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Central choline reverses hypotension caused by α-adrenoceptor or ganglion blockade in rats: the role of vasopressin

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Abstract

The effect of intracerebrovenricularly (i.c.v.) injected choline on blood pressure was investigated in rats made hypotensive by blocking peripheral α-adrenoceptors or autonomic ganglionic transmission. Choline (50–150 μg; i.c.v.) increased blood pressure in a dose-dependent manner and 150 μg of choline restored blood pressure to the resting level. The pressor response to choline was associated with an increase in plasma vasopressin levels. Pretreatment with mecamylamine (50 μg; i.c.v.), but not atropine (10 μg; i.c.v.), blocked both the pressor and vasopressin responses to i.c.v. choline. The vasopressin receptor antagonist, [β-mercapto-β,β-cyclopenta-methylene-propionyl[1],O-Me-Try[2],Arg[8]]vasopressin (10 μg/kg; i.v.), given 5 min after i.c.v. choline (150 μg), abolished the pressor effect of choline and blood pressure returned to the pre-choline levels. It is concluded that the precursor of acetylcholine, choline, can increase blood pressure and reverse hypotension in α-adrenoceptor or ganglionic transmission blocked rats, by increasing plasma vasopressin.

Keywords: Choline; Acetylcholine; Blood pressure; Hypotension; Vasopressin; Nicotinic receptor

1. Introduction

A great deal of data have accumulated over 40 years implicating brain acetylcholine in the control of blood pressure (for review see Brezenoff and Guiliano, 1982; Brezenoff, 1984; Philippu, 1988). Yet, despite this, our knowledge regarding the role of central cholinergic system in blood pressure control is incomplete. Much of the work concerning the cardiovascular effects of the central injection of drugs acting at cholinergic synapses has been done with normotensive or hypertensive animals (for review see Brezenoff and Guiliano, 1982; Brezenoff, 1984; Philippu, 1988). The role of central cholinergic neurones in hypotension has received no or little attention until the last few years. Recently, it has been reported that the centrally active cholinomimetic drugs can restore blood pressure and increase survival rate of rats in an experimental haemorrhage shock model (Guarini et al., 1989, 1990, 1991; Onat et al., 1994). Based on these observations, it has been suggested that a decrease in central cholinergic tone is involved in the complex pathophysiology of cardiovascular shock (Guarini et al., 1989).

The synthesis and release of acetylcholine can be affected by the level of its precursor, choline (for review see Tucek, 1990; Wurtman, 1992). Treatments that raise tissue choline levels produce a parallel increase in acetycholine synthesis and release (Maire and Wurtman, 1985: Ulus et al., 1989; Koshimura et al., 1990; Johnson et al., 1992; Farber et al., 1993; Marshall and Wurtman, 1993; Buyukuysal et al., 1995) and augment cholinergic transmission (Ulus and Wurtman, 1976; Ulus et al., 1977a,b, 1978). The dependency of cholinergic neurones on choline becomes more evident when the firing rate of neurones increases (Maire and Wurtman, 1985; Ulus et al., 1989; Buyukuysal and Wurtman, 1990). In such conditions administration of choline can greatly enhance cholinergic transmission (Ulus et al., 1977b, 1978).

The choline supplied to brain cholinergic neurones originates mainly from the circulation, and in limited amounts from the hydrolysis of released acetycholine and choline-containing membrane phospholipids (Tucek, 1990: Wurtman, 1992). Therefore, a continuous and adequate supply of choline from the circulation for acetycholine synthesis is a crucial factor in the maintenance of the central cholinergic tone (Wurtman, 1992). Due to a decrease in brain blood flow (Skarphedinsson et al., 1986), it is likely that the supply of free choline to brain cholinergic neurones is

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diminished during sustained hypotension. If so, a suggested decrease (Guarini et al., 1989) in brain cholinergic tone during hypotension and shock may result from the decrease in free choline supply to brain cholinergic neurones, and, provision of extra free choline to central cholinergic neurones might improve central cholinergic neurotransmission and help to reverse hypotension. Recently we tested this hypothesis in rats made hypotensive by acute haemorrhage (Ulus et al., 1995). In agreement with our expectation, central administration of choline effectively reversed haemorrhagic hypotension (Ulus et al., 1995). In our previous report (Arslan et al., 1991) we observed that central administration of choline also reverses the hypotension that occurs after peripheral α-adrenoceptor or ganglionic transmission blockade or transection of the spinal cord. These observations support further the idea that providing free choline might improve central cholinergic neurotransmission and help to reverse hypotension, but these aspects were not characterised in the previous report (Arslan et al., 1991).

So, considering the importance of the above observations the present study was undertaken to characterise the blood pressure reversing effect of choline, injected i.c.v. in rats made hypotensive by blockade of α-adrenoceptor or autonomic ganglionic transmission. This study was aimed to determine (1) the dose-response relationship and the time course of the pressor effect of i.c.v. choline in α-adrenoceptor or ganglionic transmission blocked rats, (2) which central cholinergic receptor, nicotinic and/or muscarinic, is involved in the pressor response to i.c.v. choline and (3) whether this pressor response is elicited by vasopressin secretion.

2. Materials and methods

2.1. Animals

Male Wistar rats (Experimental Animals Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) weighing 280–350 g were used in all experiments. The colony room was maintained at 20–24°C with a 12-h light-dark cycle.

