

**HEMORRHAGIC SHOCK IN DOCA-SALT HYPERTENSIVE RATS:  
EFFECTS OF OXOTREMORINE ON BLOOD PRESSURE  
AND VASOPRESSIN RELEASE**

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**Summary:**

Stimulation of central cholinergic receptors increased mean arterial blood pressure. The pressor effect of cholinomimetics has also been observed in hypovolemic shock models in which they significantly reduce mortality. In the present study, the effects of cholinergic agonist oxotremorine (50 µg/kg, i.v.) on blood pressure, 60 minute survival rate and plasma vasopressin levels were compared in normotensive and deoxycorticosteron acetate (DOCA)-salt hypertensive rats subjected to hemorrhagic shock under urethane anesthesia. Oxotremorine elevated blood pressure and decreased 60 minute mortality rate in normotensive and hypertensive rats, both effects being significantly higher in the latter group. Basal plasma vasopressin levels were significantly higher in normotensive rats and rose up to 4 fold in response to bleeding to hemorrhagic shock in both groups. A further rise in plasma vasopressin was observed after saline treatment, whereas oxotremorine prevented this increase in both groups independent of basal blood pressure, although none of these changes could reach the level of statistical significance in hypertensive rats. The results of this study reveal that the antishock effects of oxotremorine are not primarily mediated via vasopressin release and are potentiated if there is an underlying DOCA-salt hypertension. The mechanisms of this potentiation need to be further investigated.

**Key words:** Oxotremorine; Hemorrhagic shock; Vasopressin; Deoxy-corticosterone acetate (DOCA)-salt hypertension; Cholinergic; Rat.

**Abbreviations:** ACTH, adrenocorticotrophic hormone; DOCA, deoxycorticosteron acetate; MAP, mean arterial pressure; NO, nitric oxide; NOS nitric oxide synthase.

### **Introduction**

It is well known that acetylcholine plays a role in central cardiovascular regulation and stimulation of central muscarinic receptors in rats induces a vasopressor response, primarily through an increase in sympathetic outflow to the vasculature (1). Administration of centrally active cholinomimetics peripherally, directly into the cerebral ventricles and into several specific sites such as the posterior hypothalamic nucleus, the ventrolateral medullary pressor area, hippocampus, locus ceruleus, central nucleus of amygdala and C1 area of the rostral ventrolateral medulla were reported to increase arterial blood pressure (2-9).

It has been shown that cholinomimetics improved hypotension and increased survival rate in rats subjected to severe hemorrhagic shock (10,11). The antishock effects of cholinomimetics were claimed to be mediated both by central muscarinic (11) and nicotinic receptors (10-12). Interestingly, adrenocorticotrophic hormone (ACTH)-induced reversal of hemorrhagic shock was also reported to be mediated by central muscarinic receptors (13,14). On the other hand, vasopressin is known to be released in response to hypovolemia and assists to restore blood pressure by inducing water and salt retention in hypovolemic states (15). Carbachol was reported to increase circulating levels of vasopressin (16,17) and its release from the posterior pituitary gland has been shown to be controlled via nicotinic receptors (18).

Although muscarinic receptor antagonists prevent the pressor effect of oxotremorine in hemorrhagic shock, the increase in survival rate was found to be independent from the restoration of hypotension (11). The latter effect was postulated to be due to an increase in circulating blood volume in response to oxotremorine-induced vasopressin release via nicotinic receptors.

Therefore, the present study was designed to investigate whether oxotremorine-induced effects on blood pressure, vasopressin release and short-term survival in rats subjected to hemorrhagic shock would be altered by the underlying DOCA-salt hypertension in which salt and water retention is the major pathogenesis.

### **Methods**

#### *Animals*

Experiments were performed in inbred albino Sprague Dawley rats of both sexes (Marmara University School of Medicine, Experimental Research Laboratory and Animal Unit). All animals were fed with a standard rat chow and water ad libitum, and kept at room temperature ( $22 \pm 2^{\circ}\text{C}$ ) in an air-conditioned room with a 12-h light/dark cycle.

