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Effects of Ethanol on Neurohumoral Mechanisms for Blood Pressure Regulation in Hemorrhaged Conscious Rats*

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=ABSTRACT=

The role of neurohumoral mechanisms in the regulation of cardiovascular functions and the effects of ethanol (EOH) on these mechanisms were examined in hemorrhaged conscious Wistar rats. The rats were bled at a constant rate (2 ml/kg/min) through the femoral artery until mean arterial pressure (MAP) was reduced by 30 mmHg. We studied the responses to hemorrhage 1) under normal conditions (Normal), and after pretreatments with 2) neural blockade (NB), pentolinium, 3) arginine vasopressin V1-receptor antagonist (AVPX) + NB, 4) angiotensin II AT1-receptor antagonist (AngIIX) + NB, 5) combined humoral blockade (HB), and 6) neurohumoral blockade.

Intravenous administration of 30% EOH (6.3 ml/kg) attenuated the baroreceptor reflex sensitivity, and enhanced the depressor action of AngIIX.

During hemorrhage, NB produced a faster fall in MAP than Normal both in the saline and EOH groups. However, HB accelerated the rate of fall in MAP only in the EOH group. The recovery from hemorrhagic hypotension was not different between NB and Normal rats, but was attenuated in HB rats in the saline group. Under NB, AngIIX, but not AVPX, retarded the recovery rate compared with NB alone. EOH attenuated the recovery of MAP after hemorrhage in Normal rats, but completely abolished the recovery in HB rats. We conclude that 1) the maintenance of MAP during hemorrhage is mediated almost entirely by the autonomic functions, 2) angiotensin II plays an important role in the recovery from hemorrhagic hypotension, but AVP assumes little importance, 3) AVP release largely depends on the changes in blood volume, whereas renin release depends on the changes in blood pressure rather than blood volume, and 4) EOH increases the dependence of cardiovascular regulation on angiotensin II and impairs the recovery from hemorrhagic hypotension through the attenuation of autonomic functions.

Key Words: Hemorrhage, Baroreceptor reflex, Vasopressin, Angiotensin, Ethanol

INTRODUCTION

Hemorrhage elicits several neurohumoral mechanisms to maintain blood pressure (Davis & Freeman, 1976; Cowley et al, 1980; Yamazaki & Sagawa, 1985; Kato et al, 1989; Courneya et al, 1991). In this situation, the baroreceptor reflex is the most powerful and rapidly acting autonomic mechanism in maintaining blood pressure (Fejes-Tóth et al, 1988; Kato et al, 1989; Korner et al, 1990; Courneya & Korner, 1991).

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The role of vasopressor hormones in the blood pressure regulation in response to hemorrhage has been studied by using hormonal blockades, but their role is still controversial. According to the review of Schadt and Ludbrook (1991), angiotensin II (AngII) and arginine vasopressin (AVP) have no effect on the maintenance of blood pressure during hemorrhage in conscious rats, but probably contribute to the recovery of blood pressure after hemorrhage. Other investigators also have suggested an important role for AngII in the blood pressure regulation during hemorrhage in anesthetized rats (Pang, 1983) and conscious rabbits (Schadt & Gaddis, 1990), and for AVP in anesthetized rats (Zerbe et al, 1982; Pang, 1983) and conscious dogs (Schwartz & Reid, 1981).

On the other hand, Matsukawa et al. (1991) suggested that the drugs used to block AngII may result in the inconsistent findings. The converting enzyme inhibitors have effects other than the blockade of the renin-angiotensin system and the AngII receptor antagonist, saralasin, has a partial agonistic action. In addition, anesthesia has profound effects on the neural and hormonal regulation of blood pressure (Crofton & Share, 1990). It is, therefore, necessary for the study of AngII activity to use a specific AngII-receptor antagonist without agonistic action and to carry out the study in conscious animals.

