



Peripheral administration of CDP-choline, phosphocholine or choline increases plasma adrenaline and noradrenaline concentrations

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Summary

1 Intraperitoneal (i.p.) injection of 200–600 µmol/kg of cytidine-5'-diphosphocholine (CDP-choline) increased plasma adrenaline and noradrenaline concentrations dose- and time-dependently.

2 CDP-choline treatment caused several-fold increases in plasma concentrations of CDP-choline and its metabolites phosphocholine, choline, cytidine monophosphate (CMP) and cytidine.

3 Equivalent doses (200–600 µmol/kg; i.p.) of phosphocholine or choline, but not CMP or cytidine, increased plasma adrenaline and noradrenaline dose-dependently.

4 CDP-choline, phosphocholine and choline (600 µmol/kg; i.p.) augmented the increases in plasma adrenaline and noradrenaline in response to graded haemorrhage.

5 The increases in plasma adrenaline and noradrenaline induced by i.p. 600 µmol/kg of CDP-choline, phosphocholine or choline were abolished by pre-treatment with hexamethonium (15 mg/kg; i.p.), but not atropine (2 mg/kg; i.p.).

6 At 320–32 000 µM concentrations, choline, but not CDP-choline or phosphocholine, evoked catecholamine secretion from perfused adrenal gland. Choline (3200 µM)-induced catecholamine secretion was attenuated by the presence of 1 µM of hexamethonium or mecamlamine, but not atropine, in the perfusion medium.

7 Intracerebroventricular (i.c.v.) injection of choline (0.5–1.5 µmol) also increased plasma adrenaline and noradrenaline dose- and time-dependently. Pre-treatment with mecamlamine (50 µg; i.c.v.) or hexamethonium (15 mg/kg; i.p.), but not atropine (10 µg; i.c.v.), prevented i.c.v. choline (1.5 µmol)-induced elevations in plasma adrenaline and noradrenaline.

8 It is concluded that i.p. administration of CDP-choline or its cholinergic metabolites phosphocholine and choline increases plasma adrenaline and noradrenaline concentrations by enhancing nicotinic cholinergic neurotransmission in the sympatho-adrenal system. Central choline also activates the sympatho-adrenal system by increasing central nicotinic cholinergic neurotransmission.

Keywords: CDP-choline, choline, adrenaline, noradrenaline, cholinergic

Introduction

Cytidine-5'-diphosphocholine (CDP-choline) is an endogenous compound which is involved in cell membrane synthesis by being formed in the rate-limiting step (Kennedy & Weiss, 1956) of the biosynthesis of phosphatidylcholine (PC), the most abundant phospholipid. PC is formed by the reaction of diacylglycerol with CDP-choline, whose synthesis is governed by the action of CTP:phosphocholine cytidyltransferase that combines cyti-

dine triphosphate (CTP) and phosphocholine. Cytidine and uridine (via uridine triphosphate [UTP]) are the precursors of the nucleotide CTP, while phosphocholine is the phosphorylated form of choline, the precursor of the neurotransmitter acetylcholine. Exogenous administration of CDP-choline results in elevations in plasma and tissue levels of choline and one of the pyrimidines; in rats CDP-choline yields cytidine (Lopez G-Coviella *et al.*, 1987; Lopez G-Coviella *et al.*, 1995), while in humans the principal metabolite is uridine

(Wurtman *et al.*, 2000). These intermediates are then utilized for the synthesis of nucleic acids, phospholipids, and acetylcholine. Indeed, CDP-choline treatment has been shown to increase brain phospholipid synthesis (Lopez G-Coviella *et al.*, 1987; Lopez G-Coviella *et al.*, 1995), to enhance cholinergic neurotransmission in central nervous system, and to produce pharmacological effects in cholinergic nature (Savci *et al.*, 2002a,b 2003; Cavun *et al.*, 2004; Cavun & Savci, 2004). Moreover, exogenous CDP-choline has been reported to exhibit beneficial effects in clinical and experimental studies of impaired memory (Spiers *et al.*, 1996; Alvarez *et al.*, 1997), Alzheimer's disease (Cacabelos *et al.*, 1996), Parkinson's disease (Cubells & Hernando, 1988), glaucoma (Grieb & Rejdak, 2002) and in ischemia- and stroke-related conditions (for recent review, see Adibhatla & Hatcher, 2005; Davalos *et al.*, 2002; Secades and Lozano, 2006).