#### *DOCA-salt hypertension*

Deoxycorticosteron acetate (DOCA)-salt hypertension was induced in 6 week-old rats weighing 140-160 g. A left flank incision was used to remove the left kidney, with great care to avoid adrenalectomy under ketamine ( $50 \text{ mg kg}^{-1}$ , i.p.) and chlorpromazine ( $0.75 \text{ mg kg}^{-1}$ , ip) anesthesia. Rats were given standard rat chow and 1% NaCl solution as a drinking water ad libitum. Two days after the surgery, subcutaneous (s.c.) DOCA treatment was started twice a week at a dose of  $40 \text{ mg kg}^{-1}$ . Sham-operation was performed in rats received standard rat chow and tap water ad libitum, and treated by sesame oil (vehicle of DOCA;  $2.5 \text{ ml kg}^{-1}$ , s.c.) twice a week (normotensive sham-operated group). DOCA or vehicle treatment was continued for 4 weeks as described by Ormsbee & Ryan (19).

All rats were weighed daily and changes in body weight were recorded as an indicator of growth and/or water retention.

*Experimental protocol*

Experiments were performed under urethane ( $1.2 \text{ g kg}^{-1}$ , i.p.) anesthesia. Normal body temperature was maintained by continuous monitoring via a rectal thermometer and a heating pad during the experiments. All procedures were approved by the Institutional Animal Care and Use Committee.

Both iliac arteries were catheterized with a PE-10 attached to a PE-50 polyethylene tubing in order to monitor blood pressure and bleed to hemorrhagic shock. The left iliac vein was also catheterized for intravenous administration of drug solutions. All catheters were filled with 1% heparin-saline solution.

Right iliac arterial cannula was connected to a pressure transducer (Grass Model P23ID, U.S.A.) for direct measurement of blood pressure. Arterial blood pressure and heart rate were continuously recorded on a polygraph (Grass, Model 7, USA). Following the 2 h-stabilization period, basal mean arterial pressure and heart rate values were recorded. Then, rats were bled through left iliac artery in 20 min in 3 successive steps until mean arterial pressure (MAP) fell to and stabilize at approximately 25 mm Hg. A total of  $2.2 \pm 0.1 \text{ ml blood } 100 \text{ g}^{-1}$  was withdrawn that is approximately equivalent of 50% of total blood volume in rats (20).

There were 6 experimental groups: Group 1, normotensive sham-operated saline-treated; Group 2, normotensive sham-operated oxotremorine-treated; Group 3, normotensive saline-treated; Group 4, normotensive oxotremorine-treated; Group 5, hypertensive saline-

treated; and Group 6, hypertensive oxotremorine-treated rats. Since sham-operation did not significantly alter blood pressure and plasma vasopressin levels, the data obtained from Group 1 and 3, and Group 2 and 4 were pooled and presented as the results of the “normotensive saline” and “normotensive oxotremorine” groups.

After the stabilization of MAP at around 25 mm Hg, methylatropine bromide (2 mg/kg; i.v.) was injected 5 min prior to oxotremorine (50  $\mu\text{g kg}^{-1}$ ; i.v.) treatment to prevent its peripheral muscarinic effects. Instead, physiological saline (0.1 ml 100  $\text{g}^{-1}$ ; i.v.) was given 5 min prior to physiological saline (0.1 ml 100<sup>-1</sup> g, i.v.) injection in saline-treated groups. Blood pressure was monitored for 60 min after the treatment and rats survived for more than 1 hour were sacrificed by bleeding. The oxotremorine dose used in this study was reported to be effective in rat hemorrhagic shock model previously (11).

The first and the last blood samples obtained during bleeding respectively. The first (just before bleeding started) and the last (approximately 20 minutes after bleeding started) blood samples obtained during bleeding were kept on ice until processed for the measurements of basal and shock-induced plasma vasopressin levels, respectively. An additional 1.5 ml blood sample was taken to measure after-treatment vasopressin levels. In order to prevent further blood volume reduction, 1.5 ml of blood was withdrawn in a heparinized injector before the treatments, kept at room temperature and re-injected after the treatment.

#### *Vasopressin assay*

Arterial blood samples were centrifuged (2000 rpm, 10 min) and plasma aliquots were kept frozen at  $-20^{\circ}\text{C}$  until the assay. Vasopressin levels were measured by radioimmunoassay.

