British Journal of Pharmacology (1995) 116, 1911-1917

Restoration of blood pressure by choline treatment in rats made hypotensive by haemorrhage

[1]Ismail. H. Ulus, B. Yener Arslan, Vahide Savci & Burhan K. Kiran

Uludag University Medical Faculty, Department of Pharmacology, Bursa, Turkey

1 Intracerebroventricular (i.c.v.) injection of choline (25-150 μg) increased blood pressure in rats made acutely hypotensive by haemorrhage. Intraperitoneal administration of choline (60 mg kg^{-1}) also increased blood pressure, but to a lesser extent. Following i.c.v. injection of 25 μg or 50 μg of choline, heart rate did not change, while 100 μg or 150 μg i.c.v. choline produced a slight and short lasting bradycardia. Choline (150 μg) failed to alter the circulating residual volume of blood in haemorrhaged rats.

2 The pressor response to i.c.v. choline (50 μg) in haemorrhaged rats was abolished by pretreatment with mecamylamine (50 μg, i.c.v.) but not atropine (10 μg, i.c.v.). The pressor response to choline was blocked by pretreatment with hemicholinium-3 (20 μg, i.c.v.).

3 The pressor response to i.c.v. choline (150 μg) was associated with a several fold increase in plasma levels of vasopressin and adrenaline but not of noradrenaline and plasma renin.

4 The pressor response to i.c.v. choline (150 μg) was not altered by bilateral adrenalectomy, but was attenuated by systemic administration of either phentolamine (10 mg kg^{-1}) or the vasopressin antagonist [β-mercapto-β,β-cyclopenta-methylenepropionyl[1], O-Me-Tyr^2,Arg^8]-vasopressin (10 μg kg^{-1}).

5 It is concluded that the precursor of acetylcholine, choline, can increase and restore blood pressure in acutely haemorrhaged rats by increasing central cholinergic neurotransmission. Nicotinic receptor activation and an increase in plasma vasopressin and adrenaline level appear to be involved in this effect of choline.

Keywords: Choline; acetylcholine; blood pressure; haemorrhage; vasopressin; nicotinic receptors

Introduction

Considerable evidence has accumulated over the years to implicate brain acetylcholine in the control of blood pressure (Philippu, 1981; 1988; Brezenoff & Giuliano, 1982; Brezenoff, 1984). Pharmacological activation of brain cholinergic neurotransmission results in an increase in blood pressure in several vertebrate species, including rat, dog and man (Brezenoff & Guiliano, 1982). More recently, it has been reported that the centrally acting drugs, oxotremorine and physostigmine, can restore blood pressure and increased survival rate of rats in an experimental haemorrhagic shock model (Guarini *et al.*, 1989). Based on these observations it has been suggested that an increase in central cholinergic tone is involved in the development and maintenance of hypertension (Brezenoff, 1984), while a decrease in central cholinergic tone is involved in the complex pathophysiology of cardiovascular shock (Guarini *et al.*, 1989).

The rates at which neurones synthesize and release acetylcholine can be affected by the level of its precursor, choline (Wurtman *et al.*, 1980). The supply of choline to cholinergic neurones originates from the circulation, breakdown of released acetylcholine, and hydrolysis of choline-containing membrane phospholipids (Wurtman, 1992). Under appropriate circumstances, treatments that raise circulating and tissue choline levels cause a parallel increase in acetylcholine synthesis (Cohen & Wurtman, 1975) and release (Maire & Wurtman, 1985; Ulus *et al.*, 1989; Koshimura *et al.*, 1990; Johnson *et al.*, 1992) and enhance cholinergic transmission, causing functional changes in neurones and cells postsynaptic to those with elevated acetylcholine levels (Ulus & Wurtman, 1976; Ulus *et al.*, 1977; 1978). The dependency of cholinergic neurones on choline becomes more evident when the firing rate of neurones increases (Maire & Wurtman, 1985; Ulus *et al.*, 1989). In such conditions administration of choline can greatly enhance cholinergic transmission (Ulus *et al.*, 1978; Wecker & Schmidt, 1979). Thus, a continuous and sufficient supply of choline for acetylcholine synthesis is a crucial element in the maitenance of cholinergic neurotransmission (Wurtman, 1992).

Brain blood flow and metabolism are greatly altered during haemorrhagic hypotension and shock (Skarphedinsson *et al.*, 1986). It is likely that free choline supply to brain cholinergic neurones from the circulation also is diminished during haemorrhagic hypotension and shock, and a suggested decrease (Guarini *et al.*, 1989) in brain cholinergic tone during haemorrhagic shock may result from the decrease in free choline supply. If so, providing extra free choline to central cholinergic neurones might restore central cholinergic neurotransmission and help to restore blood pressure.

The present study was designed to test this hypothesis in rats made hypotensive by acute haemorrhage. The main purpose was to determine (1) whether administration of free choline alters blood pressure in rats subjected to acute haemorrhage, (2) if so which cholinoreceptors mediate the action of choline, and (3) which peripheral mechanisms are involved in choline-induced restoration of blood pressure.

Methods

Animals

Male Wistar rats (Experimental Animals Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) weighing 220-300 g were used in all experiments. Four to 6 rats were housed in hanging cages with free access to food and water. The colony room was maintained at 20-24°C with a 12 h light-dark cycle (light on 08 h 00 min-20 h 00 min).

The surgical and experimental protocols were approved by the Animals Care and Use Committee of Uludag University.

[1] Author for correspondence.