It has been documented that acute ethanol (EOH) administration depresses baroreceptor reflex function (Abdel-Rahman & Wooles, 1987; Russ et al, 1991; Sun & Reis, 1992), and aggravates the blood pressure fall in response to hemorrhage (Reves & Newman, 1972; Park et al, 1993). The underlying mechanism, however, is still unclear. Although hemorrhagic shock occurs frequently in trauma patients with acute alcoholism (Huth et al, 1983), few studies have been conducted on the effect of EOH on the cardiovascular and hormonal responses to hemorrhage. Furthermore, the experimentally repeated hemorrhages may correspond to a common clinical situation with intermittent hemorrhage, but in most studies the effects of a single hemorrhage have been evaluated.

Accordingly, we systematically evaluated the contribution of autonomic and hormonal mechanisms for the blood pressure regulation after repeated hemorrhages in conscious rats. The responses to the hemorrhage after blocking autonomic neural functions and the vasoconstrictor action of vasopressin and AngII with specific receptor antagonists were compared in saline and EOH-treated rats.

METHODS

Animals and operations

The experiments were performed in conscious male Wistar rats (285~500 g; average 365.5 g). Under anesthesia with thiopental sodium (50 mg/kg, ip), cannulae (PE50) filled with heparinized saline (50 units/ml) were inserted into the bilateral femoral veins for administration of drugs. Cannulae were also inserted into the abdominal aorta via both femoral arteries for the direct measurement of arterial pressure and blood drawing for hemorrhage. After the surgery, the rat was allowed about 3 hours to regain consciousness as it is known that the anesthetic attenuates the baroreceptor reflex (Stornetta et al, 1987) and can alter the compensatory responses to hemorrhage (Zimpfer et al, 1982). Arterial pressure was measured using a Statham P50 pressure transducer and the electronically damped mean arterial pressure (MAP) was continuously recorded on a polygraph (Grass, Model79E) throughout the experiment. Heart rate (HR) was obtained from the arterial pressure pulse.

Assessment of baroreceptor reflex sensitivity (BRS)

In order to evaluate the effect of EOH administration on autonomic mechanisms, we tested the baroreceptor reflex function using the widely used method (Abdel-Rahman & Wooles, 1987; Mitchelini et al, 1992; Park et al, 1993). The peak change in MAP and the reflex change in HR were used to calculate the ΔHR/ΔMAP ratio (expressed as beats/ min/mmHg) as a measure of BRS immediately after the pressor or depressor responses through bolus injections of 3 doses of norepinephrine (0.3, 0.5 and 1.0 μg/kg) or nitroprusside (0.5, 1.0 and 1.5 μg/kg), respectively. The BRS was assessed before and after the infusion of saline or EOH.

Experimental protocol

The rats were divided into two groups and were infused with saline or EOH. In EOH group, 6.3 ml/kg of 30% EOH (vol/vol) was infused into a femoral vein using a Harvard infusion pump (Model 22) at a rate of 0.1 ml/min, and then the rats were stabilized for 10 min. The saline group received the same volume of 0.9% NaCl instead of EOH. The animals in each group were studied under normal conditions and after pretreatment with drugs that eliminate the circulatory effects of arginine vasopressin (AVP), angiotensin II (AngII), or autonomic reflexes, in various combinations. We used six different combinations of neurohumoral blockade in each group as follows: 1) normal conditions (Normal), 2) neural blockade (NB), 3) NB and AVP blockade (NB+AVPX), 4) NB and AngII blockade (NB+AngIIX), 5) combined humoral blockade (HB:AngIIX+ AVPX), and 6) total neurohumoral blockade (NB+HB).

To prevent the vascular effects of AVP and AngII, we injected 10μg/kg of the AVP V1-receptor antagonist ([β-mercapto-b,b-cyclopentamethylene-propionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin, Sigma Chemical) and 10mg/kg of the specific AngII AT1-receptor antagonist Losartan (E.I. du Pont de Nemours and Co., Wilmington, Del). In the HB group, we gave both AVP and AngII antagonists. NB was produced with injection of 10 mg/kg of pentolinium tartrate (Sigma Chemical), a ganglionic blocker. Injection of pentolinium induced an abrupt decrease in MAP, and thus MAP was maintained close to the preinjection level by continuous infusion of norepinephrine at a constant rate throughout the experiment. In this way, autonomic reflex function was blocked without affecting the release of vasopressin and renin through the hypotensive stimulus. The effectiveness of NB was evaluated by eliciting the baroreceptor reflex, as described above. The reflex change of HR was completely abolished by NB.