Briefly, plasma samples were thawed at room temperature for about 20 minutes. Five ml ice-cold ethanol was added to each 0.5 ml-plasma sample, vortexed (Kubota, Japan) for 1 minute and centrifuged at 4°C at 1500 G for 10 minutes. Supernatants were dried in a vacuum concentrator (Juan NT, Saint-Herblain, France). The dried residues of the extracts were resuspended with 1 ml of assay buffer, and 400 µl aliquots were assayed in duplicate using a radioimmunoassay kit (Buhlmann Laboratories, Basel, Switzerland). The minimum detection level (analytical least detectable level-ALC) of the assay was 1.25 pg ml<sup>-1</sup> (1.15 pmol L<sup>-1</sup>), and the specificity was 100% for arginin vasopressin.

#### *Drugs*

Deoxycorticosteron acetate, methylatropine bromide, oxotremorine and urethane were purchased from Sigma (U.S.A.), ketamin (Katarlar), chlorpromazine (Largactil) and heparin sodium (Liquemine) were given as a gift by Eczacıbaşı Parke Davis, Eczacıbaşı Rhône-Poulenc and Roche (Turkey), respectively. All drugs were dissolved and/or diluted in saline (0.9% NaCl) except DOCA, which was dissolved in sesame oil.

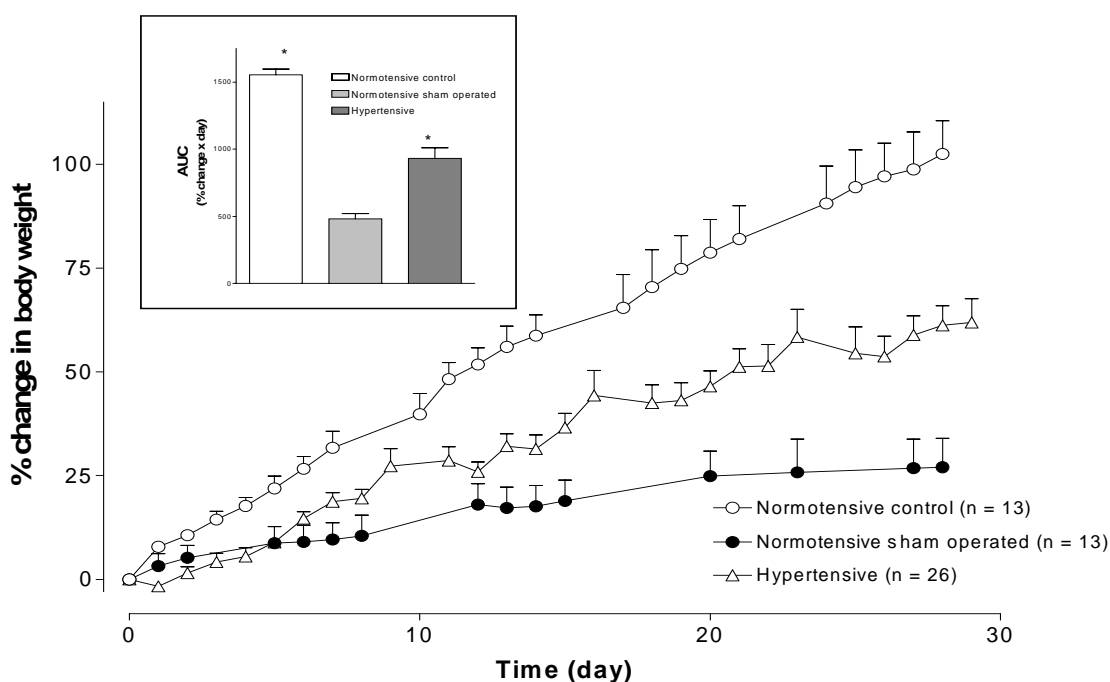
#### *Data analysis*

MAP was calculated as "1/3 pulse pressure + diastolic blood pressure" and the results were expressed as "mean ± S.E.M.". Analysis of variance (ANOVA) for repeated measures and *post-hoc* Dunnett's test were used to analyze the effects of treatments (oxotremorine and saline) on mean arterial blood pressure and weight gain. Plasma vasopressin levels in saline and oxotremorine-treated rats were expressed as pg ml<sup>-1</sup> and compared by Wilcoxon signed rank test or Mann Whitney U-test. The 60 min-survival rate data were analyzed by chi square (X<sup>2</sup>) test. The level of statistical significance was accepted as P<0.05.