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

2.2. Surgery, cannulations and blood pressure recording

Under ether anaesthesia, the left common carotid artery and left jugular vein were cannulated with polyethylene tubing filled with heparinized saline (400 U/ml). The free end of the catheters was exteriorized at the nape of the neck and sealed until use. For central drug administration, a burr hole was drilled through the skull 1.5 mm lateral to mid-line and 1.0 mm posterior to bregma. A 10 mm length of 22 gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The cannula was lowered 4.5–5.0 mm below the skull surface and fixed with acrylic cement.

Following surgery, the rats were placed in individual cages and allowed to recover from anaesthesia for 2 h. During this recovery period the rats remained calm and without evidence of pain.

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, USA). The blood pressure is reported as systolic pressure (mm Hg). The heart rate was counted from the phasic pressure tracing recorded on the polygraph and reported as beats/min.

2.3. α-Adrenoceptor blockade

To block α-adrenoceptors, a nonselective α-adrenoceptor antagonist phentolamine was given as a bolus intravenously (i.v.) via the jugular vein catheter at a dose of 10 mg/kg. At this dose, phentolamine has been shown previously to cause complete α-adrenoceptor blockade and sustained hypotension for at least 1 h (Arslan et al., 1991; Lo et al., 1991). In pilot experiments, successful blockade was verified by the absence of a pressor response to noradrenaline (250–500 μg/kg; i.v.) within 10 min after the treatment of phentolamine.

In one set of experiments, a nonselective β-adrenoceptor antagonist, propranolol, was given i.v. at a dose of 0.5 mg/kg, to block β-adrenoceptors, 15 min after the treatment with phentolamine.

2.4. Ganglion blockade

To block ganglionic nicotinic transmission, 15 mg/kg of hexamethonium was injected intraperitoneally (i.p.). Over a 8–20 mg/kg dose range, hexamethonium has been shown to produce effective ganglion blockade (Kayaalp, 1965; Krstic, 1980; Osborn et al., 1987) and sustained hypotension for at least 45 min (Arslan et al., 1991). To test the efficacy of hexamethonium as a ganglion blocker, the pressor responses to i.v. bolus injections of acetylcholine (100–1000 μg/kg) in methylatropine (5 mg/kg; i.p.) pretreated animals were measured 15 min before and after the administration of hexamethonium. Pilot experiments showed that the pressor response to a bolus i.v. injection of 100 μg/kg or 1000 μg/kg of acetylcholine was inhibited by about 87% or 80%, respectively, 15 min after the treatment with hexamethonium.

In one set of experiments, a peripheral muscarinic receptor antagonist atropine methylnitrate was given i.p at a dose of 5 mg/kg to block ganglionic muscarinic receptors, 15 min after the treatment with hexamethonium.

2.5. Plasma vasopressin measurement

In some experiments, 2-ml blood samples were removed from the arterial cannula to determine plasma levels of vasopressin. Blood samples were kept on ice and plasma was obtained by centrifugation (1500 × g, 15 min) at 4°C. Vasopressin was extracted from plasma and assayed by using a radioimmunoassay kit (Buhlmann Laboratories, Basel, Switzerland) as described previously (Ulus et al., 1995). The standard curve ranged from 2 to 80 pg/ml of vasopressin and the minimal detectable dose of vasopressin was 0.8 pg/ml. When necessary serial dilutions were made to ensure that dose of vasopressin in the sample fell within the range of the standard curve.

2.6. Drugs

The following drugs were used: choline chloride, atropine sulphate, mecamylamine hydrochloride, hexamethonium hydrochloride, [β-mercapto-β,β-cyclopentamethylene-propionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (Sigma, St. Louis, MO, USA) and phentolamine (Regitine, Ciba-Geigy, Istanbul, Turkey). The drugs were dissolved in saline (0.9% NaCl solution). The volume injected into the lateral cerebral ventricle was 10 μl. The volume injected i.p. or i.v. was 1 ml/kg.

2.7. Statistics

Data are presented as means ± standard error of the mean (S.E.M.). Paired two-tailed *t*-test was used when animals served as their own controls and an independent two-tailed *t*-test was used for testing the significance of differences between mean values from different groups of rats. Analysis of variance was performed for appropriate groups and suitable a posteriori tests were performed if significant interactions were found. A P value less than 0.05 was considered significant.

3. Results

3.1. Control experimental conditions

The baseline blood pressure and heart rate, at the end of the recovery period and prior to any drug administration, in the different groups of rats used in the present study showed a maximal variation of 12 mm Hg and 38 beats/min between groups. Prior to any drug administration, the systolic blood pressure and heart rate for all groups of animals used were 118 ± 1 mm Hg and 365 ± 8 beats/min, with no significant difference among groups.

3.2. Effects of α-adrenoceptor or ganglionic blockade

Administration of phentolamine (10 mg/kg; i.v.) or hexamethonium (15 mg/kg; i.p.) caused an immediate decrease in blood pressure (Fig. 1A). Blood pressure stabilised at about 60% (64–78 mm Hg) or 70% (73–86 mm Hg) of the baseline level within 3–5 min after the treatment with phentolamine or hexamethonium, respectively. Blood pressure remained at this low level at least for 45 min (Fig. 1A) and then tended to recover over the next 2 h (data not shown). The decrease in blood pressure was associated with an increase in heart rate in both phentolamine- and hexamethonium-treated animals (Fig. 1B).

