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European Journal of Pharmacology 468 (2003) 129–139
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Intravenously injected CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock: effect is mediated by central cholinergic activation

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Received 19 December 2002; received in revised form 4 March 2003; accepted 11 March 2003

Abstract

Intravenous (i.v.) administration of cytidine-5′-diphosphate choline (CDP-choline) (100, 250 and 500 mg/kg) increased blood pressure in normal rats and reversed hypotension in haemorrhagic shock. Choline (54 mg/kg; i.v.), at the dose equimolar to 250 mg/kg CDP-choline decreased blood pressure of rats in both conditions and caused the death of all hypotensive animals within 2–5 min. Equimolar dose of cytidine (124 mg/kg; i.v.) did not change cardiovascular parameters. Choline levels in plasma, lateral cerebral ventricle and hypothalamus increased after CDP-choline administration. Intracerebroventricular (i.c.v.) hemicholinium-3 pretreatment (20 μg), greatly attenuated the pressor effect of CDP-choline in both conditions. Atropine pretreatment (10 μg; i.c.v.) did not change the pressor effect of CDP-choline while mecamylamine (50 μg; i.c.v.) abolished the pressor response to drug. Besides, acetylcholine (1 μmol; i.c.v.) produced similar increases in blood pressure in normal and hypotensive conditions to that observed in CDP-choline given rats. CDP-choline (250 mg/kg; i.v.) increased plasma catecholamines and vasopressin levels but not plasma renin activity. Pretreatment of rats with either prazosin (0.5 mg/kg; i.v.) or vasopressin V_1 receptor antagonist, [β-mercapto,β,β-cyclopentamethylenepropionyl[1],O-Me-Tyr[2]-Arg[8]]vasopressin (10 μg/kg; i.v.), attenuated the pressor response to CDP-choline while simultaneous administration of these antagonists before CDP-choline injection completely blocked the pressor effect. Results show that i.v. CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock. Activation of central nicotinic cholinergic mechanisms by the increases in plasma and brain choline concentrations appears to be involved in the pressor effect of this drug. Moreover, the increases in plasma catecholamines and vasopressin levels mediate these effects.

Keywords: CDP-choline; Choline; Cytidine; Haemorrhagic shock; Vasopressin; Catecholamine

1. Introduction

Cytidine-5′-diphosphate choline (CDP-choline), an endogenous intermediate in phosphatidylcholine biosynthesis, has numerous beneficial physiological and pharmacological actions on cellular functions (Weiss, 1995). When administered orally or intravenously to rats and humans, CDP-choline is rapidly hydrolyzed to cytidine monophosphate and phosphocholine by phosphodiesterases in the cell wall, and is subsequently dephosphorylated to cytidine and choline (Lopez G.-Coviella et al., 1987; Wurtman et al., 2000). The final hydrolysis products, cytidine and choline, are then taken up by the cells and used for intracellular enzymatic reactions including resynthesis of CDP-choline itself (Dixon et al., 1997; Lopez G.-Coviella et al., 1995; Savci et al., 2002; Weiss, 1995). Both choline and cytidine cross the blood–brain barrier and are efficiently utilized in brain cells where they contribute to critical metabolic functions as formation of nucleic acids, proteins and acetylcholine (Dixon et al., 1997; Weiss, 1995). CDP-choline is also a drug extensively used to treat several cerebral ischemic situations (for recent review, see Adibhatla et al., 2002; D'Orlando and Sandage, 1995; Warach et al., 2000; Wurtman, 1999). It has also beneficial effects in Parkinson's disease (Cubells and Hernando, 1988), Alzheimer's disease (Amenta et al., 2001; Cacabelos et al., 1994, 2000) and glaucoma (Grieb and Rejdak, 2002). It can be given peripherally without any serious side effects and is exceptionally

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doi:10.1016/S0014-2999(03)01602-9

well tolerated in humans. In spite of the clinical interest using CDP-choline, there is no systematic investigation about the pharmacological effect of this drug. We recently demonstrated that centrally injected CDP-choline has an ability to increase blood pressure and reverse haemorrhagic hypotension in conscious rats by increasing brain choline levels and enhancing cholinergic transmission (Savci et al., 2002). Therefore, considering these data, we aimed to determine whether intravenously given CDP-choline can increase blood pressure in normal and hypotensive conditions as centrally given drug does. The present study was designed to investigate the cardiovascular effects of intravenously given CDP-choline in both conditions. Besides, the involvement of central cholinergic system and peripheral vasoactive hormones in these effects was investigated.

2. Methods

Adult male Wistar rats (250–300 g) were used throughout the study. The surgical and experimental protocols were approved by the Animals Care and Use Commitee of Uludag University.

2.1. Surgical procedures

Under ether anesthesia, the left common carotid artery and the left juguler vein of rats were cannulated with PE 60 and PE 50 tubing filled with heparinized saline (250 U/ml), respectively. For intracerebroventricular injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to midline, 1.0 mm posterior to bregma. A 21-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. This guide cannula was lowered 4.5 mm below the surface of the skull and was fixed to the skull with acrylic cement. After surgery, the rats were placed in individual cages and allowed to recover from anesthesia for 4–5 h. During this period, the rats remained calm and without evidence of pain.

2.2. Blood pressure recording

After the recovery period, the arterial cannula was connected to a volumetric pressure transducer (BPT 300) attached to a DA100B general-purpose transducer amplifier (Commat, Ankara, Turkey). Blood pressure and heart rate were recorded and analyzed using the MP100 system and AcqKnowledge software (Biopac Systems, California, USA). The blood pressure was reported as mean arterial pressure (mm Hg), and heart rate was expressed as beats/min.

2.3. Haemorrhage protocol

A total volume of 2–2.1 ml of blood per 100 g body weight was withdrawn over a period of 7–10 min to produce haemorrhagic shock. The blood pressure was then monitored for a stabilization period of 10 min to determine post-haemorrhage levels of blood pressure and heart rate. The arterial catheter was then flushed with 0.1 ml of heparinized saline and reconnected to the transducer.

After this stabilization period, rats received injections through the i.v. or i.c.v. cannula and blood pressure was then monitored for a period of 60 min.

2.4. Experimental protocol

In normotensive animals, after the connection of arterial catheter to the transducer, baseline blood pressure and heart rate measurements were recorded. Rats were allowed to be stabilized for 15 min. At the end of this period, i.v. injections were made and monitoring of cardiovascular parameters was continued for 60 min. In pretreated rats, atropine, mecamylamine and hemicholinium-3 were injected intracerebroventricularly 15 min before saline or CDP-choline administrations. Vasopressin V_1 receptor antagonist or prazosin injections were made intravenously 5 min before saline or CDP-choline injections. In hypotensive rats, 10 min after haemorrhage, saline or CDP-choline was injected and cardiovascular parameters were monitored for the next 60-min period. In pretreated rats, all pretreatments were made 5 min after the end of haemorrhage. Other procedures were the same with that performed in normotensive animals.

All antagonist doses were chosen from our previous experiments. The dose of atropine (10 μg; i.c.v.) and mecamylamine (50 μg; i.c.v.) is able to block the muscarinic or nicotinic effects of choline (150 μg; i.c.v.) (Arslan et al., 1991; Gurun et al., 1997; Savci and Ulus, 1997; Ulus et al., 1995). Hemicholinium-3 dose (20 μg; i.c.v.) is enough to abolish the pressor (Arslan et al., 1991; Ulus et al., 1995) and endocrine (Gurun et al., 1997; Savci et al., 1996) effects of choline (50–150 μg; i.c.v.). In our previous pilot experiments, 10 μg/kg dose of vasopressin V_1 receptor antagonist, [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]vasopressin, blocked the pressor response to i.v. administration of 50 and 100 ng/kg of vasopressin and 0.5 mg/kg dose of prazosin blocked the pressor effect of 5–10 μg/kg of phenylephrine (i.v.).