Surgical procedures

In animals anaesthetized with ether, the vagus nerve and the cervical sympathetic trunk were separated from the left carotid artery and the vessel was cannulated with PE 50 tubing filled with heparinized saline (400 u ml^{-1}). The catheter was exteroirized at the nape of the neck and sealed until use. For central injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to the mid-line and 1.0 mm posterior to bregma and a 10 mm length of 20 gauge stainless stell hypodermic tubing was directed through the hold toward the lateral ventricle. The cannula was lowered 4.5–5.0 mm below the surface of the skull and was fixed to the skull with acrylic cement. Lumbar bilateral adrenalectomy or a sham operation were performed in 6 or 7 rats respectively. Following the completion of these surgical and cannulation procedures the rat was placed in an individual plastic cage and allowed to recover from anaesthesia. Observations commenced about 2 h after the rats had regained consciousness. During this observation period rats were left undisturbed and did not show any sign of pain.

Haemorrhage and blood pressure recording

Following the recovery period the arterial line was connected to a pressure transducer (Statham P23) and blood pressure was recorded on a polygraph (Grass model 7D, Boston, MA, U.S.A.) continuously for 10–20 min. The heart rate was counted from the phasic pressure tracing recorded on the polygraph. After obtaining a stable baseline blood and heart rate, the arterial line was disconnected from the transducer and rats were bled, allowing a loss of 2.1–2.2 ml of blood per 100 g of body weight within 5–10 min. The arterial catheter was then flushed with 0.3 ml of heparinized saline and reconnected to the transducer. The blood pressure was then monitored for a stabilization period of 15–20 min to determine the post-haemorrhage levels of blood pressure and heart rate. After this stabilization period, rats received injections through the i.c.v. cannula. The blood pressure and heart rate were then monitored for a period of 5 to 90 min.

In one set of experiments rats were subjected to graded haemorrhage. A blood sample (0.55 ml per 100 g of body weight) was withdrawn over 10 s into a syringe. The arterial catheter was then flushed with 0.3 ml of heparinized saline and connected to the pressure transducer to monitor the blood pressure for the following 5 min. This procedure was repeated 4 times at 5 min intervals.

I.c.v. injection of drugs

For i.c.v. injection, the injection cannula (25 gauge, 11.5 mm stainless steel tubing) was inserted through the cannula guide. The injection cannula was connected by polyethylene tubing, which was filled with saline or saline containing the desired dose of the drug of interest in a 10–50 μl microsyringe. Drugs were then infused slowly within 6–8 s. In one set of experiments, rats received two i.c.v. injections at a 15 min interval.

Blood volume and haematological parameters

The volume of total circulating blood was measured according to the radioisotope dilution principle (Gibson *et al.*, 1947; Bradley & Bar, 1968) using radioiodinated albumin ([^{125}I]-albumin; Diagnostic Products Company, Los Angeles, CA, U.S.A.). Briefly, 0.3 ml of [^{125}I]-albumin solution (3×10^4 c.p.m. per rat) was injected through the arterial catheter and then the catheter was flushed with 0.7 ml of saline. Two minutes after the injcetion, a 1 ml blood sample was obtained for counting radioactivity in a gamma scintillation spectrometer (DPC Gambyt CR, Diagnastic Products Company).

Blood cells counts, haemoglobin and haematocrit were determined in a 1 ml blood sample by an automated haematology analyser (Medonic CA 610, Stockholm, Sweden).

Plasma renin, vasopressin and catecholamines

Blood samples were kept on ice and plasma was obtained by centrifugation (1800 r.p.m. for 20 min) at 4°C. Plasma renin activity was assayed by measurement of angiotensin I production at 37°C for 60 min. Angiotensin I was measured by radioimmunoassay using a commercially available kit (Clinical Assay Inc, Cambridge, MA, U.S.A.). Plasma renin levels were measured on the same day that the blood samples were obtained. Plasma renin activity is expressed as nanogram of angiotensin I produced by renin per ml of plasma during 60 min of incubation.

Plasma noradrenaline and adrenaline were determined by radioenzymatic assaay using a commercially available kit (CAT-A-KIT, Amersham International, Buckinghamshire, England). Briefly, an aliquot (50 μl) of fresh plasma was incubated with catechol-*O*-methyltransferase and tritiated S-adenosyl methionine. The reaction was stopped by addition of borate buffer (pH = 8.0) containing authentic metanephrine and normetanephrine. The amines were extracted into toluene-isoamyl-alcohol and then into 0.1 M acetic acid. The radioactive products were separated by thin layer chromatography, and the appropriate areas were scraped separately into counting vials. After periodate oxidation to vanillin, phosphor-containing toluene was added, and tritium was assayed by liquid scintillation spectrometry.

Aliquots of plasma were also frozen at −20°C for about 10 days, when they were thawed for vasopressin extraction and radioimmunoassay. Vasopressin was extracted from 0.5 ml of plasma with Sep-Pak C18 cartridges. Extracts were dried in a vacuum concentrator (Jouan NT, Saint-Herblain, France). This method of extraction provided 67 ± 4% (mean ± s.e.mean, $n = 5$) recovery of unlabelled vasopressin. The dried residues of the extracts were resuspended with 1 ml of assay buffer, and

Table 1 Pre- and post-haemorrhage values for blood pressure, heart rate and haematological parameters

Parameters	*Pre-haemorrhage*	*Post-haemorrhage*
Systolic blood pressure (mmHg)	114 ± 2	68 ± 3*
Heart rate (beats min^{-1})	372 ± 12	392 ± 18
Blood volume (ml 100 g^{-1} body weight)	5.3 ± 0.1	4.1 ± 0.2*
Red blood cells ($\times 10^6$ mm^{-3})	8.1 ± 0.1	6.7 ± 0.1*
Haematocrit (%)	43 ± 1	35 ± 1*
Haemoglobin (g 100 ml^{-1})	15.1 ± 0.2	12.4 ± 0.3*

Fifty-five rats were subjected to acute haemorrhage by allowing a loss of 2.1–2.2 ml of blood per 100 g of body weight within 5–10 min. In 35 rats blood pressure and heart rate were measured immediately before and 20 min after bleeding to determine 'pre-haemorrhage' and 'post-haemorrhage' values respectively. Pre-haemorrhage blood volume was determined in 9 rats. Post-haemorrhage blood volume was determined 20 min after bleeding was used to determine the pre-haemorrhage values of red blood cells, haemotocrit and haemoglobin. The post-haemorrhage values of these parameters were determined in the same rats, 20 min after the bleeding. Data are given as mean ± s.e.mean of 8–35 rats. Significantly different (*$P < 0.01$) from corresponding 'pre-haemorrhage' value.