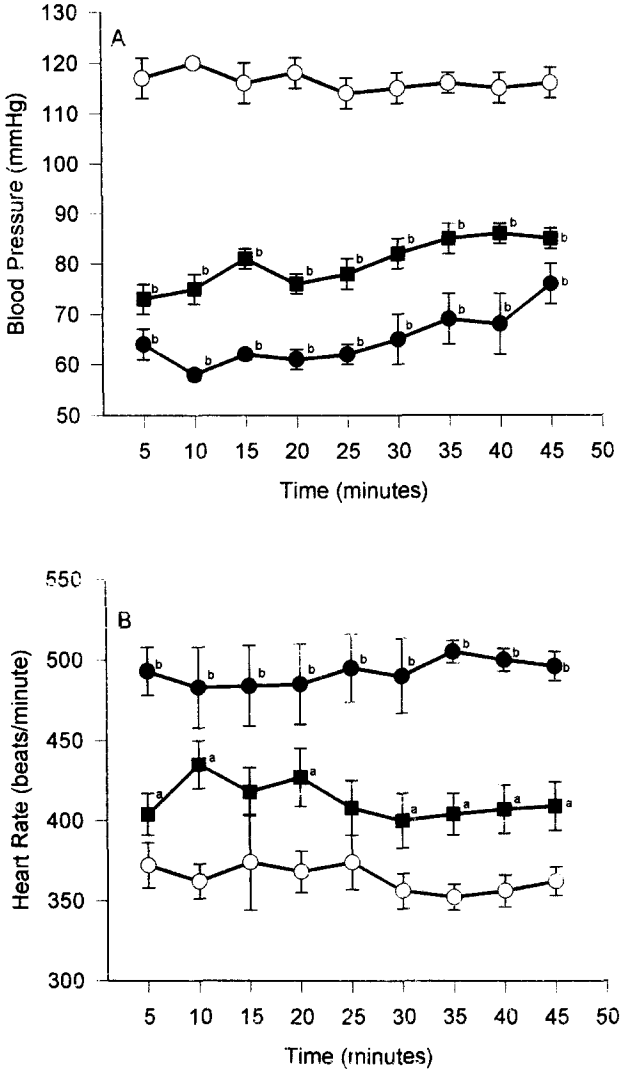

Fig. 1. Blood pressure and heart rate changes after phentolamine or hexamethonium. Rats received saline [(○) 1 ml/kg; i.v. or i.p.], phentolamine [(●)10 mg/kg; i.v.] or hexamethonium [(■) 15 mg/kg; i.p.], and blood pressure (A) and heart rate (B) changes were monitored for 45 min. Each point represents the mean ± S.E.M. (vertical bars) for 6–8 rats. Significantly ($^a P < 0.05$; $^b P < 0.01$) different from the corresponding value in the saline-treated rats.

Administration of saline (1 ml/kg; i.v. or i.p.) did not alter blood pressure and heart rate during the 45 min observation period (Fig. 1A, B).

Administration of propranolol (0.5 mg/kg; i.v.) increased blood pressure and decreased heart rate in phentolamine-treated rats: the blood pressure and heart rate sta-

bilised with in 10 min after propranolol. The blood pressure and heart rate were 70 ± 8 mm Hg ($n = 6$) and 485 ± 12 beats/min ($n = 6$) or 91 ± 2 mm Hg ($n = 6$) and 314 ± 13 beats/min ($n = 6$) immediately before and 10 min after the treatment of propranolol, respectively.

Administration of atropine methylnitrate (5 mg/kg, i.p.) did not alter the blood pressure and heart rate in hexamethonium-treated rats. The blood pressure and heart rate were 83 ± 4 mm Hg ($n = 6$) and 428 ± 28 beats/min ($n = 6$), and 86 ± 3 mm Hg ($n = 6$) and 413 ± 33 beats/min ($n = 6$) immediately before and 10 min after the treatment with atropine methylnitrate, respectively.

3.3. Effects of choline

The systolic blood pressure and heart rate values were 117 ± 4 mm Hg ($n = 15$) and 368 ± 17 beats/min ($n = 15$), 71 ± 2 mm Hg ($n = 28$) and 455 ± 15 beats/min, and 82 ± 2 mm Hg ($n = 32$) and 408 ± 12 beats/min ($n = 32$) immediately before i.c.v. saline or choline injection in the control, phentolamine- and hexamethonium-treated rats, respectively.

In control rats, i.c.v. injection of choline (50–150 μg) produced an immediate pressor response that reached a maximum within 2 min and returned to the control levels within 5–15 min after the treatment (Fig. 2A).

In phentolamine- or hexamethonium-treated rats, i.c.v. choline (50–150 μg) caused a dose-dependent increase in blood pressure (Fig. 2B, C). For all doses studied blood pressure began to increase 20–40 s after injection, reached a maximum within 5 min and then decreased gradually toward the pre-choline values over the next 15 min (Fig. 2B, C). At 150 μg of choline blood pressure rose to the resting level and remained at this level for about 10 min and then decreased slightly over the next 10 min. At lower choline doses (50 or 100 μg) blood pressure increased significantly but failed to reach the resting levels at any time point within the 20-min period after choline treatment (Fig. 2B, C).

In phentolamine + propranolol-treated rats, i.c.v. injection of choline (150 μg) elevated blood pressure from 90 ± 2 mm Hg ($n = 6$) to 111 ± 4 mm Hg, 113 ± 2 mm Hg and 109 ± 3 mm Hg, 5, 10 and 15 min after the injection, respectively. In hexamethonium + atropine methylnitrate treated rats, blood pressure rose from 81 ± 2 mm Hg ($n = 6$) to 112 ± 4 mm Hg within 5 min and remained at this level for 20 min after i.c.v. injection of choline (150 μg).