### Results

#### Weight gain (Fig. 1)

Basal body weight values were not significantly different from each other between groups. Average body weight of intact control animals increased to  $102.5 \pm 2.5\%$  of the basal value at 4 weeks after the surgery. Interestingly, sham-operation seemed to interfere with weight gain since the increase in body weight of sham-operated normotensive rats was only  $27.8 \pm 2.1\%$  ( $P < 0.01$ ). Although body weight increase in hypertensive rats ( $61.9 \pm 5.7\%$ ) was significantly lower than intact control rats ( $P < 0.01$ ), it was significantly higher than sham-operated normotensive rats ( $P < 0.01$ ), indicating water and salt retention due to DOCA-saline treatment.

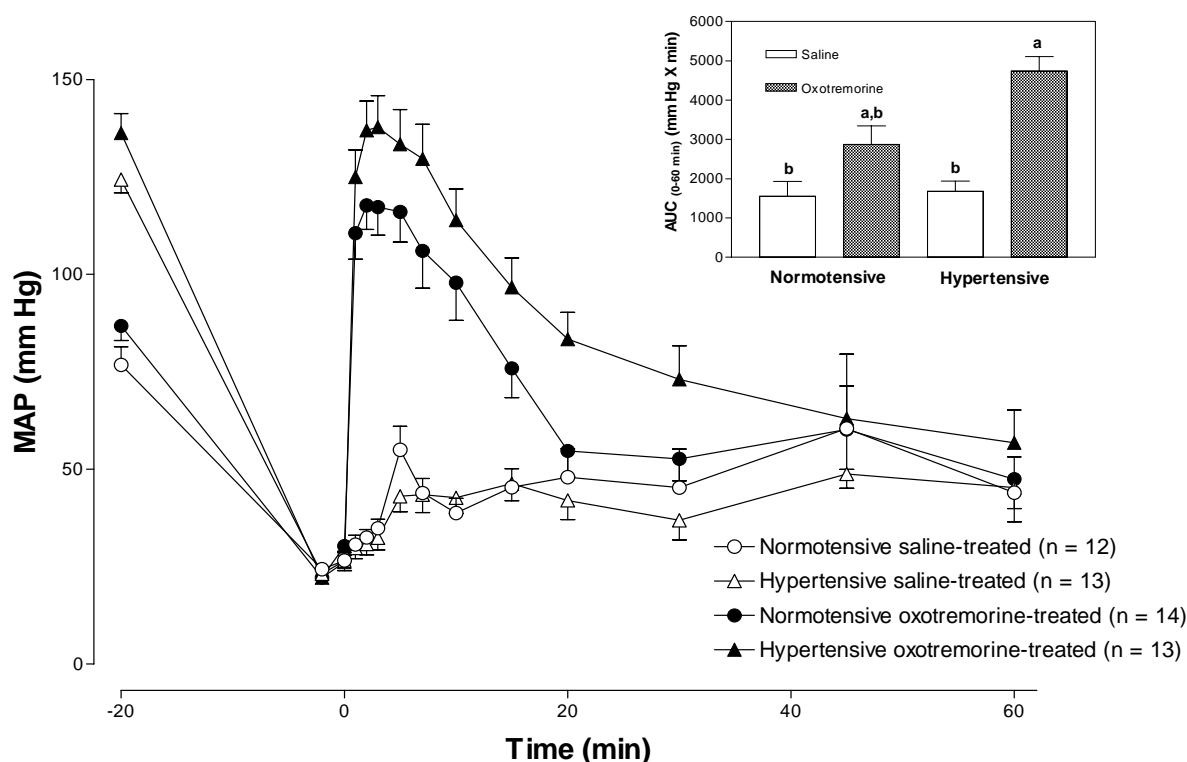


**Figure 1.** Change in body weight of rats during 30 days of saline and DOCA injection. Data are expressed as mean  $\pm$  S.E.M. Areas under the weight gain/time curves (AUC) are shown at the inset figure. \*  $P < 0.05$  vs normotensive sham-operated rats.