I.c.v. acetylcholine (1 μmol) injections were performed using the same procedures for CDP-choline injections. The dose of acetylcholine was chosen from our previous report at which CDP-choline or choline was injected centrally in molar doses (Savci et al., 2002).

2.5. Intracerebroventricular injection of drugs

For i.c.v. injection, the injection cannula (25 gauge, 11.5 mm stainless steel tubing) was inserted through the guide cannula. 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 50-

μl microsyringe. Drugs were then infused slowly within 10 s. The volume of saline or drug-containing saline for i.c.v. injection was 10 μl.

2.6. Microdialysis study

Handmade microdialysis probes were used. Rats were anaesthetized with intraperitoneal (i.p.) chloral hydrate (400 mg/kg) and placed in a stereotaxic frame. The skull was exposed and drilled over the right lateral ventricle or hypothalamus (coordinates for lateral cerebral ventricle: 1.0 mm posterior to bregma, 1.5 mm lateral to the midline and 4.5 mm vertical to the skull; for hypothalamus: 1.8 mm posterior to bregma, 1 mm lateral to the midline and 8.9 mm vertical to the skull). Probe (molecular weight cutoff of dialysis membrane was 10,000 Da and length was 1 mm for ventricle and hypothalamus) was implanted and then fixed with acrylic cement to the skull. Dialysis experiments were carried out 18–24 h after operation to avoid effects of anaesthesia. The dialysis probe was perfused with artificial cerebrospinal fluid (pH=7.4) of the following composition: 148 mM NaCl, 3.0 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1.3 mM NaH_2PO_4, 0.2 mM Na_2HPO_4. The perfusion rate was 2 μl/min. Dialysate samples were collected at 10-min intervals. Dialysis probe was perfused with artificial cerebrospinal fluid for the first 60-min stabilization period. After this period, three consecutive samples were collected and these samples were used as basal choline levels of rats.

In hypotensive animals, 24 h after the dialysis probe implantation to the hypothalamus, rats were anaesthetized with ether and left carotid arteries were catheterized to perform the haemorrhage procedure and control cardiovascular parameters. Animals were allowed to recover from anaesthesia for 4–5 h. After the recovery period, microdialysis probe was attached to perfusion pump and arterial catheter was connected to transducer. Dialysis probe was perfused with artificial cerebrospinal fluid for the first 60 min for stabilization period and then three consecutive samples for basal choline levels were obtained. After that, haemorrhage was performed within 10 min and after 10 min of cardiovascular stabilization period, drugs were injected intravenously. Collection of samples were continued during these time periods. Perfusion rate and the collection intervals were the same as performed in normotensive animals.

2.7. HPLC measurements of choline levels

Dialysate samples were injected to a high performance liquid chromatography (HPLC) system combined with an immobilized enzyme reactor and an electrochemical detector (Jasco 840 EC). Briefly, choline was separated on a cation exchange column (from B.A.S.). An enzyme reactor containing acetylcholinesterase and choline oxidase (from B.A.S.) converted choline to hydrogen peroxide. Hydrogen peroxide was then electrochemically detected with a platinum electrode at +0.500 V. The mobile phase consisting of 0.05 M Na_2HPO_4 (pH 8.5) and antibacterial Kathon (0.5%) was delivered by an HPLC pump (Jasco PU 980). The flow rate was 1.0 ml/min. Chromatograms were completed within 6 min, thereby allowing immediate analysis.

2.8. Plasma choline measurement

For measurement of plasma choline levels, blood samples (100 μl) were withdrawn from arterial catheter at the indicated time point. In normotensive rats, blood samples were collected just before and 2, 5, 10, 20 and 60 min after saline or CDP-choline injections. In hypotensive rats, samples were withdrawn before and 10 min after haemorrhage and 2, 5, 10, 20 and 60 min after saline or CDP-choline injections. Plasmas were obtained by centrifugation of blood samples at +4 °C, 1800 rpm for 10 min. Plasma choline levels were measured by radioenzymatic assay.

2.9. Determination of plasma vasopressin, catecholamines and renin activity

To determine plasma vasopressin, plasma adrenaline, noradrenaline levels and plasma renin activity, 2 ml of blood was removed from the arterial catheter 5 min after i.v. saline or CDP-choline injection. Ice-cold polypropylene tubes containing EDTA were used for sample collection. Blood samples were placed on ice immediately. After centrifugation at +4 °C, 1800 rpm, for 20 min, plasmas were separated.

Plasma noradrenaline and adrenaline were determined by using commercially available radioimmunoassay kit which is manufactured by LDN (Nordhorn, Germany) and distributed by DSL Deutschland (Sinsheim, Germany). According to the assay procedure, adrenaline and noradrenaline were first extracted using *cis*-diol-specific affinity gel, acylated to *N*-acyladrenaline or *N*-acylnoradrenaline, and then converted enzymatically during the detection procedure into *N*-acylmetanephrine or *N*-acylnormetanephrine. The assay procedure followed the basic principle of radioimmunoassays.

Aliquots of plasma were also frozen at −20 °C for about 10 days, then they were thawed for vasopressin extraction and radioimmunoassay. Vasopressin was extracted with ethanol and extracts were dried in a vacuum concentrator (Jouan NT, Saint-Herblain, France). During the extraction procedure, the recovery of vasopressin added to rat plasma was 89±2% (mean±S.E.M.) (n=16). The dried residues of the extracts were resuspended with 1 ml of assay buffer, and 400-μl aliquots were assayed in duplicate using a commercially available radioimmunoassay kit (Bühlmann Laboratories, Basel, Switzerland). The values expressed as pg/ml and were not corrected for extraction loses.

Plasma renin activity was determined by using The Gamma Coat [^{125}I] Plasma Renin Activity RIA Kit (Clinical Assay, Cambridge, MA, USA). Plasma (500 μl) was used for the measurement. The assay procedure involved an

initial incubation of plasma to generate angiotensin I, followed by quantitation of angiotensin I by radioimmunoassay. Plasma renin activity was expressed as nanogram of angiotensin I produced by renin per milliliter of plasma during 60 min of incubation.

2.10. Drugs

The following drugs were used: CDP-choline, choline, cytidine, atropine sulfate, acetylcholine, mecamylamine HCl, [β-mercapto,β,β-cyclopentamethylenepropionyl[1],O-Me-Tyr[2]-Arg[8]]vasopressin, prazosin (Sigma, St. Louis, MO, USA) and hemicholinium-3 (RBI, Massachusetts, USA). The drugs were dissolved in saline (0.9% NaCl). All doses of drugs refer to the free base.

2.11. Data and statistical analysis

Data are presented as means±S.E.M. Student's *t*-test was used to test the significance of differences between mean values from different groups of rats. Analysis of variance (one- or two-way) was performed for appropriate groups. Newman–Keuls was performed as a posteriori test if significant interactions were found. A P value of less than 0.05 was considered significant.