Injection of choline (150 μg; i.c.v.) decreased heart rate by 63 ± 12 beats/min ($n = 7$) and 84 ± 17 beats/min ($n = 8$) in control and hexamethonium-treated rats, respectively. At lower doses (50 μg or 100 μg) choline failed to alter heart rate in hexamethonium-treated rats (data not shown). In phentolamine treated rats, i.c.v. choline (50–150 μg) failed to alter heart rate in any time point (data not shown). In phentolamine + propranolol-treated rats, 150 μg of choline reduced heart rate significantly, by 79 ± 8 beats/min ($n = 6$), within 10 min. In hexamethonium + atropine methylnitrate-treated rats, i.c.v. choline (150 μg) decreased heart rate by 63 ± 10 beats/min ($n = 6$)

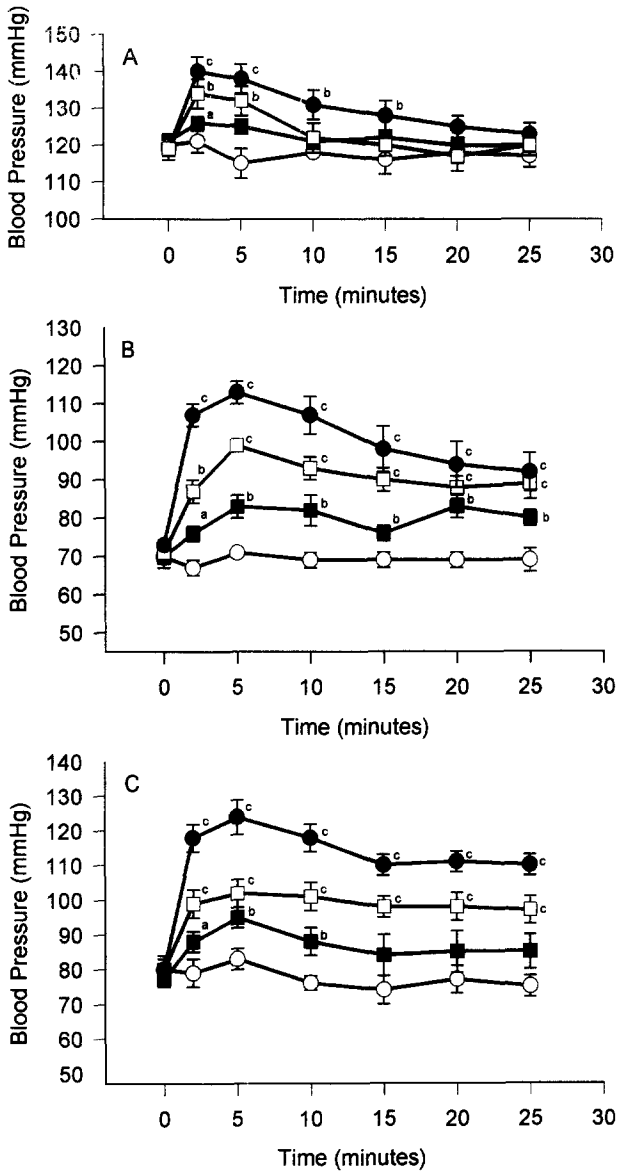

Fig. 2. Dose and time course of the pressor response to i.c.v. choline in control and phentolamine- or hexamethonium-treated rats. Rats were injected i.c.v. with saline (○) or choline [50 μg (■), 100 μg (□), 150 μg (●)] 15 min after pretreatment with saline (A), phentolamine (B) or hexamethonium (C). Each point represents the mean ± S.E.M. (vertical bars) for 5–8 rats. Significantly ($^a P < 0.05$; $^b P < 0.01$; $^c P < 0.001$) different from the corresponding value in the i.c.v. saline-treated rats.

A slow (over 30 s) infusion (via the jugular vein catheter) of choline (150 μg) did not alter blood pressure and heart rate in either control or phentolamine- or hexamethonium-treated rats.

3.4. Effects of receptor antagonism on the pressor response to choline

To determine if central muscarinic and/or nicotinic receptors mediate the pressor response to i.c.v. choline,

rats were pre-treated with the muscarinic antagonist atropine (10 μg; i.c.v.) or the nicotinic antagonist mecamylamine (50 μg; i.c.v.) 10 min before i.c.v. injection of choline (150 μg). As seen in Fig. 3, mecamylamine markedly prevented or reduced the pressor response to i.c.v. choline in phentolamine or hexamethonium-treated rats, respectively. Atropine showed no effect (Fig. 3A, B).

3.5. Effect of choline on plasma vasopressin level

To determine whether the pressor response to choline is associated with an increase in plasma vasopressin, blood pressure and plasma vasopressin levels were assessed 5 min after i.c.v. injection saline or choline. As seen in Table 1, in the control situation (i.c.v. saline treatment), α-adrenoceptor or ganglion blocked rats were hypotensive and their plasma vasopressin levels were 9.5-fold or 3-fold higher than the levels observed in intact (control), nor-