Mean arterial pressure (Fig 2)

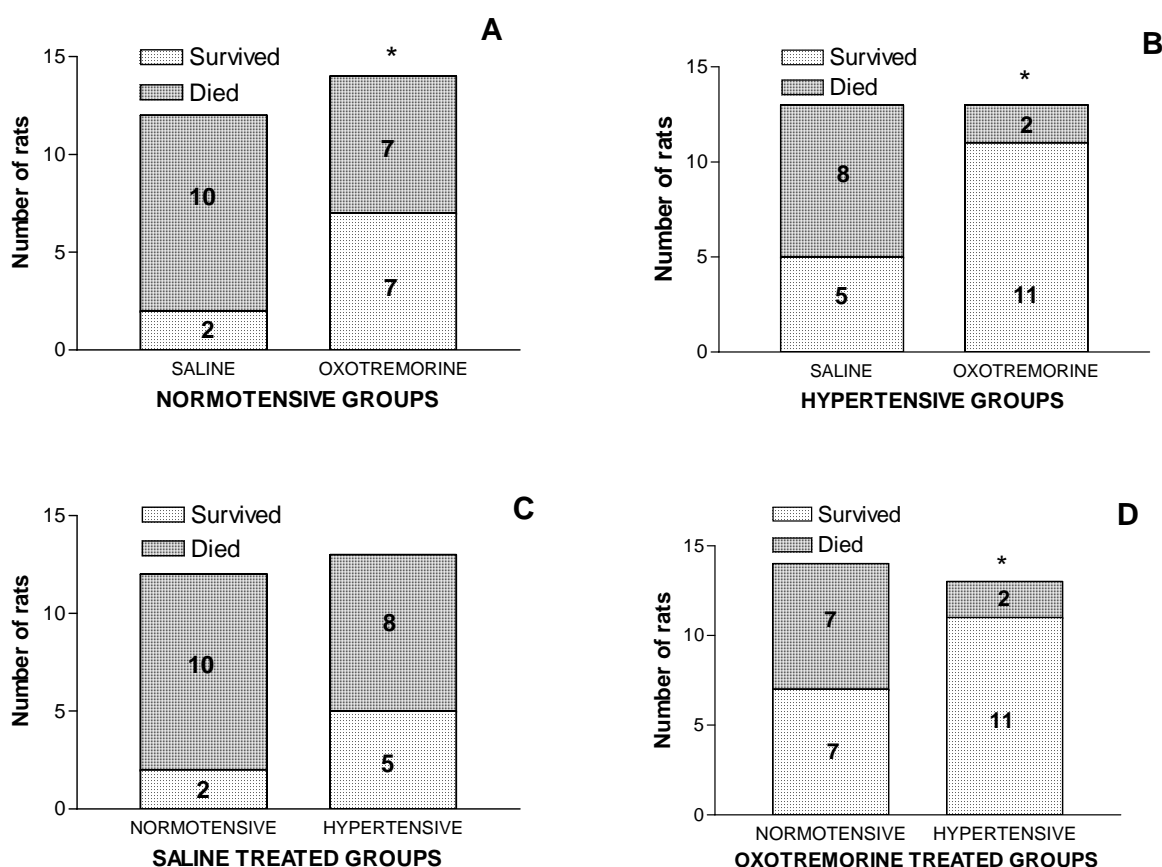
Basal MAP values were significantly different in normotensive ( $76.8 \pm 4.5$  mm Hg) and hypertensive ( $124.3 \pm 3.3$  mm Hg) rats ( $P < 0.01$ ). Despite this difference, similar amounts of blood were withdrawn to reduce MAP down to  $26.6 \pm 2$  mm Hg in normotensives ( $2.1 \pm 0.1$  ml/100 g) and to  $26.5 \pm 1.0$  mm Hg in hypertensives ( $2.4 \pm 0.1$  ml/100 g) ( $P > 0.05$ ). Oxotremorine elevated MAP to  $117.7 \pm 6.1$  mm Hg in normotensive and to  $137 \pm 7.4$  mm Hg in hypertensive rats, which was more exaggerated and sustained in the latter group. The comparison of the area under the MAP-time curves revealed a significant difference between oxotremorine-treated normotensive and oxotremorine-treated hypertensive rats ( $P < 0.01$ ).