400 μl aliquots were assayed in duplicate using a commercially available radioimmunoassay kit (Buhlmann Laboratories AG, Basel, Switzerland. The values expressed as pg ml^{-1} and were not corrected for extraction losses.

Drugs used

The following drugs were used: choline chloride, atropine sulfate, mecanylamine HCl, hemicholinium-3 bromide, [β-mercapto-β,β-cyclopenta-methylenepropionyl[1], O-Me-Tyr[2],-Arg[8]]-vasopressin (Sigma, St. Louis, MO, U.S.A.), and phentolamine hydrochloride (Ciba-Geigy, Istanbul, Turkey). The drugs were dissolved in physiological saline. All doses of drugs refer to the free base. The volume of solution injected into the cerebral ventricle was 10 μl. When choline was injected at increasing doses, isotonicity was maintained by appropriately decreasing the concentration of NaCl in the vehicle solution.

Data and statistical analysis

Data are presented as mean ± s.e.mean. Paired two-tailed *t* test was used when animals served as their own controls and an independent *t*-test was used for testing the significance of differences between mean values from different groups of rats. Analysis of variance (one- or two-way) was performed for appropriate groups. Suitable *a posteriori* test were performed if significant interactions were found.

A *P* value of less the 0.05 was considered significant.

Results

Effects of haemorrhage

Pre- and post-haemorrhage values for the animals' cardiovascular and haematological variables are shown in Table 1. The haemorrhage protocol in the present experiments, allowing a loss of 2.1–2.2 ml of blood per 100 g of body within 5–10 min, resulted in about 40% loss of the initial blood volume. This was not fatal for unanaesthetized rats; no animal died during bleeding or within 2 h after bleeding. The blood loss was restored partially, probably by entering of extravascular fluid during the stabilization period, and the residual blood volume reached 78 ± 4% of the initial volume (Table 1) 20 min after the bleeding. At this time, the red-cell count, haematocrit and haemoglobin concentration were significantly ($P<0.05$) lower than the initial values (Table 1).

The haemorrhage elicited severe and long lasting hypotension and transient bradycardia. Immediately after the bleeding, heart rate and blood pressure were significantly lower

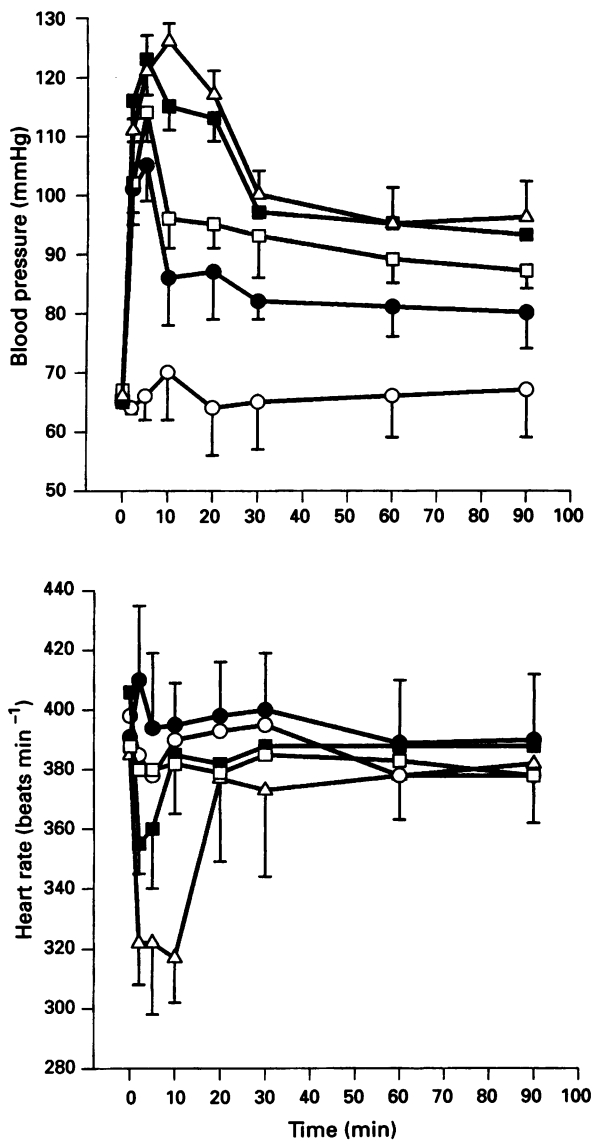

Figure 1 Increase in systolic blood pressure and decrease in heart rate in haemorrhaged hypotensive rats induced by choline i.c.v. Rats were subjected to acute haemorrhage as described in Table 1 and received i.c.v. saline (○) or choline 25 μg (●), 50 μg (□), 100 μg (■), or 150 μg (△) 20 min after bleeding. Blood pressure and heart rate were then monitored for 90 min. Values represent the mean ± s.e.mean (vertical bar) for 5–7 rats. The blood pressure values at all the time points following i.c.v. injection of 50 μg, 100 μg and 150 μg of choline were significantly ($P<0.01$) higher than the values of the saline-treated rats. The blood pressure values 2, 5, 10 and 20 min after i.c.v. injection of 25 μg of choline were also significantly ($P<0.05$) higher than the corresponding values obtained in the saline-treated rats. The heart rate values 2 and 5 min, or 2, 5 and 10 min, after i.c.v. injection of 100 μg or 150 μg of choline respectively were significantly ($P<0.05$) lower than the corresponding pre-choline values.