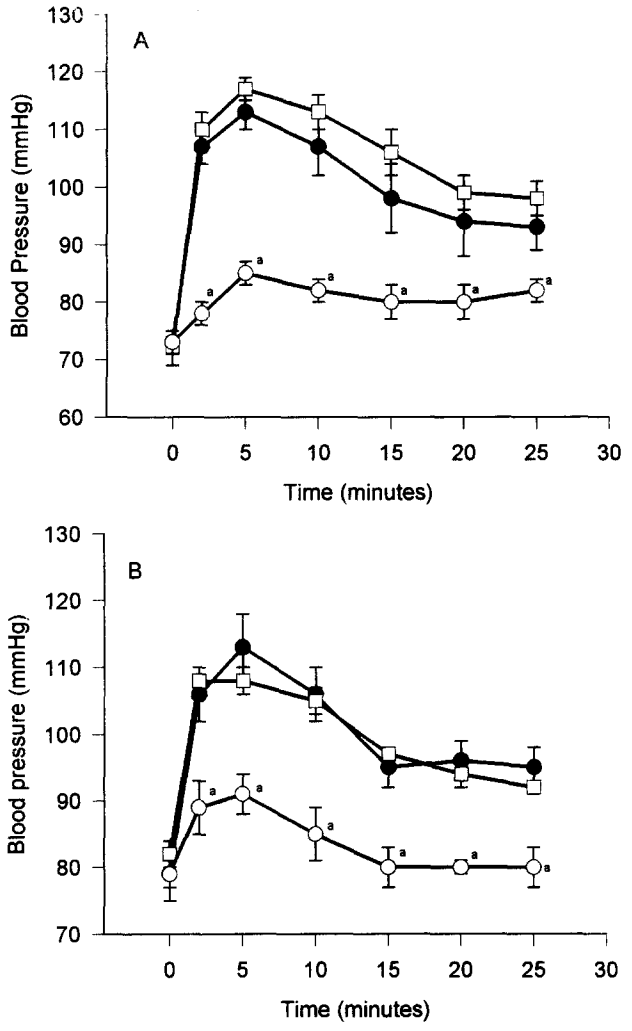

Fig. 3. Effect of atropine and mecamylamine on the pressor response to i.c.v. choline in phentolamine- or hexamethonium-treated rats. Rats were injected i.c.v. with saline [10 μl (●)], mecamylamine [50 μg (○)] or atropine [10 μg (□)] 5 min after administration of phentolamine [10 mg/kg, i.v. (A)] or hexamethonium [15 mg/kg, i.p. (B)]. Ten minutes after the i.c.v. drug pretreatment, rats were given i.c.v. choline (150 μg) and blood pressure response was monitored for 25 min. Each point represents the mean ± S.E.M. (vertical bars) for 6–8 rats. Significantly ([a] $P < 0.01$) different from the corresponding value in the i.c.v. saline pre-treated rats.

Table 1
Increases in blood pressure and plasma vasopressin levels in phentolamine- or hexamethoium-treated rats induced by i.c.v. choline

Group/i.c.v. treatment	Blood pressure (mm Hg)	Vasopressin (pg/ml)
Control		
Saline	117 ± 4	4 ± 1
Choline (150 μg)	132 ± 4 [a]	19 ± 3 [a]
Phentolamine		
Saline	69 ± 3	38 ± 9
Choline (50 μg)	83 ± 3 [a]	75 ± 2 [a]
Choline (100 μg)	99 ± 1 [a]	90 ± 5 [a]
Choline (150 μg)	113 ± 3 [a]	106 ± 8 [a]
Hexamethonium		
Saline	83 ± 3	12 ± 2
Choline (50 μg)	95 ± 3 [a]	31 ± 7 [a]
Choline (100 μg)	102 ± 4 [a]	47 ± 13 [a]
Choline (150 μg)	114 ± 5 [a]	56 ± 13 [a]

Rats were injected i.c.v. with saline or choline 15 min after administration of saline (control; i.p.), phentolamine (10 mg/kg; i.v.) or hexamethonium (15 mg/kg; i.p.). Blood pressure was measured 5 min after the i.c.v. treatment and 2 ml of arterial blood was removed and plasma vasopressin was measured by radioimmunoassay. Data are given as means ± S.E.M. Significantly ([a] $P < 0.01$) higher than the corresponding saline value.

Table 2
Effects of atropine or mecamylamine on the pressor and plasma vasopressin responses to i.c.v. choline in phentolamine- or hexamethonium-treated rats

Group/i.c.v. treatment	Blood pressure (mm Hg)	Vasopressin (pg/ml)
Phentolamine		
Saline + saline	72 ± 3	47 ± 10
Saline + choline	114 ± 2 [b]	114 ± 27 [a]
Atropine + saline	79 ± 2	48 ± 5
Atropine + choline	117 ± 3 [b]	130 ± 15 [b]
Mecamylamine + saline	79 ± 3	42 ± 6
Mecamylamine + choline	85 ± 2	54 ± 12
Hexamethonium		
Saline + saline	82 ± 3	12 ± 3
Saline + choline	112 ± 2 [b]	62 ± 7 [b]
Atropine + saline	84 ± 2	13 ± 3
Atropine + choline	108 ± 2 [b]	60 ± 6 [b]
Mecamylamine + saline	77 ± 2	10 ± 4
Mecamylamine + choline	91 ± 4 [a]	39 ± 9 [a]

Rats were injected i.c.v. with saline (10 μl), atropine (10 μg) or mecamylamine (50 μg) 5 min after administration of phentolamine (10 mg/kg; i.v.) or hexamethoniun (15 mg/kg; i.p.). Ten minutes after the first i.c.v. injection, rats were given a second i.c.v. injection of either saline (10 μl) or choline (150 μg). Blood pressure was measured 5 min after the second i.c.v. treatment and then 2 ml of arterial blood was removed and plasma vasopressin was measured by radioimmunoassay. Data are given as means ± S.E.M. for 7 rats. Significantly ([a] $P < 0.05$; [b] $P < 0.01$) higher than the corresponding saline values.

motensive rats, respectively (Table 1). In these rats, i.c.v. choline (50–150 μg) increased plasma vasopressin further in a dose-dependent manner (Table 1) and elicited a pressor response. In control rats, i.c.v. injection of 150 μg of choline also increased plasma vasopressin by about 5-fold (Table 1).

