_1 Antagonist	0.16	8.62	(Weak agonist)		∞
V_2 Antagonist	38	6.25	0.46	8.16	0.012
V_1/V_2 Antagonist	0.45	8.22	1.1	7.81	2.57
Aaa-AVP	1.2	7.75	0.53	8.11	0.44

[a] All data are from Ref. 27. Specific antagonist formulas are given in the text. The effective dose is defined as the dose that reduces the response to 2× units of agonist to equal the response to 1× unit. Estimated *in vivo* pA_2 values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 ml/kg).

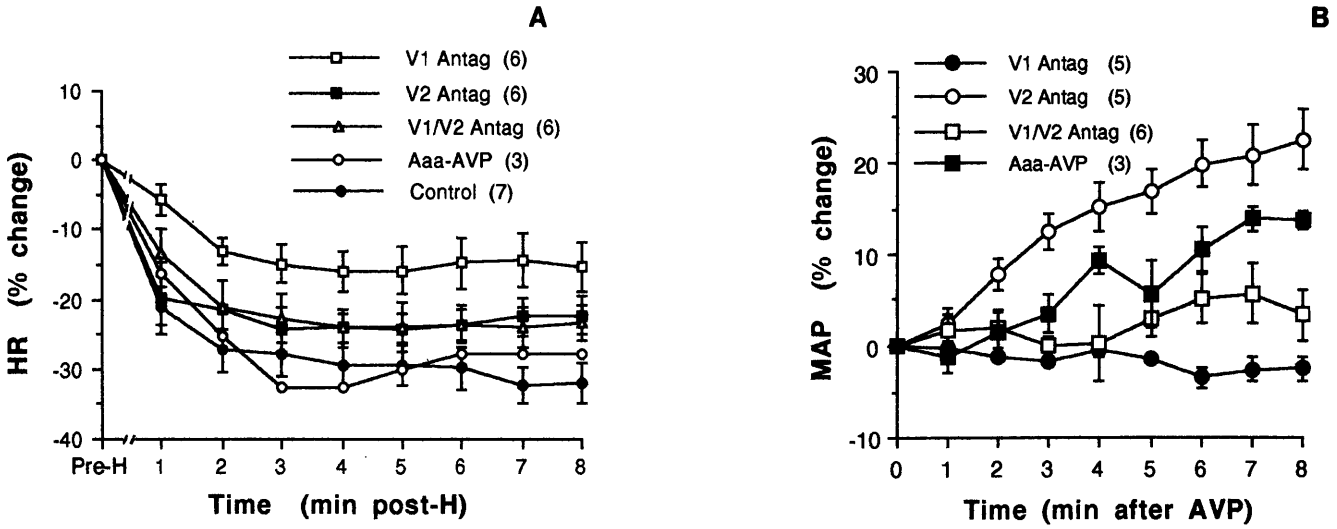

Figure 2. Effect of peripheral addition of AVP antagonists on HIB and on AVP-induced changes in MAP. Axis labels are as in Figure 1, and chemical formulas are given in the text for each specific antagonist. Data are presented as the mean ± SE. (A) HR changes are presented after first hemorrhages (H1) with Control results repeated from Figure 1A. The V_1 antagonist data represent combined data from two different procedures of drug administration in which antagonist was given either as a bolus dose at 10 nmol/kg 10 min before hemorrhage ($n = 3$) or by infusion at 2 nmol/min·kg beginning 2 min before hemorrhage and continuing for 8 min after hemorrhage ($n = 3$). The V_2 antagonist was given as a bolus dose at 10 nmol/kg 10 min before hemorrhage. The mixed receptor V_1/V_2 antagonist was given as a bolus dose at 10 nmol/kg 10 min before hemorrhage. The mixed antagonist Aaa-AVP ($n = 3$) was given by infusion at a dose of 2 nmol/min·kg begun 5 min before hemorrhage and continued throughout the 8-min posthemorrhage period. (B) AVP antagonist effects on the MAP response to infused AVP are presented. MAP refers to the MAP in the presence of AVP as the percentage of change from the value measured immediately before AVP infusion. AVP at a dose of 10 mU/min·kg was infused into rats 30 min after the blood had been replaced following the hemorrhage tests described in Figure 2A. Antagonist doses are as described in Figure 2A. V_1 and V_1/V_2 antagonists were administered once 40–50 min before AVP infusion. V_2 antagonist was administered 50 min before AVP infusion and again at 10 min before the start of AVP infusion. Aaa-AVP was administered 45 min before and again at 5 min before the start of AVP infusion.