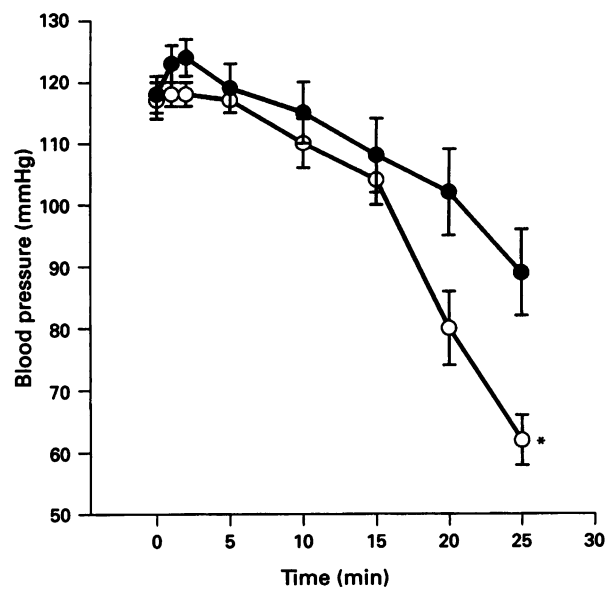

Figure 2 Systolic blood pressure response to graded haemorrhage in i.c.v. choline-treated rats. Rats were subjected to graded haemorrhage by removing 0.55 ml of blood per 100 g body weight from the arterial cannula 5, 10, 15 and 20 min after i.c.v. injection of saline (○) or choline (●, 50 μg). Each time the arterial cannula was flushed with 0.3 ml heparinized saline and blood pressure was recorded after 5 min. Values represent the mean ± s.e.mean for 6 rats. *Significantly different ($P<0.01$) from the corresponding value in the choline-treated group.

($P<0.01$; paired t test) than the pre-haemorrhage values and averaged 312 ± 18 beats min^{-1} ($n=35$) and 53 ± 5 mmHg ($n=35$), respectively. Blood pressure rose gradually during the first 10 min after the bleeding, and then stabilized at a level about 40–50 mmHg lower than the pre-haemorrhage values (Table 1). During the stabilization period, heart rate progressively increased and returned to the pre-haemorrhage values (Table 1).

Effects of i.c.v. choline

Injection of choline (25–150 μg, i.c.v.) evoked a dose-dependent increase in blood pressure. For all doses studied, blood pressure began to increase 20–40 s after injection and reached a maximum within 2–5 min (Figure 1). Following this initial peak, blood pressure began to decrease and stabilized at 80–85 mmHg or 90–95 mmHg within 10–20 min after the injection of 25 or 50 μg of choline respectively (Figure 1). At higher doses of choline (100 or 150 μg) blood pressue increased immediately to values greater than pre-haemorrhage levels, remained at this level for about 10 min and then gradually returned to the pre-haemorrhage levels (Figure 1).

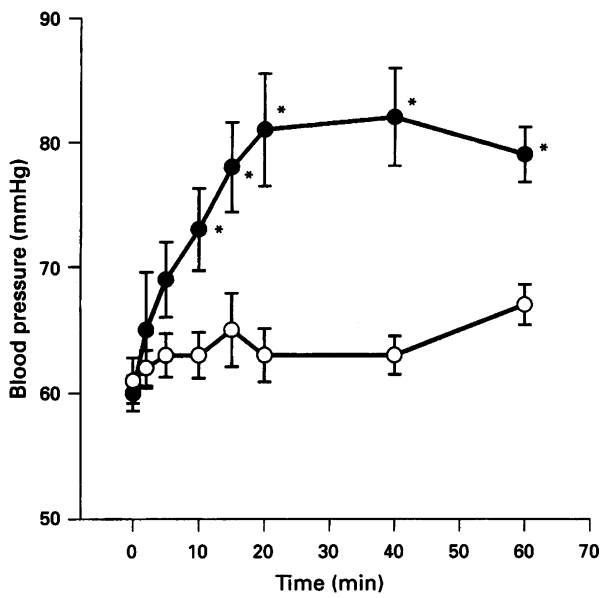

Figure 3 Increase in systolic blood pressure in haemorrhaged hypotensive rats induced by choline i.p. Rats were subjected to acute haemorrhage as described in Table 1 and received i.p. saline (○) or choline (●, 60 mg kg^{-1}) 20 min after bleeding. Blood pressure was then monitored for 60 min. Values represent the mean ± s.e.mean for 4–6 rats. *Significantly higher ($P<0.05$) than the corresponding value obtained in the saline-treated rats.

Heart rate did not change following i.c.v. injection of 25 μg or 50 μg of choline, while 100 μg or 150 μg of choline produced a short lasting bradycardia (Figure 1).