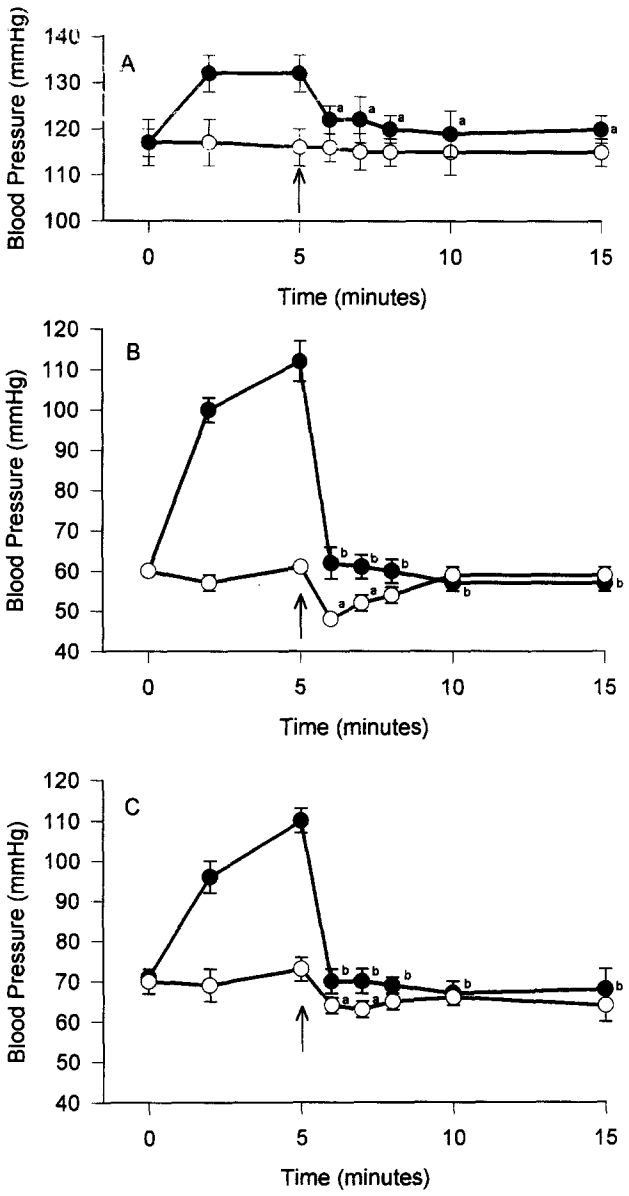

Fig. 4. Effect of vasopressin receptor antagonist on the pressor response to i.c.v. choline in phentolamine- or hexamethonium-treated rats. Rats were injected i.c.v. with saline [10 μl (○)] or choline [150 μg (●)] 15 min after administration of saline [1 ml/kg, i.v. (A)], phentolamine[10 mg/kg, i.v. (B)] or hexamethonium [15 mg/kg, i.p. (C)]. Five minutes after these i.c.v. treatments (at the arrow), rats were given a vasopressin pressor antagonist, [β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (10 μg/ kg; i.v.), and blood pressure was monitored for 10 min. Each point represents the mean ± S.E.M. (vertical bars) for 5–8 rats. Significantly (^{a} $P < 0.05$; ^{b} $P < 0.01$) lower than the corresponding pre-[β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin.

3.6. Effects of receptor antagonism on the blood pressure and plasma vasopressin responses to choline

To determine whether the blood pressure and plasma vasopressin responses to i.c.v. choline are both influenced in a similar manner by the central cholinergic receptor antagonism in phentolamine- or hexamethonium-treated rats, animals were pre-treated with i.c.v. saline (10 μl), atropine (10 μl) or mecamylamine (50 μg) 10 min prior to i.c.v. choline (150 μg). Mecamylamine abolished both responses in phentolamine-treated rats (Table 2). In hexamethonium-treated rats, both responses were reduced significantly (Table 2). Atropine failed to alter the responses to choline (Table 2). Mecamylamine and atropine given alone did not alter blood pressure or plasma vasopressin level (Table 2).

3.7. Effect of a vasopressin receptor antagonist on the pressor response to choline

To further evaluate if choline restored blood pressure via a vasopressin-dependent mechanism, the vasopressin receptor antagonist, [β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (10 μg), was given i.v. 5 min after i.c.v. choline (150 μg). As seen in Fig. 4B,C, the blood pressure of rats decreased from the increased levels after choline to the pre-choline levels in phentolamine or hexamethonium-treated rats. In control rats, blood pressure also decreased significantly after vasopressin receptor antagonist but did not return to the pre-choline levels (Fig. 4A).

Administration of vasopressin receptor antagonist did not alter blood pressure in i.c.v. saline-treated control rats (Fig. 4A) but produced a slight and transient decrease in blood pressure in i.c.v. saline-treated sympathetically blocked rats (Fig. 4B, C).