**Figure 2.** Effects of oxotremorine (50  $\mu$ g/kg, i.v.) on mean arterial pressure (MAP) in normotensive and DOCA-salt hypertensive rats subjected to hemorrhagic shock. Data are expressed as mean  $\pm$  S.E.M. Areas under the MAP/time curves (AUC) are shown at the inset figure. <sup>a</sup>:  $P < 0.05$  vs corresponding saline-treated rats; <sup>b</sup>:  $P < 0.01$  vs oxotremorine-treated hypertensive rats.

60 min-survival rates (Fig 3)

Oxotremorine decreased mortality significantly in both normotensive and hypertensive groups. Ten of 12 normotensive rats died within 60 min after saline injection, whereas 50% (7/14) survived after oxotremorine ( $P<0.05$ ). The 60 min-survival rate was 5/13 and 11/13 in hypertensive rats treated with saline and oxotremorine, respectively ( $P<0.05$ ). Interestingly, the decrease in mortality due to oxotremorine treatment was higher in the hypertensive group and the comparison of 60 min-survival rate in oxotremorine-treated normotensive (7/14) and hypertensive rats (11/13) revealed statistically significant difference ( $P<0.05$ ).



**Figure 3.** Effects of oxotremorine (50 µg/kg, i.v.) on 60 minute survival rates in normotensive and DOCA-salt hypertensive rats subjected to hemorrhagic shock. \*  $P<0.01$  vs corresponding saline-treated (A and B panels) or normotensive (D panel) rats.

*Plasma vasopressin levels (Table 1)*

Basal plasma vasopressin levels were significantly higher in normotensive rats (P<0.05). Vasopressin concentrations raised up to 4 fold in response to bleeding to hemorrhagic shock (P<0.001) in both groups and the statistically significance between hypertensives and normotensives was maintained (p<0.01). A further rise in plasma vasopressin was observed after saline treatment in both groups. This increase was statistically significant in normotensives (P<0.05), but could not reach the level of significance in hypertensives. Oxotremorine prevented this increase in both groups independent of basal blood pressure. After-treatment vasopressin concentrations were significantly lower in normotensive oxotremorine group when compared with the corresponding saline group (P<0.01). A similar, but statistically insignificant change was also seen in hypertensive rats.

**Table 1. Plasma vasopressin levels in rats subjected to hemorrhagic shock. Values are expressed as mean ± S.E.M.**

Group	n	Treatment	Vasopressin levels (pg/ml)		
			Basal	Hemorrhagic Shock	After treatment
Normotensive	12	Saline	60.9 ± 9.0	250.2 ± 31.5 <sup>c</sup>	376.1 ± 42.7 <sup>c,d</sup>
Normotensive	14	Oxotremorine			194.2 ± 37.4 <sup>c,e</sup>
Hypertensive	13	Saline	31.6 ± 5.1 <sup>a</sup>	140.1 ± 18.1 <sup>b,c</sup>	185.4 ± 31.6 <sup>c</sup>
Hypertensive	13	Oxotremorine			157.1 ± 23.6 <sup>c</sup>

<sup>a</sup> Compared with basal levels in normotensives (P<0.05); <sup>b</sup> Compared with hemorrhagic shock levels in normotensives (P<0.01); <sup>c</sup> Compared with basal levels in the corresponding group (P< 0.001); <sup>d</sup> Compared with hemorrhagic shock levels in the corresponding group (P < 0.05); <sup>e</sup> Compared with after-treatment levels in the corresponding saline group (P < 0.01-0.05)

### **Discussion**

Hemorrhagic shock may be induced by acute bleeding to very low blood pressure values in rats (10-12). In the present study, normotensive and DOCA-salt hypertensive rats were subjected to hemorrhagic shock. Despite the significant difference in basal blood pressure values, similar amounts of blood were withdrawn to reduce MAP down to approximately 25 mm Hg. The mortality rate of hemorrhagic shock was also not significantly different in normo- and hypertensive rat groups. Four-fold increase in plasma vasopressin levels was observed in both normotensive and hypertensive rats in response to bleeding, although basal levels were significantly lower in the latter group. In fact, basal plasma vasopressin levels were higher in both groups than reported for conscious animals, since general anesthetics have significant effects on vasopressin release (21). These observations indicate that rats respond to abrupt bleeding similarly independent of basal circulating blood volume and blood pressure values under general anesthesia.