3. Results

3.1. Cardiovascular effects of CDP-choline in normotensive rats and in haemorrhagic shock conditions

In normal rats, intravenous administration of CDP-choline (100, 250 and 500 mg/kg) increased mean blood pressure time-dependently (Fig. 1, top). Analysis of variance confirmed that CDP-choline produced nonsignificant dose [$F(3,24)=2.1$, $P=0.1$], but significant time [$F(7,168)=15.6$, $P<0.001$], and a dose–time interaction [$F(21,168]=2.7$, $P<0.001$] effect. At all doses injected, blood pressure reached to its maximum within 2 min and returned to control levels at around 10 min (Fig. 1, top). Interestingly, as seen in Fig. 1 (top), the increase in blood pressure observed after the injection of 500 mg/kg dose of CDP-choline was lower and short-lasting than that observed after the injection of 250 mg/kg CDP-choline. Heart rate did not change significantly; however, in 500 mg/kg dose of CDP-choline group, it tended to decrease from 378±34 to 309±37 beats/min ($P=0.05$) after 2 min of administration (data not shown).

In haemorrhagic shock conditions, intravenous administration of CDP-choline (100, 250 and 500 mg/kg) immediately increased blood pressure and reversed hypotension (Fig. 1, bottom). Analysis of variance revealed a significant effect of CDP-choline dose [$F(3,15)=7.8$, $P<0.05$], time [$F(7,105)=29.7$, $P<0.001$], and a significant treatment–time interaction [$F(21,105)=6.7$, $P<0.001$] on blood pressure. At all groups, blood pressure began to increase within 1 min

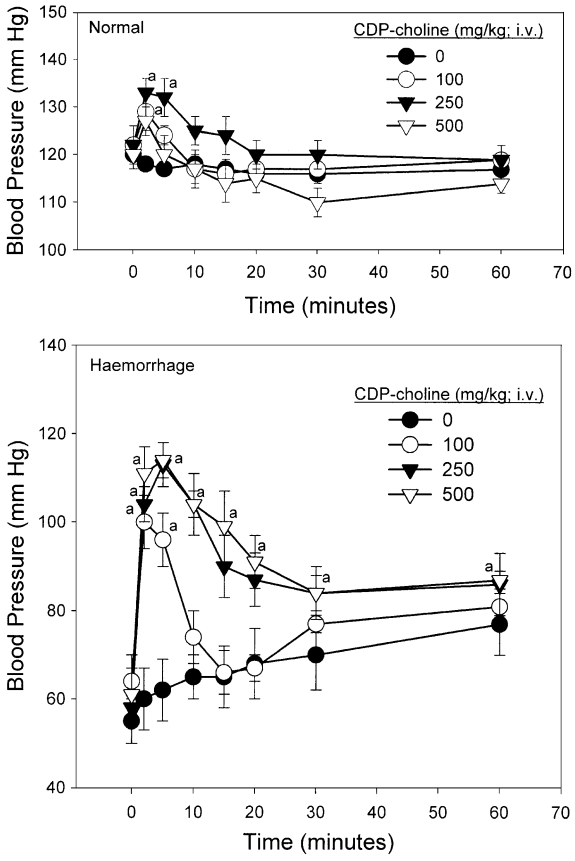

Fig. 1. Blood pressure effects of CDP-choline. Top: In normotensive rats, saline (0.1 ml/kg) or CDP-choline (100, 250 and 500 mg/kg) was administered intravenously. Blood pressure was monitored during the 60-min period after injection. Bottom: Rats were subjected to acute haemorrhage and received saline (0.1 ml/kg) or CDP-choline (100, 250 and 500 mg/kg) intravenously. "0" indicates the time point that saline or CDP-choline was injected after haemorrhage. Blood pressure was monitored for a 60-min period after i.v. injections. Data are given as means±S.E.M. of six to eight measurements. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a]$P<0.05$, significantly different from the value of the saline group.

and reached to its maximum within 5–10 min. Approximately 40 mm Hg increase in blood pressure was observed in the 100 mg/kg CDP-choline injected group while pressor response reached to 55–60 mm Hg in the highest dose of CDP-choline (500 mg/kg; i.v.) injected rats. Heart rates were not statistically different in saline or CDP-choline injected groups in these conditions (data not shown).

3.2. Cardiovascular effects of peripherally injected choline and cytidine in normal and haemorrhagic shock conditions

In order to determine whether intravenously injected equimolar doses of choline and cytidine, immediate hydrolysis products of CDP-choline, can produce independent cardiovascular effects in normal and hypotensive conditions, 54 mg/kg choline or 124 mg/kg cytidine was injected to normal or haemorrhaged rats. Doses were chosen as equimolar to 250 mg/kg CDP-choline. In normal rats, intra-

venously injected choline (54 mg/kg) decreased blood pressure from 115±1 to 77±2 mm Hg 1 min after injection. Blood pressure levels stayed around 90–100 mm Hg for the next 20 min and gradually returned to control levels (Fig. 2, top). Cytidine (124 mg/kg; i.v.) tended to decrease blood pressure from 113±2 to 106±4 mm Hg (n=10) while CDP-choline (250 mg/kg; i.v.) exerted clear pressor effect (Fig. 2, top). Analysis of variance revealed a significant effect of choline and CDP-choline treatment [F(3,22)=3.6, P<0.05], time [F(8, 176)=2.6, P<0.05], and treatment–time interaction [F(24,176)=10.8], P<0.001] on blood pressure. In haemorrhaged rats, intravenous administration of choline (54 mg/kg) immediately decreased blood pressure and caused to death of all animals within 2–5 min (Fig. 2, bottom). However, intravenous administration of equimolar dose of cytidine (124 mg/kg) did not alter hypotensive levels when compared to saline treated group (Fig. 2, bottom). On the other hand, CDP-choline (250 mg/kg; i.v.) reversed hypotension within 2 min in haemorrhaged rats (Fig. 2, bottom). Analysis of variance confirmed that CDP-choline produced significant treatment [F(2,16)=11.6, P<0.001], time [F(8, 128)=11.0, P<0.001], and treatment–time interaction [F(16,128)=5.8, P<0.001)] effect.

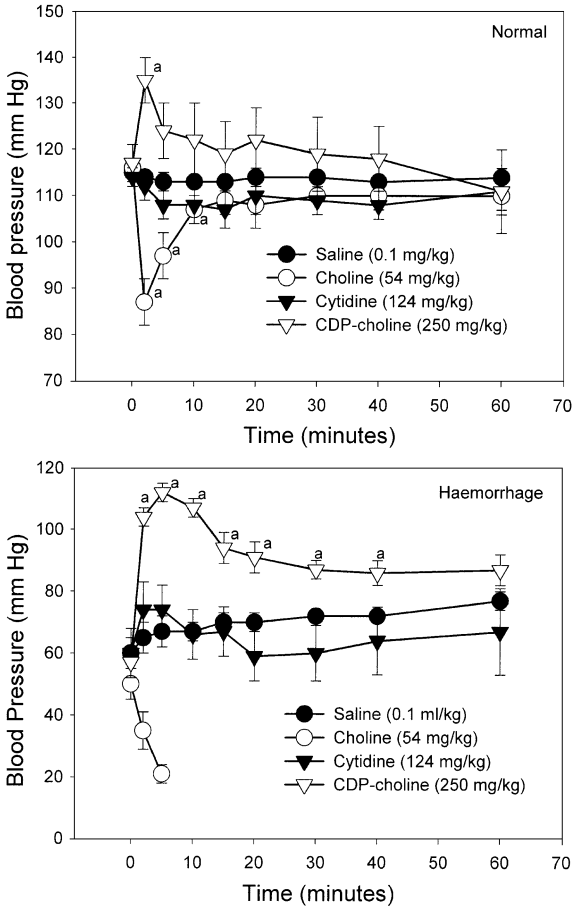

Fig. 2. Blood pressure effects of equimolar doses of CDP-choline, choline and cytidine. Top: In normotensive rats, saline (0.1 ml/kg), CDP-choline (250 mg/kg), cytidine (124 mg/kg) or choline (54 mg/kg) was administered intravenously. Blood pressure was monitored during the 60-min period after injection. Bottom: Rats were subjected to acute haemorrhage and received saline (0.1 ml/kg), CDP-choline (250 mg/kg), cytidine (124 mg/kg) or choline (54 mg/kg) intravenously. "0" indicates the time point that saline or CDP-choline was injected after haemorrhage. Blood pressure was monitored for a 60-min period after i.v. injections. Data are given as means±S.E.M. of four to eight rats. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a]P<0.05, significantly different from the value of the saline group.