conditions (Fig. 2B). The V_1 antagonist ($P < 0.0001$ by ANOVA-RM) and both mixed receptor antagonists, V_1/V_2 ($P < 0.005$ by ANOVA-RM) and Aaa-AVP ($P < 0.05$ by ANOVA-RM), completely or partially blocked the expected increase in MAP after infusion of AVP when compared with the BP response to AVP in the presence of the V_2 antagonist. Although the response of control animals without added antagonist was not tested, the V_2 antagonist was administered at a dose that was only one quarter of its reported effective dose for antivasopressor activity (Table III). Initial HR and MAP values before AVP infusion were not different among the four groups. As reported by others (21, 30), antagonist effects were long-lasting, since the MAP response to AVP was blocked for 3 to 4 hr after a single exposure to V_1 antagonist (10-min infusion at 2 nmol/min·kg).

AVP Antagonist Effects on HIB: Central Administration. The effect of central administration of AVP antagonists on HIB was compared with control bradycardias in the same rat after saline injection into the cerebral spinal fluid (CSF). First (H1) and second (H2) hemorrhages in the same rat gave similar bradycardias (Fig. 3), and HR recovery was nearly complete by 15 min. Rats with an indwelling LVC gave somewhat attenuated HR responses compared with rats without cannulae, although the differences were not significant by ANOVA-RM. In general, control bradycardias were

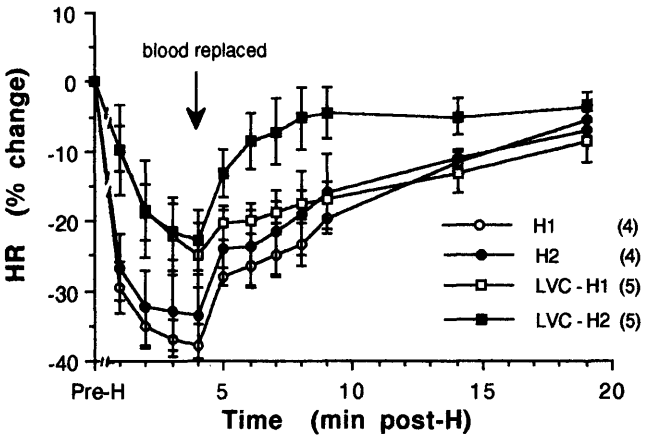

Figure 3. Control hemorrhages in rats with and without lateral ventricle cannulae. HR responses to first (H1) and second (H2) hemorrhages (1 hr after H1) are presented in rats with and without LVC. Data are given as the mean ± SE. Numbers of animals (*n*) are given in parentheses. HR recoveries after replacement of blood are also shown.

measured before testing the effects of drugs because of the unknown latencies of the drug effects in the CSF. AVP antagonists were injected at one tenth the dose given peripherally. The volume of CSF in a 300-g rat has been estimated at 580 μl (33), or about 8% of the plasma volume. The V_1 antagonist that attenuated HIB when given peripherally (Fig. 2A) had no effect when given centrally at one tenth the dose (Fig. 4A). HR decreased 14.7 ± 5.5% by 4 min after hemorrhage in the presence of V_1 antagonist, compared with 17.9 ± 3.9% in its absence. Central addition of the mixed V_1/V_2 antagonist also had no significant effect on HIB (Fig. 4B). After 4 min, HR had decreased 29.0 ± 4.4% in the presence of antagonist compared with 37.7 ± 2.9% after saline injection. A preliminary test of the V_2 antagonist also showed no effect on HIB since HR decreased 28.0% by 4 min in the presence of antagonist and 26.9% in its absence (mean data of two rats).