We examined the blood pressure and hormonal responses to repeated hemorrhage under six conditions of neurohumoral effector function. In the first hemorrhage, arterial blood was withdrawn into a heparinized plastic syringe at a constant rate of 2ml/kg/min using a Harvard withdrawal pump until MAP was reduced by 30 mmHg. The duration of hemorrhage or the removed blood volume was used as an index of the ability to maintain MAP. Fifteen minutes after the first hemorrhage, a second hemorrhage was induced by the same procedure and the withdrawn blood samples were centrifuged at 2,500 rpm for 20 min at 4℃. For AVP assay, plasma was acidified with 1N HCl and stored at -20℃. To determine plasma renin concentration (PRC), 50 μl of plasma was stored in a tube containing 50 μg of EDTA.

In another series, we studied the effects of volume loading by saline or EOH infusion and of NB on the prehemorrhagic hormonal level in five different conditions (Table 3). In order to obtain the control blood samples (Control group) without

any treatment or surgical stress, five rats were decapitated and the trunk blood was collected in a plastic tube containing heparin solution (1,000 units/ml). In the other groups, an equal volume of rat donor blood, obtained by decapitation, was given simultaneously through the venous catheter during the arterial blood sampling period to minimize the hypovolemic effect on the hormonal levels. Plasma concentrations of AVP and renin were determined using the radioimmunoassay methods described by Lee et al. (1987) and Cho et al. (1989), respectively.

Statistical analysis

All data are presented as mean ± SE. The Mann-Whitney U test was used to make comparisons between groups; the Wilcoxon signed rank test was used for paired observation. Probability levels of less than 0.05 were considered statistically significant.

RESULTS

Effects of ethanol on BRS

EOH infusion significantly attenuated the BRS measured with nitroprusside or norepinephrine injection, but saline infusion had no effect on the BRS (Table 1).

Effects of neurohumoral blockade

AngIIX greatly decreased MAP in both groups. The EOH group showed a significantly greater decrease in MAP after AngIIX than the saline group (19.9 ± 1.2 vs. 10.3 ± 1.4 mmHg, $p<0.01$). However, AVPX exerted little depressor effect (2.8 ± 0.8 vs. 3.3 ± 1.0 mmHg).

After injection of the ganglionic blocker pentolinium, we infused norepinephrine to maintain MAP close to the pre-pentolinium level. The four saline-infused NB groups required similar infusion doses of norepinephrine (average 13ng/ kg/min). In the EOH-infused NB groups, however, significantly larger infusion doses of norepinephrine were required in the rats with AngIIX (NB+AngIIX and NB+HB; 38.9 ± 3.2 and 31.2 ± 3.6 ng/kg/min, respectively) than in NB and NB+AVPX rats (11.9 ± 3.2 and 14.9 ± 2.6 ng/kg/min, respectively).

The stabilized or adjusted baseline values of MAP and HR before hemorrhage are summarized in Table 2. All groups, except NB+AngIIX in the saline group and HB in both groups, were similar in the values of MAP and HR just preceding the hemorrhage procedure. Lower MAP and higher HR in HB rats was due to the depressor effect of

Table 1. Baroreceptor reflex sensitivity measured with nitroprusside or norepinephrine injected before (Pre) and after (Post) infusion of saline or ethanol in rats

Drug	Saline			Ethanol		
	N	Pre (beats/min/mmHg)	Post (beats/min/mmHg)	N	Pre (beats/min/mmHg)	Post (beats/min/mmHg)
Nitroprusside	8	−1.12±0.15	−1.14±0.17	10	−1.22±0.16	−0.78±0.04*
Norepinephrine	9	−1.58±0.11	−1.44±0.12	10	−1.18±0.11	−0.96±0.11**

Baroreceptor reflex sensitivity is expressed as the ΔHR/ ΔMAP ratio of the responses to nitroprusside or norepinephrine.
N indicates the number of rats.
All values are mean ± SE.
*$p<0.05$, **$p<0.01$ significantly different from Pre values.

hormonal blockade.