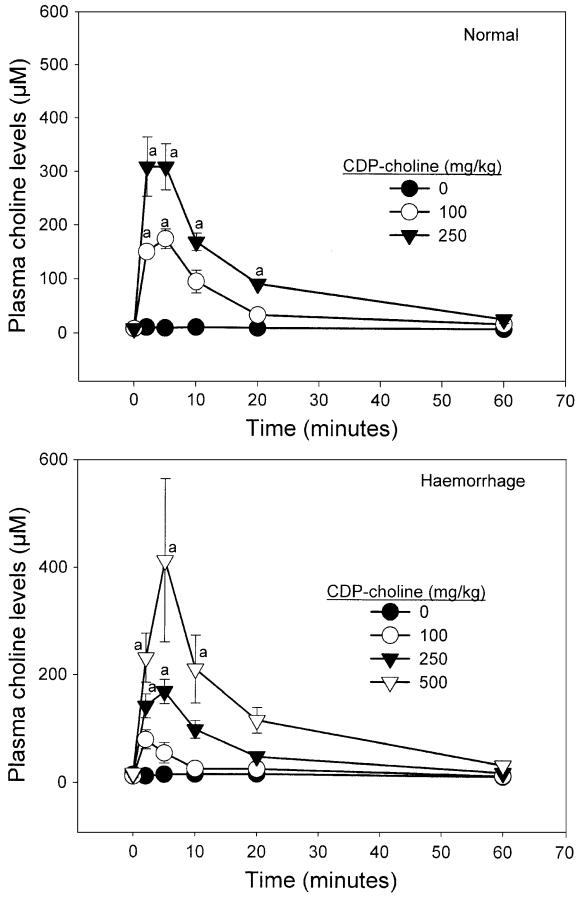

Fig. 3. Increase in plasma choline levels after i.v. CDP-choline administration. Top: In normotensive rats, blood samples were taken at different time intervals after CDP-choline (250 and 500 mg/kg; i.v.) administration. Bottom: Rats were subjected to haemorrhage and CDP-choline was injected (100, 250 and 500 mg/kg) intravenously. Blood samples were taken at 2, 5, 10, 20 and 60 min after injection. Plasmas were separated and used for choline measurement. Data are given as means±S.E.M. of five to six measurements. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a]P<0.05, significantly different from the value of the saline group.

3.3. Increases in plasma choline concentrations after i.v. CDP-choline administration

In this part of the study, we aimed to determine whether plasma choline levels change after i.v. administration of CDP-choline when given at the doses where it produced pressor effects. Basal plasma choline concentration of rats

were 8±2 μM. Haemorrhage itself did not affect the basal levels. Fig. 3 shows the increases in plasma choline levels of normotensive (top) and haemorrhaged (bottom) rats treated with i.v. CDP-choline (100, 250 and 500 mg/kg). Increments of plasma choline concentrations were also dose- and time-related in normal {treatment [$F(2,10)=64.0$, $P<0.001$), time [$F(85,50)=56.6$, $P<0.001$], treatment–time [$F(10,50)=18.9$, $P<0.001$]} and hypotensive {treatment [$F(3,15)=8.4$, $P<0.05$], time [$F(6,90)=15.0$, $P<0.001$], treatment–time [$F(18,90)=5.1$, $P<0.001$]} rats.

3.4. Increases in lateral cerebral ventricle and hypothalamus choline concentrations after peripheral CDP-choline administration

Our recent report demonstrated that the activation of central cholinergic neurons by presynaptic mechanisms are involved in the pressor effect of i.c.v. CDP-choline (Savci et al., 2002). In order to investigate the role of central cholinergic activation by increasing brain choline levels after i.v. CDP-choline, we first determined whether choline concentrations in lateral cerebral ventricle change after peripheral administration of CDP-choline. Basal choline in ventricular perfusate was 1.7±0.3 pmol/10 min. About fourfold increase in lateral cerebral ventricle choline concentrations was observed 10 min after CDP-choline injection (250 mg/kg; i.v.) and choline levels in CDP-choline injected group were significantly higher than those of saline injected rats at all time points during the next 40 min (Fig. 4).

Our previous report also showed that the increases in plasma vasopressin and adrenaline levels mediate the pressor response to i.c.v. CDP-choline (Savci et al., 2002). Considering hypothalamus as a control center for both the regulation of vasopressin secretion and the central sympathoadrenal outflow, we next determined if hypothalamic choline levels can change after i.v. CDP-choline administration. Basal choline levels in hypothalamic perfusates were 1.85±0.71 pmol/10 min. CDP-choline administration (250 mg/kg, i.v.) produced a two to threefold increase in extracellular choline levels in this area. Haemorrhage procedure tended to increase choline levels at hypothalamic perfusates

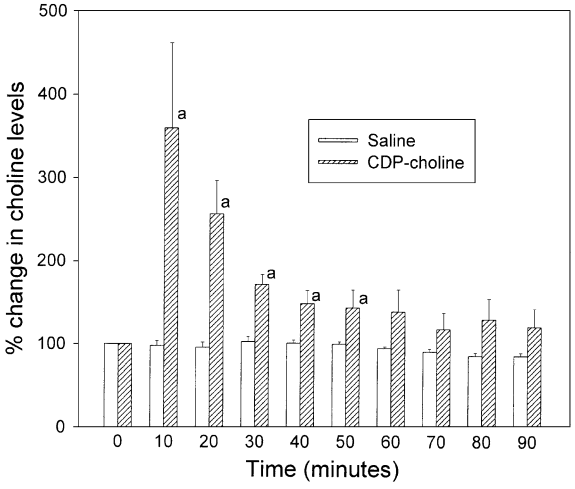

Fig. 4. Changes in the lateral cerebral ventricle choline levels after i.v. CDP-choline administration. At the beginning of the experiment, rats were dialysed 60 min for stabilization period, then saline (0.1 ml/kg) or CDP-choline (250 mg/kg) was injected intravenously. Samples were collected at 10-min intervals before and after injections. Data are given as means± S.E.M. of four to five measurements. Statistical analysis was performed by using one-way ANOVA with post hoc Newman–Keuls test. [a]$P<0.05$, significantly different from the saline group.