Spiperone Effect on HIB: Central Administration. Spiperone, a $5HT_1$, $5HT_2$, D_2, and α_2-antagonist, was tested at a dose reported to block the HR bradycardia involved in the Bezold-Jarisch reflex induced by peripheral administration of phenyl biguanide (34). The ability of spiperone to block the phenyl biguanide-induced bradycardia is confirmed in the present study (Table IV), thus establishing that the central administration procedure was delivering drug into the CSF as expected. Spiperone also significantly increased the bradycardia induced by hemorrhage, causing a HR change of −25.5 ± 2.0% ($P \leq 0.005$ by ANOVA-RM) (Fig. 5). The HR decrease of only 13.6 ± 3.2% for the control hemorrhage after injection into the CSF of 0.4 *M* lactic acid (the diluent for spiperone) was considerably less than that seen in other rats with a LVC.

Discussion

Studies in rats (2–5, 10–12, 21–23, 35), rabbits (8, 16, 17, 24), dogs (20, 36), and humans (1, 9) have examined the effects of hemorrhage on cardiovascular parameters. Although specific protocols vary with regard to the pattern and rate of blood loss and the anesthetic state, it is clear that two immediate consequences of hypotensive hemorrhage are a marked bradycardia and a decrease in renal sympathetic nerve activity.

The suggestion of a role for AVP in HIB originally came from the fact that AVP levels rise markedly after hypotensive hemorrhage (11, 16–23). Actual AVP levels in Wistar rats anesthetized with nitrous oxide have been reported to rise from 22 pg/ml to 118–973 pg/ml

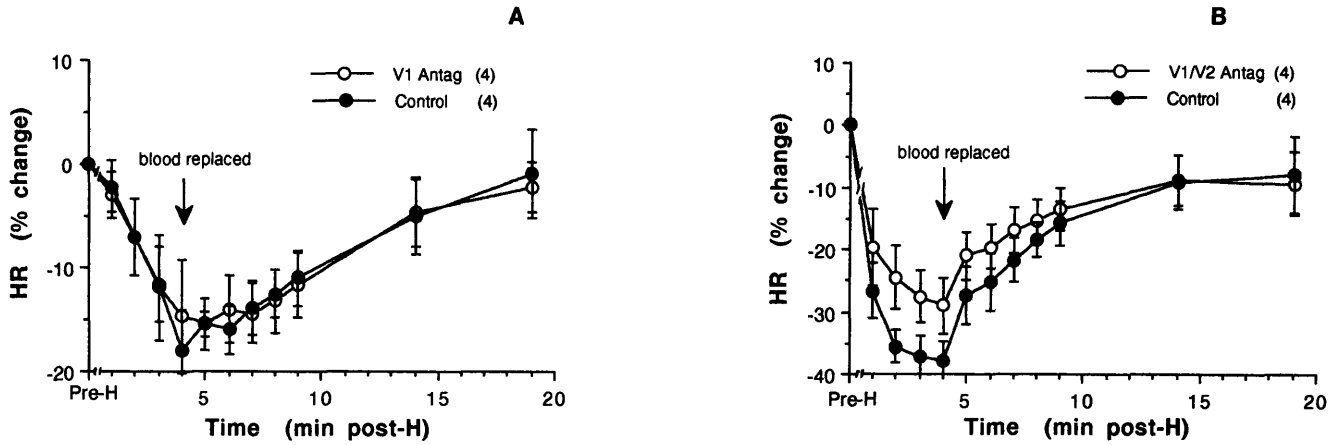

Figure 4. Effect of central administration of AVP antagonists on HIB. Data are presented as the mean ± SE. Control hemorrhages (H1) were tested first, then hemorrhages (H2) in the presence of AVP antagonist were carried out 1 hr later in the same rat. Antagonists were injected into the lateral ventricle 5 min before hemorrhage at a dose of 1 nmol/kg. After MAP had reached ≤50 mm Hg, HR was monitored for 4 min, then the blood was replaced and HR was monitored during recovery. (A) V_1 antagonist and (B) mixed V_1/V_2 antagonist effects on HIB are presented.