Measured 5 min after i.c.v. choline (150 μg), the residual blood volume (4.3 ± 0.3 ml 100 g^{-1} body weight), the red-cell count ($6.9\pm0.1\times10^6$ mm^{-3}), haematocrit ($36\pm1\%$) and haemoglobin content (12.8 ± 0.3 g 100 ml^{-1}) were not different from values (4.2 ± 0.2 ml 100 g^{-1} body weight; $6.5\pm0.2\times10^6$ mm^{-3}; $34\pm2\%$; 10.5 ± 0.5 g 100 ml^{-1}, respectively) in saline-treated rats ($n=6$).

Some rats were first given i.c.v. choline (50 μg) and then subjected to graded haemorrhage. Choline caused a slight transient increase in systolic blood pressure (Figure 2). Injection of saline (10 μl, i.c.v.) failed to alter blood pressure. Five minutes after the i.c.v. injection of choline or saline, rats were subjected to graded haemorrhage. This resulted in a transient fall in systolic blood pressure immediately after each haemorrhage. After each of the first two haemorrhages, blood pressure returned to values not significantly different from pre-haemorrhage in both choline- and saline-treated rats. Subsequent haemorrhages resulted in sustained and highly significant ($P<0.01$; paired t test) reductions in blood pressure in saline-treated rats. Choline-treated rats tended to have smaller reductions in systolic blood pressure after each haemorrhage step in these rats, blood pressure was significantly higher than in saline-treated rats following the last haemorrhage (Figure 2).

Effect of peripherally administered choline

In the one set of experiments the haemorrhaged rats were injected with choline (60 mg kg^{-1}) or saline i.p. and blood pressure was monitored for 60 min. Blood pressure began to increase within 2–5 min and stabilized 15–20 min after the choline injection (Figure 3). The maximal increase in blood pressure was about 20 mmHg and in no animal did the blood pressure reach pre-haemorrhage levels (Figure 3). Intraperitoneal injection of lower doses (0.5–10 mg kg^{-1}) of choline failed to alter blood pressure in haemorrhaged rats (data not shown).

Interaction of i.c.v choline with atropine, mecamylamine and hemicholinium-3

To determine if central muscarinic and/or nicotinic cholinoceptors mediated the pressor effect of choline in haemorrhage-induced hypotension, rats were pretreated with atropine or mecamylamine 5 min after bleeding or 15 min before i.c.v. injection of choline (50 μg). Mecamylamine, but not atropine,

Table 2 Effect of atropine, mecamylamine or hemicholinium-3 on the pressor response to i.c.v. choline in haemorrhaged rats

	Systolic blood pressure (mmHg)		
Treatments	*Pre-haemorrhage*	*Post-haemorrhage*	*After second i.c.v treatment*
Saline + saline	118 ± 4	64 ± 5	66 ± 9
Saline + choline	116 ± 3	60 ± 4	103 ± 11*
Mecamylamine + saline	117 ± 4	66 ± 6	70 ± 9
Mecamylamine + choline	117 ± 4	67 ± 5	68 ± 8
Atropine + saline	119 ± 4	60 ± 8	56 ± 14
Atropine + choline	113 ± 4	61 ± 6	99 ± 10*
Hemicholinium-3 + saline	115 ± 3	66 ± 3	73 ± 4
Hemicholinium-3 + choline	114 ± 2	63 ± 2	70 ± 3

Rats were subjected to acute haemorrhage as described in Table 1. They received i.c.v. either saline (10 μl), atropine (10 μg), mecamylamine (50 μg) or hemicholium-3 (20 μg) 5 min after bleeding, followed by i.c.v. saline (10 μl) or choline (50 μg) 15 min after the first i.c.v. treatment. Blood pressure was measured immediately before haemorrhage, 20 min after the haemorrhage and 5 min after the second i.c.v. injection ('pre-haemorrhage', and 'post-haemorrhage' and 'after second i.c.v. treatment', respectively). Data are given as mean ± s.e.mean of 5–9 rats. * Significantly different ($P<0.01$) from corresponding 'post-haemorrhage' value.

Table 3 Effects of i.c.v. choline on blood pressure, plasma levels of vasopressin, renin and catecholamines in haemorrhaged rats

Parameters	*Basal levels*	*After bleeding and i.c.v. saline*	*After bleeding and i.c.v. choline*
Blood pressure (mmHg)	115±4	67±3	121±8*
Vasopressin (pg ml^{-1})	3±1	10±1	68±12*
Renin (ng Ang-1 h^{-1} ml^{-1})	7±2	39±3	35±4
Catecholamines			
Noradrenaline (pg ml^{-1})	247±18	415±43	490±45
Adrenaline (pg ml^{-1})	311±25	1495±176	5506±347*
Dopamine (pg ml^{-1})	no data	75±13	230±25*

Rats were subjected to acute haemorrhage as described in Table 1. The first 1.5 ml of blood was collected to determine the 'basal levels' of vasopressin, renin and catecholamines. Rats were injected i.c.v. with saline or choline (150 μg) 20 min after haemorrhage, 5 min later blood pressure was monitored and then 1.5 ml of blood was taken for measurement of post-haemorrhage plasma levels of vasopressin, renin and catecholamines. Data are given as mean±s.e.mean of 7–16 measurements. Significantly different (*$P<0.01$) from the value of the saline group.