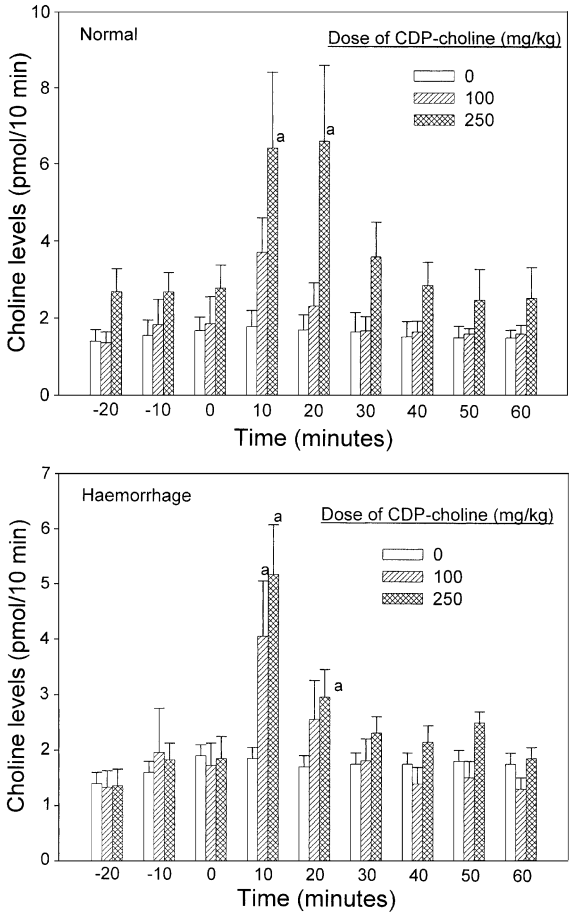

Fig. 5. Extracellular choline levels in hypothalamus after CDP-choline administration in normotensive (top) and hypotensive (bottom) conditions. Top: Rats were dialysed 60 min for stabilization period, then saline (0.1 ml/kg) or CDP-choline (100 and 250 mg/kg) was injected intravenously. Samples were collected at 10-min intervals. "−20", "−10" and "0" indicate the last three samples collected after the stabilization period, and "0" indicates the time point at which saline or CDP-choline was injected. Bottom: Samples were collected at 10-min intervals before and after haemorrhage and injections. "−20" indicates the last sample collected before the beginning of haemorrhage procedure. "−10" indicates the sample collected during haemorrhage and "0" indicates the sample collected during the 10-min stabilization period after the end of haemorrhage. Saline or CDP-choline injection was made at the end of this period. Data are given as means±S.E.M. of four to five measurements. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a]$P<0.05$, significantly different from the value of the control group.

but these increases did not reach a significant value ($P>0.05$). CDP-choline administration after haemorrhage increased extracellular hypothalamic choline levels two to three times. Increases were detected in the first 10-min dialysate after injection and continued to be high in the next two samples. Choline levels returned to basal levels 30–40 min after CDP-choline injection (Fig. 5). Analysis of variance confirmed that CDP-choline produced significant effect on hypothalamic choline levels in normal {treatment [$F(2,9)=4.2$, $P=0.05$], time [$F(6,54)=9.9$, $P<0.001$] and treatment–time interaction [$F(12,54)=3.8$, $P<0.001$]} and hypotensive {treatment [$F(2,9)=10.8$, $P<0.001$], time [$F(6,63)=11.1$, $P<0.001$] and treatment–time interaction [$F(12,63)=2.9$, $P<0.05$]} conditions.

3.5. Effects of hemicholinium-3 pretreatment on the pressor effect of CDP-choline

In order to examine whether CDP-choline increases blood pressure by increasing central cholinergic neurotransmission, we pretreated rats with hemicholinium-3 (20 μg; i.c.v.), a high affinity neuronal choline uptake inhibitor. Hemicholinium-3 pretreatment greatly abolished the increase in blood pressure and reversal of hypotension by CDP-choline given i.v. (250 mg/kg) in normal (Table 1) and hypotensive rats (Fig. 6, top), respectively.

3.6. Effect of acetylcholine on blood pressure

Subsequently, we aimed to investigate if centrally injected acetylcholine can produce similar pressor responses to that observed in CDP-choline injected rats. In normal rats, i.c.v. administered acetylcholine (1 μmol) increased blood pressure from 108±3 to 131±3 mm Hg within 2–5 min. In hypotensive rats, haemorrhage decreased blood pressure from 109±3 to 43±2 mm Hg and then acetylcholine injection (1 μmol; i.c.v.) restored blood pressure to 113±2 mm Hg.

Table 1
Blood pressure response to CDP-choline (250 mg/kg; i.v.) in normotensive rats pretreated by atropine, mecamylamine or hemicholinium-3 intracerebroventricularly

Groups	Increase in blood pressure (mm Hg)
Saline+saline	2.1±0.4
Saline+CDP-choline	12.8±0.8[a]
Atropine+CDP-choline	10.2±1.1[a]
Mecamylamine+CDP-choline	2.6±1.2
Hemicholinium-3+CDP-choline	2.6±1.4

After baseline blood pressure monitorization, saline (10 μl; i.c.v.), atropine (10 μg; i.c.v.), mecamylamine (50 μg; i.c.v.) or hemicholinium-3 (20 μg; i.c.v.) was injected. Fifteen minutes after pretreatment, saline (1 ml/kg) or CDP-choline (250 mg/kg) was administered intravenously. Blood pressure of rats were recorded during 60 min after second injections. Data represent the peak values of blood pressure obtained after second injections. Data are given as means±S.E.M. of six to seven rats.

[a] $P<0.05$, significantly different from the saline+saline group.

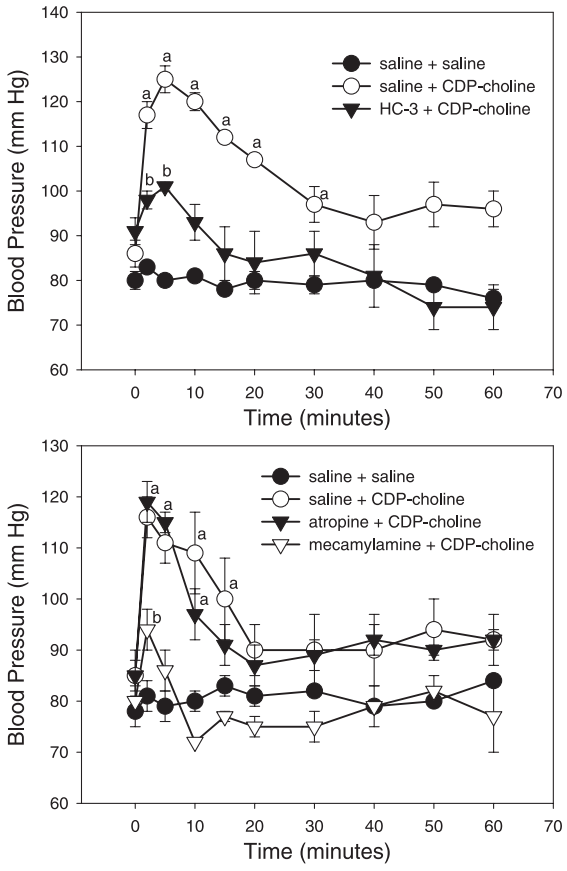

Fig. 6. Effects of hemicholinium-3 and cholinergic antagonist pretreatments on the pressor effects of peripherally injected CDP-choline in hypotensive rats. Top: Saline (10 μl; i.c.v.) or hemicholinium-3 (20 μg; i.c.v.) was injected 5 min after haemorrhage. Fifteen minutes after pretreatment, as indicated by the time point "0", saline (1 ml/kg) or CDP-choline (250 mg/kg) was administered intravenously. Blood pressures of rats were recorded during 60 min after second injections. Bottom: Saline (10 μl; i.c.v.), atropine (10 μg; i.c.v.) or mecamylamine (50 μg; i.c.v.) was injected 5 min after haemorrhage. Fifteen minutes after pretreatment, saline (1 ml/kg) or CDP-choline (250 mg/kg) was administered intravenously. Blood pressures of rats were recorded during 60 min after second injections. Data are given as means±S.E.M. of six to seven rats. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a] $P<0.05$, significantly different from the value of the saline+saline group; [b] $P<0.05$, significantly different from the value of the saline+saline and saline+CDP-choline group.