Table IV. Central Spiperone Effect on Phenyl Biguanide-Induced Bradycardia

Test[a]	*n*	Central addition	HR change (% decrease)
Saline	3	None	1.1 ± 0.2
PBG	3	Lactic acid	18.3 ± 2.4
PBG	3	Spiperone	10.6 ± 3.6
PBG	3	None	15.7 ± 4.1
PBG (vagotomy)	3	None	0.8 ± 0.2

[a] Tests were performed in the sequence given above. Lactic acid (0.4 *M*) or spiperone dissolved in lactic acid (100 μg/kg) was injected into the lateral cerebral ventricle 5 min before intravenous injection of phenyl biguanide (PBG) 10 μg/kg. After the spiperone test, the animals were allowed to recover for 1 h before the effects of PBG were retested. A bilateral vagotomy was then performed and the PBG response was tested again 30 min later.

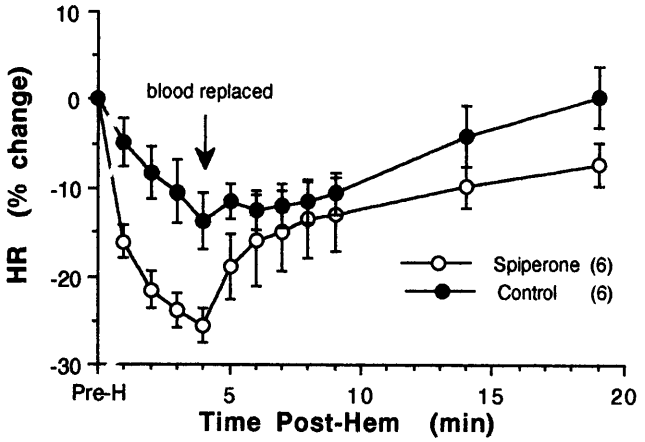

Figure 5. Effect of central administration of spiperone on HIB. Data are presented as the mean ± SE. Control hemorrhage (H1) was tested first, then hemorrhage (H2) in the presence of 100 μg/kg of spiperone was carried out 1 hr later in the same rat. Spiperone was injected into the lateral ventricle 5 min before hemorrhage.

after hemorrhage (19). In conscious Wistar rats, AVP levels rose from 5 to 108 pg/ml after 45 min (21). It is interesting that in the rabbit, major increases in AVP do not occur until 35% or more of the blood volume is lost (16), yet in the dog (20) and rat (19, 21, 22), major increases occur after only 20–25% of blood volume is removed. A second reason for implicating AVP in HIB is that AVP infusion in nonhemorrhaged animals causes a marked bradycardia (10, 21), even in the presence of total autonomic blockade (20). A third reason for suggesting a role for AVP in HIB is that in one study with Brattleboro rats, no bradycardia or decreased RSNA was observed during hemorrhage unless the animals were administered AVP (5). Finally, one study had reported that treatments that would be expected to reduce circulating AVP levels, including hydration, alcohol infusion, and hypophysectomy, reduced the extent of the HR bradycardia in rats (10).

Alcohol anesthesia, which is believed to interfere with AVP release from the neurohypophysis (18), reduced HIB in the present study in a manner similar to that previously reported by Sjöstrand (10) (Fig. 1A). This provides evidence that peripheral release of AVP may be involved; however, interpretation of the alcohol results is difficult because alcohol anesthesia may have central effects that differ from those of pentobarbital, and these effects may modify the cardiovascular response to hemorrhage independently of changes in plasma AVP levels. It has been reported that whereas alcohol blocks AVP release in response to osmotic stimuli and blocks oxytocin release in response to suckling, it may not block AVP release in the case of a strong stimulus such as hemorrhage (18). There is also evidence that alcohol may, in certain conditions, stimulate AVP release rather than inhibit it (37, 38).