Compared with reports on CDP-choline's actions in the central nervous system, relatively little is known on the effects of CDP-choline administration on sympatho-adrenal system functions. More than 20 years ago, Lopez G-Coviella *et al.* (1986) have shown that oral administration of CDP-choline to humans and rats increases urinary excretions of noradrenaline metabolite, 3-methoxy-4-hydroxyphenylglycol. Recent studies from our laboratory have shown that intravenous (Savci *et al.*, 2003) or i.c.v. (Savci *et al.*, 2002a) administration of CDP-choline elevates circulating levels of catecholamines in rats by activating the sympatho-adrenal system. We also demonstrated that increase in plasma catecholamines evoked by central (Savci *et al.*, 2002a) or peripheral (Savci *et al.*, 2003) CDP-choline administration is involved in the pressor response in normal rats or in rats made hypotensive by haemorrhage. Neither peripheral nor central cholinergic receptors that mediate this plasma catecholamine response to CDP-choline have been identified. Furthermore, it is not known whether CDP-choline's cholinergic metabolites (i.e. phosphocholine and choline) and pyrimidinergic metabolites (i.e. cytidine monophosphate [CMP] and cytidine) affect plasma catecholamine concentrations.

Thus, the present study was undertaken to characterize the mechanisms responsible for the increase in plasma catecholamine concentrations produced by peripheral and central CDP-choline administration in conscious rats. The objectives were to (1) determine the dose–response relationship and the time-course of plasma catecholamine response to CDP-choline, (2) determine the involvements of CDP-choline metabolites, phosphocholine, choline, CMP and cytidine in plasma catecholamine responses to CDP-choline, and (3) identify the central and peripheral cholinergic receptors that mediate the effect of CDP-choline

and its metabolites on plasma catecholamine concentrations. In addition, we tested whether choline, a metabolite of CDP-choline and the precursor of the neurotransmitter acetylcholine, enhanced plasma catecholamine response to graded haemorrhage.

Methods

Animals

Female Wistar rats (Experimental Animals Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) weighing 250–275 g were used in all experiments. Four 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).

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

Surgical procedures

For blood sampling, left carotid arteries of rats were cannulated using PE 60 tubing filled with heparinized saline (250 IU/ml) as described previously (Ulus *et al.*, 1995). An additional tubing (PE 50) was inserted into peritoneal space using an 18G injector tip as a guide to ensure i.p. injections. The tips of both tubings were sealed and exteriorized at the neck of the rat. In some experiments, for intracerebroventricular injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to mid-line and 1.0 mm posterior to bregma and a 10 mm length of 21-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The cannula was lowered 4.5 mm below the surface of the skull and was fixed to the skull with acrylic cement. Following the completion of these surgical and cannulation procedures, the rats were placed in individual cages and allowed to recover from anaesthesia for 4 h. During this period, rats remained undisturbed and calm and showed no evidence for pain.

Perfusion of the adrenal gland and measurement of catecholamine release

The left adrenal gland of rat was isolated and perfused as described previously (Ulus *et al.*, 1988). Briefly, rats were anesthetized with ketamine (60 mg/kg, i.p.), the abdomen was opened by a mid-line incision, left adrenal gland was exposed and a perfusion cannula (PE 50) was inserted into the distal end of the renal vein after all the branches of the adrenal vein, the renal vein and the vena cava were ligated. A small slit was made in the adrenal

cortex just opposite the entrance of the vein, and the adrenal gland, along with the ligated blood vessels and the cannula, was carefully removed and perfused with Krebs-Ringer buffer (pH 7.4) of the following composition (mM): NaCl, 120; KCl, 3.7; CaCl₂, 2.5; MgSO₄, 1.20; KH₂PO₄, 1.20; NaHCO₃, 25.0; and glucose, 11.0. The perfusion rate was 0.5 ml/min; the medium was bubbled continuously with 95% O₂/5% CO₂ and was maintained at 37 °C.