Cardiovascular responses to repeated hemorrhages

Bleeding volumes (bleeding time) to decrease MAP by 30 mmHg are shown in Figure1. During the first hemorrhage, the four NB groups showed a markedly faster fall in MAP than the Normal rats in both the saline and EOH groups. The saline-infused HB rats showed a similar response with Normal rats, but the EOH-infused HB rats showed a faster fall in MAP during hemorrhage than Normal rats. During the second hemorrhage, MAP decreased more rapidly compared with the first hemorrhage. Unlike the first hemorrhage, bleeding volume of the second hemorrhage was somewhat similar in all the saline and EOH groups except NB+AVPX and HB rats in the EOH group.

HR increased during hemorrhage only in Normal and HB rats, both with intact autonomic function

Table 2. Prehemorrhage baseline values of mean arterial pressure (MAP) and heart rate (HR) in saline- or ethanol-treated rats with six combinations of neurohumoral blockade

Group	Saline			Ethanol		
	N	MAP (mmHg)	HR (beats/min)	N	MAP (mmHg)	HR (beats/min)
Normal	5	130.2±2.2	422.0± 3.7	6	124.3±6.3	430.3± 9.4
NB	5	128.8±2.8	406.8± 9.2	6	123.5±6.8	422.3±15.0
NB+AVPX	5	126.0±2.5	396.0±15.7	5	124.6±3.3	407.2±10.9
NB+AngIIX	5	120.6±3.1*	405.6±10.3	6	118.7±6.5	447.3±15.4
HB	6	111.5±4.5*	471.3± 8.7**	7	109.9±6.1	462.9± 5.8*
NB+HB	5	123.8±6.2	420.0± 5.5	7	114.9±5.7	454.9±13.4

Abbreviation for each group is shown in the text.
N indicates the number of rats.
All values are mean ± SE.
*p<0.05, **p<0.01 significantly different from the corresponding Normal rats.

Table 3. Prehemorrhage baseline plasma concentrations of AVP (pAVP) and renin (PRC) in Wistar rats

Group	N	pAVP (pg/ml)	PRC (ng AngI/ml/hr)
Control	5	5.9±0.7	46.9±2.8
Saline Normal	5	6.9±1.0	42.4±1.8
NB	5	5.7±0.5	43.8±2.0
EOH Normal	5	7.9±1.1	41.8±2.2
NB	5	6.4±0.8	41.6±2.6

Abbreviation for each group is shown in the text.
Control means no treatment.
N indicates the number of rats.
All values are mean ± SE.

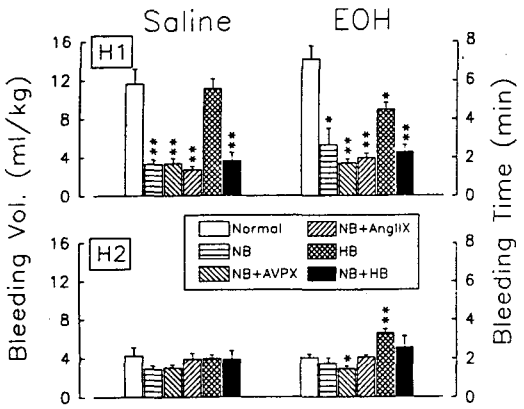

*Fig. 1. Effects of neurohumoral blockade on the bleeding volume or bleeding time to reduce mean arterial pressure by 30 mmHg during the first (H1) and the second (H2) hemorrhage in rats. Values are mean ± SE. *p<0.05, **p<0.01, significantly different from the corresponding Normal rats. Abbreviation for each group is shown in the text. Number of rats are shown in Table 2.*