4. Discussion

In the present study, we have demonstrated that i.c.v. choline (50–150 μg) increases blood pressure and reverses the hypotension induced by acute blockade of peripheral α-adrenoceptors or autonomic ganglionic transmission. Since i.v. injections of choline (150 μg) did not increase blood pressure, it is apparent that the present results were not due to leakage of choline from its central site of injection into the periphery. The pressor response to i.c.v. choline was associated with an increase in plasma levels of vasopressin. The blockade of both effects by the nicotinic receptor antagonist, mecamylamine, indicates that central nicotinic receptors are involved in both responses to i.c.v. choline.

The present results confirm and extend our report that i.c.v. choline administration increases blood pressure in the normal rat (Fig. 2A) and reverses the hypotension induced

by phentolamine (Fig. 2B) or hexamethonium (Fig. 2C). The hypotension seen after phentolamine or hexamethonium is probably mainly due to complete antagonism of α-adrenoceptor or ganglionic nicotinic transmission, respectively. This view is evidenced by the absence of an α-adrenoceptor-mediated pressor response to noradrenaline in phentolamine-treated rats and a ganglionic nicotinic receptor-mediated pressor response to acetylcholine in hexamethonium-treated rats. The decrease in blood pressure observed after α-adrenoceptor blockade was greater than the decrease seen after ganglion blockade (Fig. 1A). It has been shown (Gardiner and Bennett, 1988a,b) that the decrease in blood pressure after α-adrenoceptor blockade results partly from peripheral vasodilatation as a consequence of stimulation β_2-adrenoceptors due to an increase in circulating catecholamines. Ganglion blockade eliminates the influence of the sympatho-adrenal catecholamines on blood pressure mediated by both α- and β-adrenoceptors. Thus, the greater decrease seen in phentolamine-treated rats likely resulted from β_2-adrenoceptor stimulation in addition to α-adrenoceptor blockade. This view is supported by the increase in blood pressure seen following subsequent administration of propranolol, a β-adrenoceptor antagonist, in phentolamine-treated rats.

There were differences between the pressor response to i.c.v. choline in normal rats and α-adrenoceptor- or ganglion-blocked rats. First, in the α-adrenoceptor- or ganglion-blocked rats, the maximal blood pressure increase in response to a given dose of choline was slower in onset than the observed response in the normal rats (Fig. 2). In the normal rats, the pressor response to i.c.v. choline (50–150 μg) began 20–40 s after injection and reached a maximum within 2 min (Fig. 2A) as shown previously (Arslan et al., 1991). After α-adrenoceptor or ganglion blockade, the blood pressure increase in response to choline rose to a maximum level within 5 min (Fig. 2B, C). This finding may indicate that the immediate rise in blood pressure in normal rats is mediated by an increase in sympatho-adrenal system activity, which is eliminated after the blockade of ganglion or α-adrenoceptors.

Second, in the α-adrenoceptor or ganglion blocked rats, the increase in blood pressure in response to choline was greater in magnitude and longer lasting than the increase in normal rats (Fig. 2). The maximal increase in blood pressure after the same dose (150 μg) of choline in α-adrenoceptor or ganglion blocked rats was about 3 or 2.5 times higher, respectively, than the observed increase in normal rats (Fig. 2). Moreover, in normal rats the blood pressure returned to the pre-choline levels within 15 min after i.c.v. choline (Fig. 2A), while a considerable and significant pressor response was observable even 25 min after i.c.v. choline in α-adrenoceptor- or ganglion-blocked rats (Fig. 2B, C). These results clearly show that the pressor response to i.c.v. choline is augmented in α-adrenoceptor- or ganglion-blocked rats. This finding is in good accordance with our recent report (Ulus et al., 1995) that the pressor response to i.c.v. choline is enhanced in haemorrhaged, hypotensive rats. The enhanced pressor response to i.c.v. choline in α-adrenoceptor- or ganglion-blocked hypotensive rats can be explained by two different, peripheral and/or central, mechanisms. The peripheral mechanism is that the increase in sensitivity to the pressor agent(s) is due to a very low starting blood pressure. The central mechanism is that the demand for choline increases in some central cholinergic neurones during sustained hypotension, and the normal supply of choline to these cholinergic neurones does not keep pace with the demand for acetycholine synthesis. When this requirement is satisfied, these neurones synthesise and release more acetycholine, which allows maximal functioning at postsynaptic sites. In this way, a greater amount of the pressor agent is released into the circulation and the blood pressure is reversed and maintained at normal or near normal levels (Fig. 2B, C). Our results indicate that the pressor response to i.c.v. choline is mediated by vasopressin (see below) and that the plasma vasopressin response to i.c.v. choline is increased in sympathetically blocked rats. There is also evidence that the pressor sensitivity to vasopressin increases in sympathetically blocked rats (Burnier et al., 1983; Osborn et al., 1987; Savci and Ulus, unpublished observation). Thus, the plasma vasopressin levels and the pressor sensitivity to vasopressin were both much greater in the sympathetically blocked rats than in the control animals. Taken together, it is reasonable to assume that the peripheral and central mechanisms are both involved in the enhanced pressor response to i.c.v. choline in the sympathetically blocked hypotensive rats.