3.7. Effects of atropine and mecamylamine pretreatments on the pressor effect of CDP-choline

We also examined the type of the cholinergic receptors involved in the pressor effect of CDP-choline. In both conditions, mecamylamine, a nonselective nicotinic receptor antagonist, pretreatment blocked the pressor response to CDP-choline injected i.v. (250 mg/kg), while atropine, a nonselective muscarinic receptor antagonist, failed to change the effect (Table 1; Fig. 6, bottom).

3.8. Effect of CDP-choline on plasma renin activity, catecholamine and vasopressin levels

CDP-choline caused an increase in plasma noradrenaline and vasopressin levels in normotensive rats (Table 2). Haemorrhage itself caused significant increases in plasma levels of catecholamines and vasopressin and plasma renin activity (Table 2). I.v. administration of CDP-choline (250 mg/kg) produced additional increases in plasma adrenaline and vasopressin levels (Table 2). Plasma noradrenaline levels also tended to increase after CDP-choline administration in haemorrhaged rats; however, these increases were not statistically significant ($P>0.05$). Plasma renin activity did not change after CDP-choline administration in both conditions (Table 2).

3.9. Effects of prazosin and vasopressin V_1 receptor antagonist pretreatment on the pressor effect of CDP-choline

In order to investigate the involvement of catecholamines or vasopressin in the pressor effect of CDP-choline, pretreatment with prazosin, α_1-adrenoceptor antagonist (0.5 mg/kg; i.v.) or [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]vasopressin, vasopressin V_1 receptor antagonist (10 μg/kg; i.v.) was performed in normal and haemorrhaged rats. In normal rats, vasopressin V_1 receptor antagonist pretreatment itself did not change blood pressure but attenuated the pressor effect of CDP-choline (Fig. 7, top). Prazosin pretreatment decreased blood pressure from 114±3 to 78±5 mm Hg and greatly blocked the pressor response to i.v. CDP-choline (Fig. 7, top). Administration of

Table 2
Effects of i.v. administered CDP-choline on plasma renin activity, vasopressin and catecholamine levels

Treatment	Plasma renin activity (ng Ang-I/h/ml)	Vasopressin (pg/ml)	Noradrenaline (pg/ml)	Adrenaline (pg/ml)
Normotensive conditions				
Saline	4±1	4±1	39±12	121±24
CDP-choline	5±2	10±2[a]	135±37[a]	130±31
Hypotensive conditions				
Saline	21±4	26±1[a]	155±43[a]	1319±365[a]
CDP-choline	20±2	58±5[b]	278±56	3625±721[b]

In normotensive conditions, rats were injected saline (0.1 ml/kg) or CDP-choline (250 mg/kg). Five minutes after injections, blood samples were collected. In hypotensive rats, rats were subjected to acute haemorrhage. Saline (0.1 ml/kg) or CDP-choline (250 mg/kg) injections were made 5 min after the end of haemorrhage. Blood samples were collected 5 min after injections for measurement of hormones and plasma renin activity. Data are given as means±S.E.M. of six to eight measurements.
Ang-I: angiotensin I.
[a] Significantly different ($P<0.05$) from the value of the saline group of normotensive animals.
[b] Significantly different ($P<0.05$) from the value of the saline group of hypotensive animals.

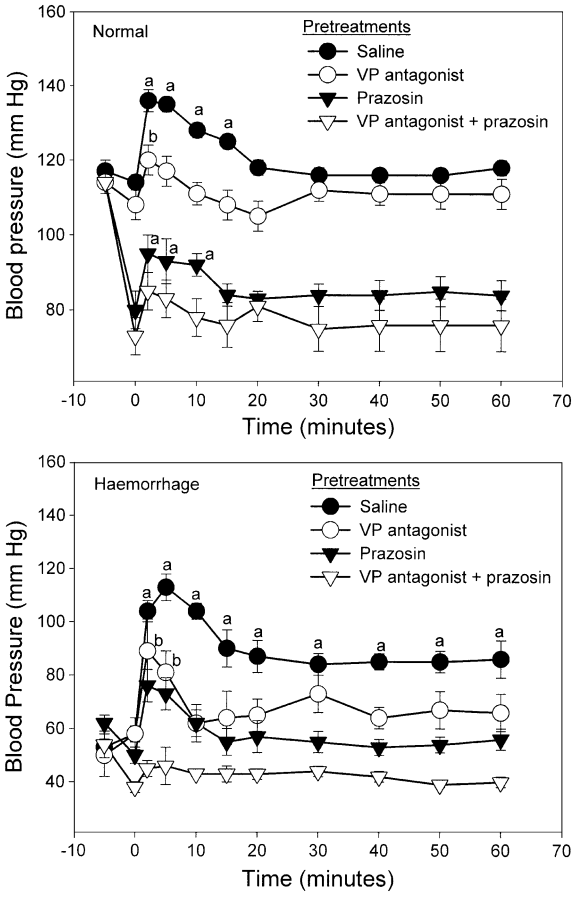

Fig. 7. Effect of α_1-adrenoceptor and vasopressin V_1 receptor antagonist pretreatments on the pressor effect of CDP-choline in normotensive (top) and hypotensive (bottom) rats. Top: After 15 min of monitorization for basal levels, rats were injected saline (0.1 ml/kg; i.v.), prazosin (0.5 mg/kg; i.v.), [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]-vasopressin (10 μg/kg; i.v.) or prazosine (0.5 mg/kg; i.v.) plus [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]vasopressin (10 μg/kg). Five minutes after injection, rats were given CDP-choline (250 mg/kg, i.v.). Blood pressure was monitored during the 60-min period after CDP-choline injection. Bottom: Rats were subjected to haemorrhage. At the end of the stabilization period, they were injected saline (1 ml/kg; i.v.), prazosin (0.5 mg/kg; i.v.), [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]vasopressin (10 μg/kg; i.v.) or prazosin (0.5 mg/kg; i.v.) plus [β-mercapto,β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2-Arg^8]vasopressin (10 μg/kg; i.v.). Five minutes after injection, rats were given CDP-choline (250 mg/kg; i.v.). Blood pressure was monitored during the 60-min period after CDP-choline injection. Data are given as means±S.E.M. of five to six rats. Statistical analysis was performed by using two-way ANOVA with post hoc Newman–Keuls test. [a]$P<0.05$, significantly different from the value of the saline group; [b]$P<0.05$, significantly different from the value of the saline and CDP-choline group. VP antagonist: vasopressin V_1 receptor antagonist.

α_1-adrenoceptor and vasopressin V_1 receptor antagonists together to normal rats caused great blockade in blood pressure (Fig. 7, top). In haemorrhaged rats, either of these pretreatments greatly attenuated the pressor effect of CDP-choline (Fig. 7, bottom). Simultaneous administration of these antagonists in haemorrhaged rats blocked the blood pressure response to CDP-choline (Fig. 7, bottom).

4. Discussion

These data show that intravenous administration of CDP-choline increases blood pressure in normal rats and effectively reverses hypotension in haemorrhagic shock. Also, peripheral administration of CDP-choline increases plasma and brain choline levels. Hemicholinium-3, a neuronal high affinity choline uptake inhibitor, and mecamylamine, a non-selective nicotinic receptor antagonist, pretreatments greatly attenuate the pressor effect. Plasma vasopressin and catecholamine levels increase after CDP-choline and both vasopressin V_1 receptor antagonist and α_1-adrenoceptor antagonis treatments abolish the pressor effect of the drug.