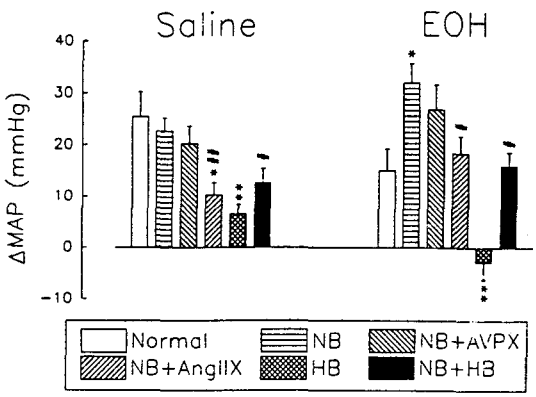

*Fig. 3. Effects of neurohumoral blockade on the recovery of mean arterial pressure (⊿MAP) from the first 15 min hemorrhagic hypotension in rats. Values are mean ± SE. *p<0.05, **p<0.01, significantly different from the corresponding Normal rats. #p<0.05, ##p<0.01, significantly different from NB rats within the group subjected to the neural blockade. Abbreviation for each group is shown in the text. Number of rats are shown in Table 2.*

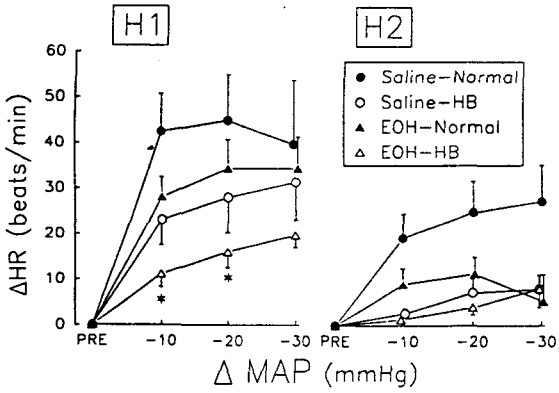

*Fig. 2. Changes in heart rate (⊿HR) in response to hemorrhagic hypotension during the first (H1) and the second (H2) hemorrhage in the rats with normal effector functions (Normal) and hormonal blockade (HB). Values are mean ± SE. *p<0.05, significantly different from the corresponding Normal rats. Number of rats are shown in Table 2.*

(Fig. 2). Tachycardic response to hemorrhagic hypotension was attenuated in the rats treated with EOH and/or HB. During the first hemorrhage, EOH-infused HB rats showed about one half of the tachycardic response of the EOH-infused Normal rats and about one fourth of that of saline-infused Normal rats. Tachycardic responses to the second hemorrhage were attenuated compared with the first hemorrhage.

Recovery of MAP after hemorrhage

Figure 3 shows the recovery of MAP at 15 min after hemorrhage. After 15 min, MAP of the salineinfused rats with intact AngII (Normal, NB, and NB+AVPX) had almost fully recovered. However, the three groups with the AngII antagonist (NB+ AngIIX, HB, and NB+HB) showed a significantly retarded recovery compared with the intact AngII groups.

In the EOH group, Normal rats showed only partial recovery of MAP compared with saline-infused Normal rats. Unlike the saline group, the MAP of HB rats had not recovered at all. The NB+AngIIX and NB+HB rats also showed a sig-