Centrally acting cholinomimetic drugs were demonstrated to induce a prompt and sustained reversal of an otherwise rapidly fatal hemorrhagic shock in rats (10,11), as shown in this study. Intracerebroventricular administration of the muscarinic receptor antagonist atropine prevented the pressor effect of oxotremorine in hemorrhagic shock but not the increase in survival rate (11). These findings indicate that the hypertensive effect of cholinomimetics is not primarily responsible in their antishock effects, since all animals treated by oxotremorine survived though atropine pretreatment prevented the pressor action. On the contrary, ACTH was reported to improve survival in rats with severe hemorrhagic shock via muscarinic receptor stimulation (14,22,23).

Interestingly, inhibition of NOS was reported to potentiate the cardiovascular and respiratory effects of ACTH and the pressor effect of oxotremorine in rat hemorrhagic shock model indicating that the NO overproduction contributes to hypotension (24-26). However, NOS inhibition has failed to potentiate the effect of oxotremorine on the mortality rate (26) supporting the idea that the antishock effect of oxotremorine is not related to the restoration of hypotension.

Hemorrhage significantly increased plasma vasopressin levels in both normotensive and hypertensive rats, as expected, since it is well known that hypotension is one of the major stimuli for vasopressin release (15). It was suggested that central injection of acetylcholine increased blood pressure of dogs through both an increase in sympathetic outflow and vasopressin release (27). However, there are contradictory data about the cholinergic link in the reflex release of vasopressin by hypotension (17,28). Cholinomimetic-induced increase in vasopressin release was found to be mediated via muscarinic receptors in the paraventricular nucleus and nicotinic receptors in the supraoptic nucleus (29). Supporting the nicotinic mechanism, i.v. injection of nicotine was shown to reverse hemorrhagic shock and this reversal was prevented by bilateral vagotomy (30).

Although oxotremorine is known as a muscarinic receptor agonist, since mecamylamine inhibited its antishock effect and the pressor effect does not seem to be primarily responsible in this regard, oxotremorine-induced vasopressin release via nicotinic receptors was postulated to prevent death of rats due to hemorrhagic shock, by increasing the volume of circulating blood (10,11). However, the present study demonstrated that oxotremorine returned blood pressure up to prebleeding levels and increased survival rate not

only in normotensive but also DOCA-salt hypertensive rats subjected to severe hemorrhagic shock, although circulating blood volume should be higher in hypertensive animals as indicated by a faster body weight gain compared with sham-operated normotensive rats. A similar mortality rate due to hemorrhagic shock in both normo- and hypertensive rats despite significantly lower basal vasopressin levels in the latter group is another finding against an important protective role of vasopressin in this model.

Bleeding stimulated vasopressin release, but oxotremorine stopped further increase in both normo- and hypertensive rats. It seems likely that, oxotremorine abolishes the compensatory increase in plasma vasopressin levels by restoring hypotension, since hypotension and hypovolemia are the major stimuli for vasopressin release (15). Unexpectedly, both effects of oxotremorine on MAP and mortality were significantly more prominent in hypertensive rats. Excessive intravascular and interstitial volumes were reported in DOCA-salt hypertension model (19). The compensatory redistribution of the interstitial fluid into the intravascular compartment in response to hypotension (31) and/or probably higher basal sympathetic tone due to DOCA-salt hypertension (32) might explain the exaggerated responses to oxotremorine in DOCA-salt hypertension group.

Collectively, the results of this study revealed that oxotremorine raised blood pressure to prebleeding levels and increased 60 minute survival rate, in normotensive rats subjected to severe hemorrhagic shock, although it abolished the need for a continuing compensatory increase in vasopressin release. Increased body water, high basal blood pressure and low basal vasopressin level due to DOCA-salt treatment made the animals neither resistant nor prone to death from severe hemorrhage, but the antishock effects of oxotremorine were potentiated by preexisting DOCA-salt hypertension despite lower vasopressin levels,

indicating that vasopressin did not play a major role in these effects. However, the fact that severe hypotension might reduce renal blood flow to a point at which any action of released vasopressin would be compromised, might be kept in mind.

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