In normotensive rats, pressor effect was small and short-lasting. Approximately 10–15 mm Hg increase in blood pressure was observed after intravenous injection of CDP-choline (Fig. 1). However, the increase in blood pressure was smaller in 500 mg/kg CDP-choline injected rats than that of 250 mg/kg injected group. It is possible that the high choline and cytidine component of 500 mg/kg dose of CDP-choline may lead to a perturbation in the pressor effect of the drug. Because intravenous injection of 54 mg/kg dose of choline which is equimolar to 250 mg/kg CDP-choline decreases blood pressure and although it is not statistically significant ($P>0.05$), equimolar dose of cytidine (124 mg/kg) also tends to decrease blood pressure. This hypothesis was further supported by the findings from our laboratory that the peripheral injection of higher doses of choline (60 and 90 mg/kg; intraperitoneal) and cytidine (250 and 500 mg/kg; intravenous) produced dose-dependent decreases in blood pressure under normal conditions (unpublished data). On the other hand, clear dose–response relationship was observed after CDP-choline administration (100, 250 and 500 mg/kg, i.v.) in haemorrhaged rats (Fig. 1, bottom). As in our previous reports (Savci and Ulus, 1997, 1998; Savci et al., 2001, 2002; Ulus et al., 1995), the pressor effect of the drug was enhanced (by about 55 mm Hg) in hypotensive conditions. These data confirm and extend our previous reports suggesting that the enhancement of central cholinergic transmission and choline demand for acetylcholine synthesis by hypotensive conditions can be satisfied by the treatments that increase plasma and brain choline levels. Thus, the administration of choline or CDP-choline, which have been shown here to increase brain choline levels, allows the maximal cholinergic functioning at the postsynaptic cholinergic receptors under hypotensive conditions.

The blood pressure effects of peripherally administered CDP-choline and choline were opposite to each other in both conditions. It has previously been reported that oral or intravenous administration of equivalent doses of CDP-choline or choline produced dissimilar effects such as choline exerted signs of cholinergic crisis, but CDP-choline did not show any of those symptoms (Agut et al., 1983). Besides, it has recently been published that peripherally given CDP-choline and its two major metabolites, cytidine and choline, have opposite behavioral effects in rats (Carlezon et al., 2002). In that report, cytidine had antidepressant effects, choline had prodepressant effects while the parent drug, CDP-choline, had no effect on behaviour. Our findings are in great agreement with those reports and prove that peripherally administered CDP-choline generates differentiated metabolic consequences from those generated by peripheral choline administration.

In the present study, in accordance with the previous papers showing that orally or intravenously administered CDP-choline increases plasma choline levels (Lopez G.-Coviella et al., 1987, 1995; Wurtman et al., 2000), we demonstrated once again that i.v. administration of CDP-choline increases plasma choline levels dose- and time-dependently. The increases in choline levels were almost 25- to 40-fold in both normotensive and hypotensive conditions. We also demonstrated that i.v. administration of CDP-choline can produce 3- to 4-fold increase in choline levels in lateral cerebral ventricle and hypothalamus. Weiss (1995) has previously reviewed the pharmacokinetics of CDP-choline in experimental animals. The paper reports that only very small amount of the total CDP-choline administered orally or intravenously is taken up as choline and cytidine by the brain (Weiss, 1995). Our findings are in great accordance with this knowledge since only three to four times increase in brain choline levels were observed although plasma choline levels increased almost 40 times after CDP-choline administration (Figs. 3 and 4). Interestingly, the magnitude and time profile of plasma and hypothalamic choline increases observed after peripheral CDP-choline administration were quite similar in normal and hypotensive conditions, but the pressor effect of CDP-choline in hypotensive conditions was much bigger than that observed in normal conditions. These findings nicely correspond with the relationship between neuronal activity and choline responsiveness which has been repeatedly demonstrated in several studies, implicating that in cholinergic neurons, the extent of acetylcholine synthesis and postsynaptic changes when provided with additional choline is related to its physiological activity. Briefly, our observation confirms the hypothesis suggesting that increased cholinergic neuronal activity enhances choline responsiveness (Buyukuysal et al., 1995; Savci et al., 1996, 1998, 2002; Ulus et al., 1989).

Pressor effect of intravenously injected CDP-choline appears to be mediated by the activation of central cholinergic system because (i) peripheral injection of CDP-choline's metabolic products, choline or cytidine, failed to produce similar cardiovascular effects while CDP-choline dramatically increased blood pressure as observed when CDP-choline and choline were injected centrally (Savci et al., 2002); (ii) brain choline levels increased after i.v. CDP-choline; (iii) the blockade of neuronal high affinity choline uptake by i.c.v. hemicholinium-3 pretreatment greatly attenuated the pressor effects of peripherally injected CDP-choline; (iv) the blockade of central cholinergic nicotinic receptors by i.c.v. mecamylamine pretreatment abolished the

pressor effect; and, finally, (v) i.c.v. administration of acetylcholine produced comparable increases in blood pressure in both normal and hypotensive conditions.

The abolition of the pressor response by central hemicholinium-3 pretreatment implicates that the presynaptic cholinergic mechanisms in the brain mainly are involved in the pressor effect. Moreover, central nicotinic cholinergic receptors seem to be involved in the pressor effect of this drug since mecamylamine pretreatment attenuated the pressor effects of CDP-choline, but atropine failed to change the response. These observations greatly correspond with our previous papers reporting the mediation of central nicotinic receptors in the cardiovascular effects of i.c.v. choline and CDP-choline (Arslan et al., 1991; Savci et al., 2002; Ulus et al., 1995). Another evidence supporting our conclusion is that the magnitude of the increase in blood pressure by central administration of acetylcholine was very similar to that observed in CDP-choline injected animals.

The pressor response to intravenous CDP-choline was associated with a several fold increase in the plasma levels of catecholamines—noradrenaline in normal conditions and adrenaline in haemorrhagic shock—and vasopressin but not in plasma renin activity. We know that the increases in circulation and brain choline levels, following oral administration either of choline chloride (Scally et al., 1978) or CDP-choline (Lopez G.-Coviella et al., 1986), result with an increase in the release of catecholamines from adrenal medulla and sympathetic nerves as a consequence of increase in acetylcholine release from preganglionic cholinergic nerves of the sympathetic ganglia and the adrenal medulla. We previously reported the increase in plasma catecholamines, particularly adrenaline, and vasopressin after central administration of choline and CDP-choline (Arslan et al., 1991; Savci et al., 2002; Ulus et al., 1995). Thus, in one hand, our findings are in good accordance with these reports. On the other hand, in the present study we demonstrated that plasma noradrenaline instead of adrenaline levels were increased after i.v. CDP-choline in normal conditions. This contradiction can be explained by the activation of different parts of the sympathoadrenal system by the difference of the drug injection ways. Briefly, we can suggest that in normal conditions intravenously administered CDP-choline primarily activates the sympathetic nerves but centrally given drug mostly increases the adrenomedullary outflow through the stimulation of central cholinergic system. It is very well known that central cholinergic system plays an important role in the regulation of vasopressinergic system. Hence, we can suggest that CDP-choline, at least by increasing central cholinergic transmission, can increase plasma vasopressin levels.

Our data clearly demonstrate that the increases in plasma catecholamines and vasopressin mediate the pressor effect of peripherally injected CDP-choline in rats because α_1-adrenoceptor antagonist and vasopressin V_1 receptor antagonist pretreatments attenuated the pressor response to CDP-choline in addition to the increase in plasma levels of these hormones. Also, simultaneous pretreatment with both antagonists completely blocked the pressor effect of the drug.