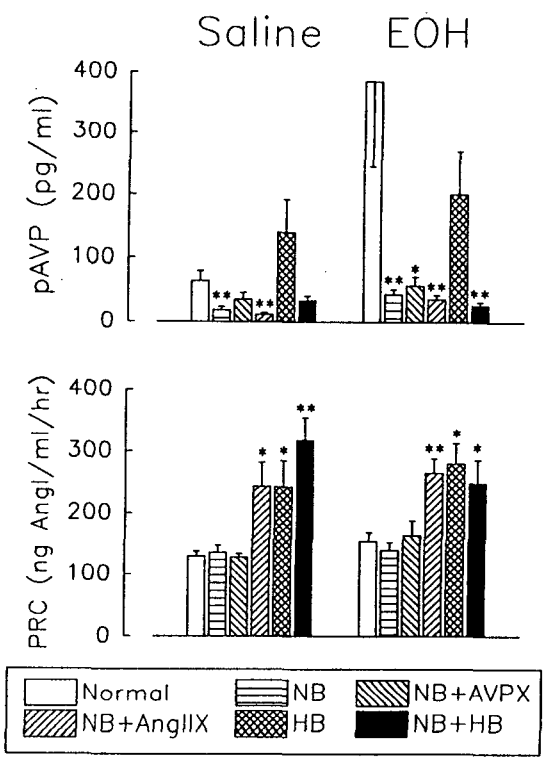

Fig. 4. Changes in the plasma concentrations of vasopressin (pAVP) and renin (PRC) after hemorrhage in rats. Values are mean ± SE. $^{*}p<0.05$, $^{**}p<0.01$, significantly different from the corresponding Normal rats. Abbreviation for each group is shown in the text. Number of rats are shown in Table 2.

nificantly retarded recovery of MAP compared with NB alone.

Hormonal responses to repeated hemorrhages

Table3 shows the basal plasma concentrations of AVP and renin in five different conditions. None of the employed procedures, including volume loading, EOH *per se*, or neural blockade, affected the basal plasma concentrations of AVP and renin.

The plasma concentrations of AVP and renin were elevated after hemorrhage (Fig. 4). Both the saline and EOH groups with intact autonomic function (Normal and HB) showed remarkably higher AVP levels than the four NB groups. The three groups pretreated with AngII antagonist (i.e., NB+AngIIX, HB, and NB+HB) showed significantly higher PRC levels compared with the three intact AngII groups. Renin responses to hemorrhage were similar both in the saline and EOH groups.

DISCUSSION

The present results showed that EOH administration attenuated the baroreceptor reflex in response to either nitroprusside or norepinephrine injection. The reduced tachycardic response to hemorrhagic hypotension in EOH-treated rats also shows a depressed baroreceptor reflex. These findings are in accordance with other previous reports (Abdel-Rahman & Wooles, 1987; Russ et al, 1991; Sun & Reis, 1992). The underlying mechanism is not entirely clear at present, but Sun and Reis (1992) have suggested that the depressed baroreceptor reflex function by EOH may be due, at least in part, to interference in medullary GABAergic and glutaminergic transmissions involved in the central control of the cardiovascular system.

AngII but not AVP seems to participate in the maintenance of basal MAP: AngIIX significantly decreased the basal MAP, whereas AVPX exerted little depressor effect. Johnson et al. (1988) and Katoh et al. (1989) also reported that the intravenous administration of V1 antagonist (10μg/kg) had no effect on MAP and HR in conscious rats. Oliver et al. (1990) reported that the threshold for hormonally mediated vasoconstriction corresponded to plasma levels of about 9~12 pg/ml for AVP and of about 7~10 ng AngI/ml/hr for PRC in rabbits. In spite of the species difference, the basal renin concentration (i.e., AngII production) in our experiment in rats was much higher than the vasoconstrictive values, whereas AVP levels were

below the threshold for vasoconstriction (Table 3).

EOH markedly enhanced the depressor action of AngIIX. Moreover, when the AngIIX was added to NB, the required infusion dose of norepinephrine to maintain basal MAP was remarkably increased in the EOH group. These results suggest that AngII becomes more important for maintaining basal MAP in EOH-treated rats. Although PRC was not changed after administration of EOH and/or neural blockade, there is a possibility that EOH may alter AngII- receptor binding or the actual AngII level in the vascular tissues. Whatever the mechanism is, however, EOH seems to increase the dependence on AngII for the maintenance of basal MAP in compensation for the attenuated baroreceptor reflex function.

The present study shows that the blood pressure regulation during hemorrhage was mediated almost entirely by autonomic functions (Fig. 1). During the first hemorrhage, HB did not impair MAP regulation, while NB produced a markedly faster fall in MAP despite continuous infusion of norepinephrine. These results are in good agreement with other reports in conscious rabbits (Quail et al, 1987; Korner et al, 1990; Oliver et al, 1990; Courneya et al, 1991) on the role of autonomic reflex functions in cardiovascular regulation during hemorrhage, despite the differences in the animal species and techniques employed. Collectively, the autonomic mechanisms help maintain MAP during hemorrhage by minimizing the reduction in cardiac output and counteracting the local vasodilator effects.