Third, central muscarinic and nicotinic receptors both mediate the pressor response to i.c.v. choline in normal rats (Arslan et al., 1991), whereas the pressor response to i.c.v. choline in α-adrenoceptor- or ganglion-blocked rats is apparently mediated mainly, if not solely, by central nicotinic receptors (Fig. 3). Other studies clearly show that the central muscarinic receptor-mediated pressor response to centrally acting cholinergic drugs is involved in sympatho-adrenal activation (for rewiev see Brezenoff and Guiliano, 1982). In the present study the role of the sympatho-adrenal system in the pressor response was eliminated. This, perhaps, leaves the nicotinic receptors as a primary means for the pressor response to i.c.v. choline. Or a different central cholinergic mechanism is involved in the regulation of blood pressure in hypotensive and normotensive rats. This view is supported by recent reports showing that choline (Ulus et al., 1995), oxotremorine, physostigmine and nicotine (Guarini et al., 1989, 1990, 1991) increase blood pressure and reverse hypotension by activating central nicotinic receptors only in haemorrhaged, sympatho-adrenal system intact rats.

The reductions in heart rate after choline in normal and hexamethonium-treated rats, but not in phentolamine-treated rats, were in accordance with previous results (Arslan et al., 1991). The sustained tachycardia in phentol-

amine-treated rats was blocked by subsequent administration of propranolol and these rats showed a reduction in their heart rate in response to choline, as normal or hexamethonium-treated rats did. These data indicate that the sustained tachycardia in phentolamine-treated rats results mainly from the reduction in heart rate in response to choline.

Plasma levels of vasopressin found in the present study in α-adrenoceptor- or ganglion-blocked rats were several-fold higher than the levels in normal rats (Table 1). These data, in agreement with previous reports (Burnier et al., 1983; Hashemzadeh-Gargari et al., 1988; Lo et al., 1991), show that acute elimination of the pressor action of the sympatho-adrenal system results in an increase in vasopressin release. The slight and transient reduction in blood pressure following vasopressin receptor antagonist was in accordance with previous reports (Burnier et al., 1983; Winn et al., 1985; Gardiner and Bennett, 1988a,b; Lo et al., 1991) showing that the increase in plasma vasopressin plays a minor role in maintaining blood pressure in sympathetically blocked rats if the renin-angiontensin system is intact. Our results show that the plasma vasopressin level increased further following i.c.v. choline (Table 1). Plasma levels of vasopressin encountered during the pressor response to i.c.v. choline were well above the concentrations required for the pressor effect of vasopressin (Osborn et al., 1987). Choline-induced changes in blood pressure and plasma vasopressin were both abolished by pre-treatment with the nicotinic receptor antagonist mecamylamine (Table 2), and the pressor response to i.c.v. choline was reversed by a vasopressin antagonist (Fig. 4). Taken together, these observations support the conclusion that the pressor response to i.c.v. choline in α-adrenoceptor- or ganglion-blocked rats is secondary to an increase in plasma vasopressin.

While vasopressin secretion appears to play a major role in mediating the pressor response to i.c.v. choline in both α-adrenoceptor-blocked and in ganglion-blocked rats, the relationship between the increase in levels of vasopressin and the magnitude of the observed pressor response is not simple. In hexamethonium-treated rats, the increase in vasopressin levels in response to a given dose of choline was relatively less than the increase observed in phentolamine-treated rats (Table 1). But the observed increase in blood pressure in both groups of animals after the same dose of choline was comparable (Table 1). It has been shown that the pressor sensitivity to vasopressin increases by about 2-fold after phentolamine (Burnier et al., 1983; Savci and Ulus, unpublished observation) and 60-fold after hexamethonium (Osborn et al., 1987). Thus, this discrepancy may be explained by a difference in the pressor sensitivity to vasopressin in hexamethonium- and phentolamine-treated rats.

The increase in plasma vasopressin in response to i.c.v. choline was in good accordance with our previous reports (Arslan et al., 1991; Ulus et al., 1995). There is considerable evidence that acetylcholinergic-vasopressinergic linkages exist, which substantiates these findings. Vasopressinergic neurones in the hypothalamic paraventricular and supraoptic nuclei receive a dense cholinergic innervation (Mason et al., 1983; Armstrong, 1985; Hatton and Mason, 1985) and there is evidence for cholinergic receptors in these structures (Mason, 1985; Meeker et al., 1986; Michels et al., 1986). Acetylcholine increases the firing frequency of vasopressin neurones (Dreifuss and Kelly, 1972), resulting in vasopressin release (Sklar and Schrier, 1983; Ivoino and Steardo, 1985; Iitake et al., 1986) by activation of nicotinic receptors. Choline increases nicotinic neurotransmission by acting presynaptically (Ulus et al., 1977a,b, 1978), by increasing the synthesis and release of acetylcholine (Ulus et al., 1989; Koshimura et al., 1990; Johnson et al., 1992; Buyukuysal et al., 1995), as well as by acting post-synaptically as an agonist for nicotinic receptors (Ulus et al., 1988). It has been shown that choline is present in various brain regions (Buccafusco, 1982), including the hypothalamus, and increases acetycholine levels in extracellular medium in the striatum (Koshimura et al., 1990) and in the hypothalamus (Ulus, unpublished observation). When viewed collectively, it is reasonable to assume that the increase in plasma vasopressin levels after i.c.v. choline is caused by an increased nicotinic cholinergic transmission to hypothalamic vasopressin secreting neurones, due to increased acetycholine release and/or direct activation of nicotinic receptors. The possibility that choline increases plasma vasopressin by acting on other brain sites, however, cannot be ruled out by the present results.

In summary, the present data show that i.c.v. administration of choline increases blood pressure and reverses hypotension in α-adrenoceptor or ganglionic transmission blocked rats. Central nicotinic receptor activation and elevation of plasma levels of vasopressin mediate the pressor action of choline.

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