A second experiment designed to interfere with AVP release into the plasma, neurohypophysectomy, failed to confirm a role for AVP in HIB. This experiment involved removal of the source of plasma AVP during hemorrhage. HIB was not affected by neurohypophysectomy (Fig. 3). An earlier study by Sjöstrand (10) showed that removal of the entire pituitary gland decreased HIB in pentobarbital-anesthetized rats; however, hemorrhage tests were carried out immediately after hypophysectomy, whereas in the present study, experimental measurements were taken after recovery from surgery. In the present study, removal of the posterior lobe of the pituitary gland succeeded in reducing peripheral AVP secretion, as judged by increased water consumption after lobectomy. The doubling of water consumption seen after posterior lobectomy was even greater than a 55% increase reported 1 week after complete hypophysectomy in Wistar rats (39). It is possible that a strong stimulus such as hemorrhage is able to trigger AVP release from the cut and healing ends of the axons of the posterior lobe; however, in the absence of plasma AVP measurements, this question remains unanswered. Another consideration is why HIB was attenuated in sham-operated animals (19%) compared with unoperated controls (32%). This difference may be due to the effects of surgery 3–4 days earlier to remove the neurohypophysis, since implantation of an LVC also reduced the extent of bradycardia (Fig. 3). Another explanation is that the younger age of the rats selected for the neurohypophysectomy experiments may have affected their cardiovascular responses to hemorrhage.

The lack of effect of neurohypophysectomy on HIB suggests that if AVP is involved in the bradycardia, its site of release and target receptors may not be peripheral, but central. The reduced HIB response in alcohol-treated rats does not necessarily contradict this conclusion, since it may reflect an alcohol effect on central AVP levels as well as peripheral. There is ample evidence from other studies that extrahypothalamic vasopressinergic neurons exist in the brain (40). Thus, neuronal release of AVP within the central nervous system

may have been more important in the development of HIB than release of AVP into the plasma. AVP levels in the cerebral spinal fluid have been measured (11–22 pg/ml basal) and have been shown to increase after hemorrhage, even though they appear to fluctuate independently of plasma AVP levels (41).

Blockade of peripheral or central AVP receptors should attenuate the HR response to hemorrhage if AVP is involved. Evidence that V_1 receptor blockade does not alter HIB in conscious female Long-Evans rats (22) and conscious rabbits (8) and that a V_2 agonist DDAVP can reduce HR and increase baroreceptor sensitivity in nonhemorrhaged Long-Evans rats (13) suggested that V_2 receptors might be involved in the HR response to hypotensive hemorrhage. The potent and selective V_2 antagonist used in the present study, however, had no effect on HIB (Fig. 2A) at a dose (10 nmol/kg) well above the reported effective dose of 0.46 nmol/kg (Table III). Although urine production was not measured, most animals treated with this drug began producing copious quantities of relatively clear urine, which suggests effective blockade of renal V_2 receptors (unpublished observations). The mixed antagonist with predominantly antidiuretic selectivity (Aaa-AVP) also failed to block HIB. These results together suggest that a V_2 receptor is not involved in the HR response to hemorrhage.

Peripheral addition of the selective and potent V_1 antagonist $d(CH_2)_5Tyr(Me)^2AVP$ caused a marked reduction in HIB, although it did not abolish it completely. This result is in direct conflict with the reported lack of effect of the same V_1 antagonist in hemorrhaged, conscious, female Wistar rats (21), female Long-Evans rats (22), male Sprague-Dawley rats (23), and conscious rabbits (8). In the present study, V_1 receptor blockade did not, however, affect the amount of blood loss required to generate the hypotensive state, as it has been reported to do in other studies (6, 15, 23). These conflicting results may be explained by differences in the strain of rat or the species used (2), differences between sexes (6, 25), or by differences between conscious and anesthetic states (1, 2, 9, 18, 41–43). Pentobarbital anesthesia has been shown to elevate baseline AVP levels in mammals (39, 41), although in rats it seems to have no effect on neurophysin release from vasopressinergic neurons after hemorrhage (42).