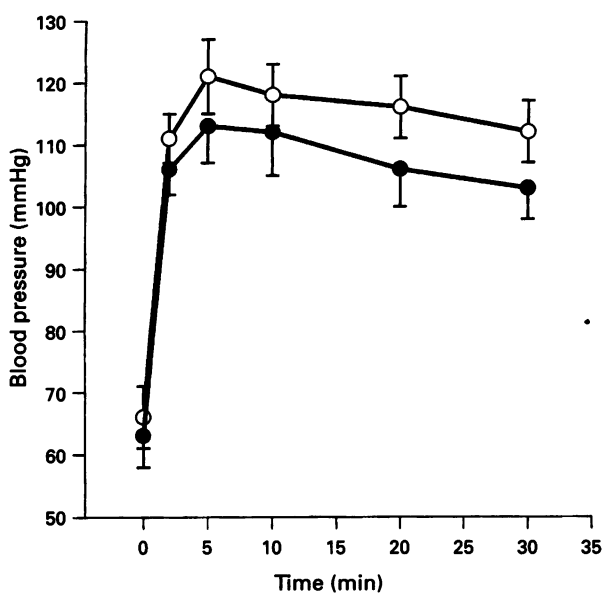

Figure 4 Effect of adrenalectomy on the pressor response to i.c.v. choline in haemorrhaged rats. Bilaterally adrenalectomized (●) or sham-operated (○) rats were subjected to haemorrhage as described in Table 1. Twenty min after the haemorrhage they were injected i.c.v. with choline (150 μg) and blood pressure was monitored for 30 min. Values represent the mean±s.e. for 6–7 rats.

abolished the effect of choline on blood pressure in haemorrhaged rats (Table 2). Given alone, these compounds did not produce any significant change in blood pressure (Table 2).

In order to determine whether the effect of i.c.v. choline was mediated through a presynaptic mechanism, rats were pretreated with the high affinity choline uptake blocker hemicholinium-3 i.c.v. 5 min after bleeding, 15 min before i.c.v. injection of choline (50 μg). The pretreatment blocked the effect of i.c.v. choline (Table 2).

Plasma vasopressin, catecholamines and renin

The haemorrhage protocol employed in this study resulted in a several fold increase in plasma levels of vasopressin, adrenaline, noradrenaline and renin (Table 3). Injection of choline (150 μg, i.c.v.) increased further, 6.8 and 3.7 fold, plasma levels of vasopressin and adrenaline, respectively (Table 3). Choline failed to alter the increased levels of noradrenaline and renin (Table 3).

Influence of bilateral adrenalectomy on the effect of i.c.v. choline

Bilateral adrenalectomy or a sham operation were performed in groups of 6 or 7 rats, respectively. Two hours after the operation animals were bled. Systolic blood pressure averaged 113±3 mmHg, or 114±5 mmHg before haemorrhage in sham operated or bilaterally adrenalectomized rats, respectively. Values 20 min after bleeding were 66±5 mmHg or 63±5 mmHg in sham-operated or bilaterally adrenalectomized rats,

Table 4 Effect of vasopressin antagonist and phentolamine on the pressor response to i.c.v. choline haemorrhaged rats

	Systolic blood pressure (mmHg)		
Pretreatments	*Pre-haemorrhage*	*Post-haemorrhage*	*After i.c.v. choline*
Saline	121±4	65±7	121±5*
Vasopressin antagonist	122±2	62±4	84±3*
Phentolamine	121±1	52±4	93±6*
Vasopressin antagonist + phentolamine	121±2	38±1	40±2

Rats were subjected to acute haemorrhage as described in Table 1. They received i.a. either saline (1 ml kg^{-1}), the vasopressin antagonist β-mercapro-β,β-cyclopenta-methylenepropionyl[1], O-Me-Tyr[2],Arg[8] -vasopressin (10 μg kg^{-1}), phentolamine (10 mg kg^{-1}) or both about 5 min after the bleeding and received i.c.v. choline (150 μg) 15 min after the i.a. treatment. Blood pressure was measured immediately before haemorrhage, 20 min after the haemorrhage and 5 min after i.c.v. injection of choline ('pre-haemorrhage', 'post-haemorrhage' and 'after i.c.v. choline', respectively). Data are given as mean±s.e.mean of 5–7 rats. * Significantly different ($P<0.01$) from corresponding 'post-haemorrhage' value.

respectively. In both groups of animals, i.c.v. injected choline (150 μg) caused an immediate and large increase in blood pressure (Figure 4). The increase in adrenalectomized animals was slightly but not significantly lower than in sham-operated rats (Figure 4).

Interaction of i.c.v. choline with α-adrenoceptor and vasopressin antagonists

To determine if plasma catecholamines and/or vasopressin mediated the pressor effect of choline in haemorrhage-induced hypotension, rats were pretreated with phentolamine, [β-mercapto-β,β-cyclopento-methylenepropionyl[1],O-Me-Tyr[2],Arg[8]]-vasopressin or both through the arterial cannula 5 min after bleeding and 15 min before i.c.v. injection of choline (150 μg). Phentolamine showed a tendency to decrease blood pressure in haemorrhaged rats and attenuated the pressor response to i.c.v. choline (Table 4). The vasopressin antagonist, β-mercapto-β,β-cyclopento-methylenepropionyl[1],O-Me-Tyr[2],Arg[8]-vasopressin itself did not alter the post-haemorrhage blood pressure but attenuated the pressor effect of choline (Table 4). When rats were pretreated with both of the drugs, the blood pressure further decreased and the pressor response to i.c.v. choline was prevented (Table 4).

Discussion

These data show that the central administration of choline (25–150 μg) raises and restores blood pressure in acutely hypotensive rats. Since blood pressure was only partially restored by the i.p. administration of a much higher dose (60 mg kg^{-1}) of choline, the effect of choline appears to be centrally mediated.