The absence of increase in plasma renin activity following CDP-choline administration in normal and hypotensive conditions indicates that plasma renin activity does not actually mediate these effects. These observations are also in great accordance with our previous reports showing no involvement of plasma renin activity in the pressor responses to choline in normal and hypotensive conditions (Arslan et al., 1991; Ulus et al., 1995). There are evidence that cardiogenic shock or haemorrhagic shock generates selective mesenteric ischemia by producing a disproportionate mesenteric vasospasm that is one of the most important reasons to decrease survival in these conditions. Moreover, it has been shown that this mesenteric vasoconstrictive response is mediated primarily by the renin–angiotensin axis (Toung et al., 2000). Therefore, we can suggest that the lack of CDP-choline's effect on plasma renin activity can be an advantage for hypovolemic shock. Indeed, in our preliminary studies, we observed the increase in survival of CDP-choline treated groups in hameorhagic shock (Savci et al., unpublished data).

In conclusion, intravenously given CDP-choline can effectively increase blood pressure and reverse hypotension in rats. Activation of central nicotinic cholinergic receptors through the activation of presynaptic cholinergic system by increasing brain choline levels are involved in the pressor effects. Besides, the increase in plasma vasopressin and catecholamines mediate the pressor response.

Acknowledgements

This study was supported by a grant from the Research Foundations of Uludag University (1998/5) and the Scientific Research Council of Turkey (SBAG-1883). The authors thank chemist Sami Aydin for his excellent technical support during the preparation of microdialysis probes and whole microdialysis study.

References

Adibhatla, R.M., Hatcher, J.F., Dempsey, R.J., 2002. Citicoline: neuroprotective mechanisms in cerebral ischemia. J. Neurochem. 80, 12–23.

Agut, J., Font, E., Sacristan, A., Ortiz, J.A., 1983. Dissimilar effects in acute toxicity studies of CDP-choline and choline. Arzneim.-Forsch. 33 (7A), 1016–1018.

Amenta, F., Parnetti, L., Gallai, V., Wallin, A., 2001. Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors. Ineffective treatments or inappropriate approaches? Mech. Ageing Dev. 16, 2025–2040.

Arslan, B.Y., Ulus, I.H., Savci, V., Kiran, B.K., 1991. Effects of intracerebroventricularly injected choline on cardiovascular functions and sympathoadrenal activity. J. Cardiovasc. Pharmacol. 17, 814–821.

Buyukuysal, R.L., Ulus, I.H., Aydin, S., Kiran, B.K., 1995. 3,4-Diaminopyridine and choline increase in vivo acetylcholine release in rat striatum. Eur. J. Pharmacol. 281, 179–185.

Cacabelos, R., Alvarez, X.A., Franco-Maside, A., Fernandez-Novoa, L., Caamano, J., 1994. Effect of CDP-choline on cognition and immune function in Alzheimer's disease and multiinfarct dementia. Ann. N.Y. Acad. Sci. 16, 211–218.

Cacabelos, R., Alvarez, X.A., Fernandez-Novoa, L., Lombardi, V.R., 2000. A pharmacogenomic approach to Alzheimer's disease. Acta Neurol. Scand., Suppl. 176, 12–19.

Carlezon Jr., W.A., Pliakas, A.M., Parow, A.M., Detke, M.J., Cohen, B.M., Renshaw, P.F., 2002. Antidepressant-like effects of cytidine in the forced swim test in rats. Biol. Psychiatry 51, 882–889.

Cubells, J.M., Hernando, C., 1988. Clinical trial on the use of cytidine diphosphate choline in Parkinson's disease. Clin. Ther. 10, 664–671.

Dixon, C.E., Ma, X., Marion, D.W., 1997. Effects of CDP-choline treatment on neurobehavioral deficits after TBI and on hippocampal and neocortical acetylcholine release. J. Neurotrauma 14, 161–169.

D'Orlando, K.J., Sandage Jr., B.W., 1995. Citicholine (CDP-choline): mechanism of action and effects in ischemic brain injury. Neurol. Res. 17, 264–281.

Grieb, P., Rejdak, R., 2002. Pharmacodynamics of citicoline relevant to the treatment of glaucoma. J. Neurosci. Res. 67, 143–148.

Gurun, M.S., Savci, V., Ulus, I.H., Kiran, B.K., 1997. Centrally administered choline increases plasma prolactin levels in conscious rats. Neurosci. Lett. 232, 79–82.

Lopez G.-Coviella, I., Agut, J., Wurtman, R.J., 1986. Effect of cytidine (5′)-diphosphocholine (CDP-choline) on the total urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG) by rats and humans. J. Neural Transm. 66, 129–134.

Lopez G.-Coviella, I., Agut, J., Von Borstel, R., Wurtman, R.J., 1987. Metabolism of cytidine (5′)-diphosphocholine (CDP-choline) following oral and intravenous administration to the human and the rat. Neurochem. Int. 11, 293–297.

Lopez G.-Coviella, I., Agut, J., Savci, V., Ortiz, J.A., Wurtman, R.J., 1995. Evidence that 5′-cytidinediphosphocholine can affect brain composition by increasing choline and cytidine plasma levels. J. Neurochem. 65, 889–894.

Savci, V., Ulus, I.H., 1997. Cardiovascular effects of central choline during endotoxin shock in the rat. J. Cardiovasc. Pharmacol. 30, 667–675.

Savci, V., Ulus, I.H., 1998. Choline administration reverses hypotension in spinal cord transected rats: the involvement of vasopressin. Neurochem. Res. 23, 737–745.

Savci, V., Gurun, M.S., Ulus, I.H., Kiran, B.K., 1996. Intracerebroventricular injection of choline increases plasma oxytocin levels in conscious rats. Brain Res. 709, 97–102.

Savci, V., Gurun, M.S., Cavun, S., Ulus, I.H., 1998. Cardiovascular effects of centrally injected tetrahydroaminoacridine in conscious normotensive rats. Eur. J. Pharmacol. 346, 35–41.

Savci, V., Cavun, S., Gurun, M.S., Ulus, I.H., 2001. Reversal of haemorrhagic shock in rats by tetrahydroaminoacridine. Pharmacology 62, 36–44.

Savci, V., Cavun, S., Goktalay, G., Ulus, I.H., 2002. Cardiovascular effects of intracerebroventricularly injected CDP-choline in normotensive and hypotensive animals: the involvement of cholinergic system. Naunyn-Schmiedeberg's Arch. Pharmacol. 365, 388–398.

Scally, M.C., Ulus, I.H., Wurtman, R.J., 1978. Choline administration to the rat increases urinary catecholamines. J. Neural Transm. 43, 102–112.

Toung, T., Reilly, P.M., Fuh, K.C., Ferris, R., Bulkley, G.B., 2000. Mesenteric vasoconstriction in response to hemorrhagic shock. Shock 13 (4), 267–273.

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.

Ulus, I.H., Arslan, B.Y., Savci, V., Kiran, B.K., 1995. Restoration of blood pressure by choline treatment in rats made hypotensive by haemorrhage. Br. J. Pharmacol. 116, 1911–1917.

Warach, S., Pettigrew, L.C., Dashe, J.F., Pullicino, P., Lefkowitz, D.M., Sabounjan, L., Harnett, K., Schwiderski, U., Gammans, R., 2000. Effect of citicoline on ischemic lesions as measured by diffusion-weighted magnetic resonance imaging. Citicoline 010 investigators. Ann. Neurol. 48, 713–722.

Weiss, G.B., 1995. Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. Life Sci. 56, 637–660.

Wurtman, R.J., 1999. Neuronal death mechanisms in cerebral ischemia: laboratory at the clinic. Rev. Neurol. 29, 524–526.

Wurtman, R.J., Regan, M., Ulus, I.H., Yu, L., 2000. Effect of oral CDP-choline on plasma choline and uridine levels in humans. Biochem. Pharmacol. 60, 989–992.