Given the V_1 sensitivity of HIB, it was surprising to find a lack of attenuation, with peripheral addition of the mixed V_1/V_2 antagonist having predominantly antivasopressor (V_1) selectivity $d(CH_2)_5D$-$Tyr(Et)^2Val^4AVP$. This antagonist failed to block HIB (Fig. 2A) despite effective inhibition of the BP rise after AVP infusion in the absence of hemorrhage (Fig. 2B). It is difficult to explain the difference in effects on HIB between the selective V_1 antagonist and the mixed V_1/V_2 antagonist, since both were clearly blocking peripheral V_1 receptors. It may be that this inconsistency is due to a nonspecific action of the V_1 antagonist at the high dose given, or that the receptor involved in the HIB response is not the classical V_1 or V_2 subtype but represents a third type of receptor with different specificities for the particular antagonists used. There is already evidence that a least two subpopulations of V_1 receptor exist, V_{1a} and V_{1b}, although the V_{1b} receptor has only been found in anterior pituitary cells (27, 44). The V_1/V_2 antagonist used in the present study has been shown to have a very low binding affinity for V_{1b} relative to V_{1a} (44); unfortunately, no information is available on the V_{1b} selectivity of the V_1 antagonist used in the present study. A third possible explanation for the inconsistent V_1 antagonist effects could have been that the site of action of AVP was central rather than peripheral (1, 21, 40, 41). If this were true, the effectiveness of the antagonist may depend not only on its potency for the V_1 receptor, but also on its ability or the ability of biologically active fragments of the antagonist to cross the blood-brain barrier. Administration of AVP antagonists directly into the CSF, however, failed to provide support for a role of central AVP in HIB (Fig. 4). The V_1 antagonist that attenuated HIB when added peripherally was ineffective when added centrally. Hence, the possibility remains that a unique AVP receptor subtype is involved at a peripheral site, although this seems unlikely since three other studies in conscious rats of a different strain failed to show V_1 sensitivity of HIB using the same V_1 antagonist (21–23). One of these studies, however, showed that intracerebroventricular administration of this same V_1 antagonist reduced BP recovery after hemorrhage (21). The success of the method for CSF delivery of antagonist was established by dye distribution on autopsy and by the fact that centrally administered spiperone, a mixed serotonin, dopamine, and catecholamine antagonist, potentiated the HR decrease in response to hemorrhage (Fig. 5) as well as attenuated the vagally mediated bradycardia induced by peripheral administration of phenyl biguanide (Table IV). It has been postulated that the decreased HR and RSNA observed during hypotensive hemorrhage is initiated by cardiac sensory receptors in the left ventricle involved in the Bezold-Jarisch reflex (1, 4, 45). The presence of a cardiac sensory component in the hemorrhage-induced changes is supported by the fact that vagotomy partially blocks HIB in rats by 50% or more (2, 4, 5, 7) and, in some cases, also blocks the decrease in RSNA (4). Cardiac afferent connections may be linked via central opioid receptors (1, 8, 9, 45) or serotonin receptors (4, 9, 45).

The most logical explanation for the results of the present study is that AVP release, either centrally or peripherally, is not a major component of HIB. The attenuation of the bradycardia by alcohol anesthesia or

by peripheral V_1 receptor antagonism was suggestive of a role of peripherally released AVP in the response, although the neurohypophysectomy results and the lack of peripheral effect of the mixed V_1/V_2 antagonist did not support this conclusion. Other nonspecific explanations for these effects were also possible. The results of the tests involving central administration of AVP antagonists provided no evidence for a role of central AVP release or central V_1 or V_2 receptors in the HR response to hemorrhage. Finally, the potentiation of the HIB response by spiperone indicates that central receptors for serotonin ($5HT_1$ or $5HT_2$), dopamine (D_2), or catecholamine (α_2) may be involved in the bradycardia induced by hypotensive hemorrhage.

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