Administration of the same doses of choline to non-bled rats also increases blood pressure (Caputi & Brezenoff, 1980; Arslan *et al.*, 1991). In non-bled rats, the increase in blood pressure in response to a given dose of choline is much smaller in magnitude and shorter lasting (Caputi & Brezenoff, 1980; Arslan *et al.*, 1991) than the observed response in haemorrhaged rats (Figure 1). Indeed, in our experiments, i.c.v. injection of 50 μg of choline to normal rats caused only a slight (by about 4–8 mmHg) and transient (lasting 5 min) increase in blood pressure (Figure 2). The same dose of choline produced much higher increases (by about 35–55 mmHg) in blood pressure in haemorrhaged rats and the increase was maintained at least for 90 min (Figure 1). Also, the same dose of choline (50 μg) protected to some extent against the haemorrhage-induced decrease in blood pressure at a time when its effect on blood pressure was no longer observable in non-bled rats (Figure 2). Moreover, injection of 25 μg of choline (i.c.v.) which does not increase blood pressure in normal rats (Ulus *et al.*, unpublished observation), increased blood pressure in haemorrhaged rats (Figure 1). These data show that the blood pressure response to i.c.v. injected choline is enhanced in haemorrhaged rats. One interpretation is that the demand for choline increases in some cholinergic neurones during haemorrhage, and the normal supply of choline to those cholinergic neurones does not keep pace with the demand for acetylcholine synthesis. When this requirement is satisfied, these neurones synthesize and release more acetylcholine which allows maximal functioning at postsynaptic nicotinic receptors. In this way, the blood pressure is restored and maintained at normal or near normal levels (Figure 1). Another interpretation of the results is that any slight enhancement of central cholinergic transmission is more efficient in increasing blood pressure when blood pressure is very low.

In normotensive, non-bled animals the pressor response to i.c.v. choline involves both central muscarinic and nicotinic mechanisms (Arslan *et al.*, 1991). In the present study, the pressor effect of choline was not influenced by pretreatment with atropine but prevented by mecamylamine (Table 2). These data show clearly that the effect of i.c.v. choline in the haemorrhaged rats was mediated solely by central nicotinic receptors. It has recently been reported that intravenous administration of oxotremorine or physostigmine restores blood pressure and increases the survival rate in haemorrhaged rats (Guarini *et al.*, 1989), an effect also mediated by central nicotinic receptors. The present results are in good accordance with this report. It seems, therefore, that a different central cholinergic mechanism is involved in the regulation of blood pressure in haemorrhaged and normal rats. This view is further supported by the differences in the heart rate in response to i.c.v. choline in haemorrhaged hypotensive rats and normotensive animals. In normotensive, non-bled rats the decrease in heart rate in response to a given dose of choline is much greater and longer lasting (Caputi & Brezenoff, 1980; Arslan *et al.*, 1991) than the observed response in haemorrhaged rats (Figure 1). The reduction in heart rate in normotensive rats is independent of the blood pressure change (Caputi & Brezenoff, 1980; Arslan *et al.*, 1991). On the other hand, in haemorrhaged rats the reduction in heart rate is apparently mediated via a baroreceptor reflex in response to the increase in blood pressure.

In addition to its ability to increase nicotinic neurotransmission by a presynaptic mechanism, choline also activates nicotinic receptors as a direct agonist (Ulus *et al.*, 1988). The choline concentration necessary to interact with the brain nicotinic receptors (K_i = 379–1167 μM) is considerably higher than the choline concentration (10–60 μM) required to increase acetylcholine release from brain cholinergic neurones (Maire & Wurtman, 1985; Ulus *et al.*, 1989). The failure of 50 μg of choline to restore blood pressure in haemorrhaged rats pretreated with the high affinity choline uptake blocker, hemicholinium-3 (20 μg), strongly suggests that the effect of choline, at doses of 25 or 50 μg, results from its presynaptic action as a precursor of acetylcholine. The observed increase in blood pressure following i.c.v. injection of higher doses (100 and 150 μg) of choline may also result, to some extent, from its direct agonist action at central nicotinic receptors.

The pressor response to i.c.v. choline was associated with a several fold increase in plasma levels of adrenaline and vasopressin but not of noradrenaline and plasma renin activity (Table 3). This lack of effect on plasma noradrenaline agrees with the previous observations that central cholinergic stimulation primarily activates the adrenomedullary pathway of the sympatho-adrenal system (Ulus & Wurtman, 1979; Arslan *et al.*, 1991). The failure of choline to increase plasma renin activity (Table 3) is in good agreement with our previous report (Arslan *et al.*, 1991) and rules out the possibilty that the peripheral renin-angiotensin system plays a role in the response to i.c.v. choline in haemorrhaged rats. The increases in plasma levels of adrenaline and vasopressin strongly suggest that these two hormones may mediate the pressor effect of i.c.v. choline in haemorrhaged rats. In fact, both phentolamine and a vasopressin antagonist attenuated the pressor response to i.c.v. choline. However, both antagonists had to be given simultaneously to block the effect of choline entirely. These observations support the conclusion that the elevated levels of plasma vasopressin and adrenaline both contribute to the pressor action of i.c.v. choline in haemorrhaged rats. On the other hand, the failure of the bilateral adrenalectomy to alter the pressor response to i.c.v. choline is not in favour of this conclusion. One possibility is that the observed pressor response to i.c.v. choline in the adrenalectomized rats was achieved by enhanced contributions by vasopressin and/or noradrenaline. This possibility was not tested in the present experiments but is currently under investigation.

In conclusion, the present data show that i.c.v. administration of choline restores blood pressure in haemorrhaged rats by restoring central nicotinic transmission. The availability of choline for some cholinergic neurones appears to play an important role in blood pressure regulation. The observations

suggest that choline might be clinically useful for treatment of haemorrhagic hypotension and for prophylactic therapy prior to extensive surgery, all the more so when it is borne in mind that choline increases the resistance to acute haemorrhage and has reticuloendothelial system-stimulating activity (Altura, 1978) which might confer trauma protection.