It is still a matter of debate whether the vasopressor hormones contribute to the blood pressure regulation during acute blood loss. Some investigators have suggested an important role for AngII in anesthetized rats (Pang, 1983) and conscious rabbits (Schadt & Gaddis, 1990), or for AVP in anesthetized rats (Zerbe et al, 1982; Pang, 1983) and conscious dogs (Schawartz & Reid, 1981; Liard, 1988) in the blood pressure regulation during hemorrhage. However, others have failed to reveal any contribution of AngII in anesthetized (Katoh et al, 1989) and conscious rabbits (Korner et al, 1990; Matsukawa et al, 1991) or AVP in conscious rats (Fejes-Tóth et al, 1988; Crofton & Share, 1990), anesthetized (Katoh et al, 1989) and conscious rabbits (Korner et al, 1990; Schadt & Hasser, 1991). This disparity in results may reflect species differences, the use of different preparations (anesthetized or conscious), or a different effect of Losartan (AngII receptor blocker) as compared with captopril (converting enzyme inhibitor) or saralasin (competitive AngII antagonist). Courneya and Korner (1991) found that the bleeding time of HB rabbits was similar to that of control rabbits. Also in our experiment, the bleeding volume of saline- infused HB rats was similar to that of Normal rats (Fig. 1). The present results suggest, therefore, that the vasopressor hormones are not vital in the maintenance of blood pressure during hemorrhage in conscious rats.

In the EOH group, the vasoactive hormones in addition to the autonomic reflex functions became more important for controlling MAP during hemorrhage (Fig. 1). During the first hemorrhage in the EOH group, HB produced a significantly faster decline in MAP compared with the saline group. The tachycardic responses during hemorrhage were reduced in HB rats and/or EOH groups (Fig. 2). The reason is not entirely clear at present, but the reduced tachycardic response in the EOH group is probably due to the attenuated baroreceptor reflex function by EOH. Brooks and Hatton (1991) observed that pretreatment with AVP antagonist attenuated the tachycardic response to nitroprusside-induced hypotension. This result suggests that AVPX may decrease sympathetic activity, but further studies are required to elucidate this question.

The second hemorrhage caused a more rapid decrease in MAP than the first hemorrhage in all

groups regardless of the neural and/or humoral blockade (Fig. 1). In addition, the tachycardic responses to the second hemorrhage were greatly reduced or almost absent compared with the first hemorrhage, and the trend was much more prominent in the EOH group (Fig. 2). These results may be due to a sympathetic inhibition after severe hemorrhage as suggested by Brizzee et al. (1991). They found that a mild hemorrhage increased HR and total peripheral resistance without hypotension, whereas a severe hemorrhage decreased them with hypotension. Thus, they proposed that a severe hemorrhage produces sympathetic inhibition rather than activation. In addition, an involvement of endogenous opiate peptides has been suggested in the control of vascular resistance during acute hemorrhagic hypotension (Burke & Dorward, 1988). Schadt et al. (1984) observed that the hemorrhage-induced hypotension could be reversed by opiate-receptor blockade in conscious rabbits. Certainly, further studies are required to prove this notion.

The vasoconstrictor action of AngII seems to be the major mechanism for recovery from hemorrhagic hypotension. MAP of Normal and NB rats were almost fully recovered to the basal level after 15 min, while MAP of HB rats was only slightly recovered (Fig. 3). AngIIX, but not AVPX, significantly attenuated the recovery of MAP. Many investigators also have found that posthemorrhagic hypotension is aggravated by captopril in conscious rats (Fejes-Tóth et al, 1988) and anesthetized rabbits (Katoh et al, 1989) or by saralasin in anesthetized rats (Pang, 1983) and conscious monkeys (Cornish et al, 1990). Collectively, these results, including the present study, suggest that the renin-angiotensin system is responsible for the partial recovery of MAP in autonomic nerve blocked animals.