This study was supported in part by a grant from The Research Fund of Uludag University (1985/1). The authors thank Ahmet Demirbilek for his technical assistance.

References

ALTURA, B.A. (1978). Role of spleen in choline stimulation of reticoendothelial system and resistance to acute haemorrhage. *Proc. Soc. Exp. Biol. Med.*, **158**, 77–80.

ARSLAN, B.RY., ULUS, I.H., SAVCI, V. & KIRAN, B.K. (1991). Effects of intracerebroventricular injected choline on cardiovascular functions and sympathoadrenal activity. *J. Cardiovasc. Pharmacol.*, **17**, 814–821.

BRADLEY, E.C. & BAR, J.W. (1968). Determination of blood volume using indocyanine green (Cardio-green) dye. *Life Sci.*, **7**, 1001–1007.

BREZENOFF, H.E. (1984). Cardiovascular regulation by brain acetylcholine. *Fed. Proc.*, **43**, 17–20.

BREZENOFF, H.E. & GIULIANO, R. (1982). Cardiovascular control by cholinergic mechanism in the central nervous system. *Annu. Rev. Phramacol. Toxicol.*, **22**, 341–381.

CAPUTI, A.P. & BREZENOFF, H.E. (1980). Cardiovascular effects produced by choline injected into the lateral cerebral ventricle of the unanaesthetized rats. *Life Sci.*, **26**, 1029–1036.

COHEN, E.L. & WURTMAN, R.J. (1975). Brain acetylcholine: increase after systemic choline adminstration. *Life Sci.*, **16**, 1095–1102.

GIBSON, J.G., SELIGMAN, A.M., PEACOCK, W.C., FINE, J., AUB, J.C. & EVANS, R.D. (1947). The circulating red cell and plasma volume and the distribution of blood in large and minute vessels in experimental shock in dogs, measured by radioactive isotopes of iron and iodine. *J. Clin. Invest.*, **26**, 126–144.

GUARINI, S., TAGLIAVINI, S., FERRARI, W. & BERTOLINI, A. (1989). Reversal of haemorrhagic shock in rats by cholinomimetic drugs. *Br. J. Pharmacol.*, **98**, 218–224.

JOHNSON, D.A., ULUS, I.H. & WURTMAN, R.J. (1992). Caffeine potentiates the enhancement by choline of striatal acetylcholine release. *Life Sci.*, **51**, 1547–1601.

KOSHIMURA, K., MIWA, S., LEE, K., HAYASHI, Y., HASEGAWA, H., HAMAHATA, K., FUJIWARA, KIMURA, M. & ITOKAWA, Y. (1990). Effects of choline administration on in vivo release and biosynthesis of acetylcholine in rat striatum as studied by in vivo brain microdialysis. *J. Neurochem.*, **54**, 533–539.

MAIRE, J.-C.E. & WURTMAN, R.J. (1985). Effects of electrical stimulation and choline availability on the release and contents of acetylcholine and choline in perfused slices from rat striatum. *J. Physiol. (Paris)*, **80**, 189–195.

PHILIPPU, A. (1981). Involvement of cholinergic system of the brain in the central regulation of cardiovascular functions. *J. Autonom. Pharmacol.*, **1**, 321–330.

PHILIPPU, A. (1988). Regulation of blood pressure by central neurotransmitters and neuropeptides. *Rev. Physiol. Biochem. Pharmacol.*, **111**, 1–115.

SKARPHEDINSSON, J.O., STAGE, L. & THOREN, P. (1986). Cerebral function during hypotensive haemorrhage in spontaneously hypertensive rats and Wistar Kyoto Rats. *Acta. Physiol. Scand.*, **128**, 445–452.

ULUS, I.H., HIRSCH, M.J. & WURTMAN, R.J. (1977). Trans-synaptic induction of adrenomedullary tyrosine hydroxylase activity by choline: evidence that choline administration can increase cholinergic transmission. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 798–800.

ULUS, I.H., MILLINGTON, W.R., BUYUKUYSAL, L.R. & KIRAN, B.K. (1988). Choline as an agonist: determination of its agonistic potency on cholinergic receptors. *Biochem. Pharmacol.*, **37**, 2747–2755.

ULUS, I.H., SCALLY, M.C. & WURTMAN, R.J. (1978). Enhancement by choline of the induction of adrenal tyrosine hydroxylase by phenoxybenzamine, 6-hydroxydopamine, insulin or exposure to cold. *J. Pharmacol. Exp. Ther.*, **204**, 676–682.

ULUS, I.H. & WURTMAN, R.J. (1976). Choline administration: activation of tyrosine hydroxylase in dopaminergic neurons of rat brain. *Science*, **194**, 1060–1061.

ULUS, I.H. & WURTMAN, R.J. (1979). Selective response of rat peripheral sympathetic nervous system to various stimuli. *J. Physiol*, **293**, 513–523.

ULUS, I.H., WURTMAN, R.J., MAURON, C. & BLUSZTAJN, J.K. (1989). Choline increases acetylcholine release and protects against the stimulation induced decrease in phosphatide levels within membranes of rat corpus striatum. *Brain Res.*, **484**, 217–227.

WECKER, L., SCHMIDT, D.E. (1979). Central cholinergic function: relationship to choline administration. *Life Sci.*, **25**, 375–384.

WURTMAN, R.J. (1992). Choline metabolism as a basis for the selective vulnerability of cholinergic neurons. *Trends Neurosci.*, **15**, 117–122.

WURTMAN, R.J., HEFTI, F. & MELAMED, E. (1980). Precursor control of neurotransmitter synthesis. *Pharmacol. Rev.*, **32**, 315–335.

(Received August 1, 1994
Revised May 1, 1995
Accepted May 23, 1995)