However, it is still uncertain whether AVP contributes to the spontaneous recovery of MAP from hemorrhagic hypotension. Some investigators observed that AVP antagonist (or AVP deficient Brattleboro rats) attenuated the recovery in anesthetized rats (Zerbe et al, 1982; Pang, 1983) and dogs (Cowley et al, 1980), or in conscious rabbits (Korner et al, 1990; Schadt & Hasser, 1991). On the other hand, Johnson et al. (1988) and Crofton and Share (1990) suggested that AVP is particularly important for blood pressure compensation to hemorrhage in female rats. Therefore, it appears that the role of AVP in MAP recovery after hemorrhage may vary with the differences in sex (1990), use of anesthetics and experimental protocol (Brizzee et al, 1991).

There is another consideration required to evaluate the role of AVP in the spontaneous recovery. Share (1988) suggested that the evaluation of the relative roles of neurohumoral mechanisms by the sequential administration of drugs that block each of them is difficult to interpret because of the compensatory reactions of the other system. All regulatory systems act in a coordinated manner to maintain arterial pressure in the face of hemorrhage. In the present experiment, the MAP recovery rate was not different between Normal, NB and NB+AVPX rats, but it was significantly attenuated in NB+AngIIX rats and was further attenuated in HB rats. The similar recovery rates in NB+AVPX and NB rats are presumably because of the compensatory action of the reninangiotensin system. Further attenuation of the recovery in HB rats suggests a contribution of AVP to blood pressure compensation to hemorrhagic hypotension when AngII is blocked. Our data may suggest that the humoral mechanisms are more important than autonomic neural mechanism in the recovery from the hemorrhagic hypotension, and the effectiveness of AVP is less than AngII in conscious male rats. In the EOH group, unlike the saline group, Normal rats showed a slower recovery than NB rats. This finding had led to a hypothesis that the EOH-

induced attenuation of autonomic reflex may contribute to the retarded recovery in Normal rats. NB+AngIIX significantly attenuated the recovery compared to NB, and HB led to no recovery of MAP for 15 min. Therefore, the attenuated autonomic reflex can account for a retarded recovery in EOH-infused Normal rats. In addition, EOH-induced cardiac contractile dysfunction (Horton, 1986; Capasso et al, 1991) may be a partial cause.

Quail et al. (1987) reported that AVP steeply increased only after more than 25% blood volume had been removed, and this response was entirely mediated by cardiac receptors. However, increases in PRC occurred at a blood loss greater than 15% and were largely independent of the baroreceptor. According to the review of Davis and Freeman (1976) and the recent study of Katoh et al. (1989), the renin release is controlled by the 'renal barostat' (i.e., pressure changes in renal vasculature). In the present study, the increase in AVP release after hemorrhage was significantly greater in the Normal and HB rats than in the four NB rats. This result seems to be due to greater blood loss to reduce MAP by 30 mmHg in Normal and HB rats. The increase in PRC after hemorrhage was not different among the three groups with intact AngII, although the bleeding volumes were different. This seems to be because of the same reduction of MAP (probably renal arterial pressure) in all groups. The three groups with AngII antagonist showed higher PRC than the other groups with intact AngII. This finding agrees with a suggestion made by Rose et al. (1987) that blockade of converting enzyme activity causes an enhanced increment in plasma renin activity following hemorrhage because of removal of feedback inhibition. Taken together, we suggest that AVP release largely depends on the changes in blood volume, whereas renin release depends on the changes in blood pressure rather than blood volume.

In conclusion, the maintenance of MAP during moderate hemorrhage is mediated entirely by the autonomic mechanisms. During the recovery phase, AngII plays an important role, but AVP seems to be of little importance. Ethanol administration increases the dependency on AngII for the maintenance of blood pressure during hemorrhage and impairs the recovery of MAP from hemorrhagic hypotension probably through the attenuation of autonomic reflex functions.

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