Graded haemorrhage protocol

Rats were subjected to graded haemorrhage as described previously (Ulus *et al.*, 1995; Savci *et al.*, 2003). Briefly, a blood sample (0.55 ml per 100 g body weight) was withdrawn over 10 s from the arterial catheter into a chilled plastic syringe containing 0.1 ml of sodium EDTA solution (50 mg/ml). The catheter was then flushed with 0.3 ml of heparinized saline and connected to the pressure transducer and arterial pressure was monitored for the following 5 min. This procedure was repeated four times at 5-min intervals. The cumulative blood loss in each animal consisted of volumes equal to 0.55, 1.10, 1.65 and 2.20 ml per 100 g of body weight. Blood samples, designated S1 through S4, were centrifuged (1500 g for 10 min), plasmas were separated and kept in –80 °C for future catecholamine assays.

Experiments

To minimize non-specific increases and variations in plasma catecholamine levels in response to experimental interventions (i.e. handling and i.p. or i.c.v. injection) and blood withdrawal, each rat was used once in all of the experiments described below with an exception of the second series of experiments in which rats subjected to the graded haemorrhage protocol.

In the first series of experiments, the time- and dose-relations of plasma catecholamine concentrations in response to i.p. CDP-choline and its metabolites were determined in five related studies as follows:

Study 1. Eighty-four rats were divided equally into two groups; the first group received 1 ml/kg of saline i.p., the second group received 600 µmol/kg of i.p. CDP-choline dissolved in 1 ml/kg of saline. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 5, 10, 20, 30, 45 and 60 min after the i.p. injection from six rats at each of six time points for plasma catecholamines, CDP-choline, phosphocholine, choline, CMP and cytidine measurements. Blood samples were also obtained from six rats in each group before injection of any of the treat-

ments (time 0) to determine basal values of plasma catecholamines.

Study 2. Twenty-four rats were divided equally into four groups. The first group received 1 ml/kg of saline i.p., while the second, the third and the fourth groups received 200, 400 and 600 µmol/kg of i.p. CDP-choline, respectively. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 10 min after i.p. injections for plasma catecholamine measurements.

Study 3. Eighty-four rats were divided equally into two groups. The first group received 1 ml/kg of saline i.p., while the second group received 600 µmol/kg of i.p. choline dissolved in 1 ml/kg of saline. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 5, 10, 20, 30, 45 and 60 min after the i.p. injections for plasma catecholamine measurements. Blood samples were obtained from six rats in each group before any treatment (time 0) to determine basal values of plasma catecholamines.

Study 4. Twenty-four rats were divided equally into four groups. The first group received 1 ml/kg of saline i.p. The second, the third and the fourth groups received 200, 400 and 600 µmol/kg of i.p. choline, respectively. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 10 min after i.p. injections for plasma catecholamine measurements.

Study 5. Sixty rats were divided equally into 10 groups. The first group received 1 ml/kg of saline i.p. The second, the third and the fourth groups received 200, 400 and 600 µmol/kg of phosphocholine i.p., respectively. The fifth, sixth, and seventh groups received 200, 400 and 600 µmol/kg of CMP i.p., respectively. The eighth, ninth and tenth groups received 200, 400 and 600 µmol/kg of cytidine i.p., respectively. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 10 min after i.p. injections for plasma catecholamine measurements.

In the second series of experiments, effects of i.p. CDP-choline, phosphocholine and choline on plasma catecholamine response to graded haemorrhage were studied in 24 rats. Rats were divided equally into four groups. The first, second, third and fourth groups received i.p. saline (1 ml/kg), CDP-choline (600 µmol/kg), phosphocholine (600 µmol/kg) and choline (600 µmol/kg), respectively. Rats were then subjected to graded haemorrhage protocol (as described above) by removing blood samples (0.55 ml/100 g of per body weight)

sequentially at 5, 10, 15 and 20 min after i.p. injections. Blood samples were designated S1 through S4 and they were maintained on ice for plasma catecholamine measurements.

In the third series of experiments, we determined the effect of blockade of peripheral cholinergic receptors on plasma catecholamine responses to i.p. CDP-choline, phosphocholine and choline. Rats were pre-treated i.p. with either saline (1 ml/kg), the muscarinic receptor antagonist, atropine methylnitrate (2 mg/kg) or the ganglionic nicotinic receptor antagonist, hexamethonium (15 mg/kg), 15 min before the i.p. injection of saline (1 ml/kg), CDP-choline (600 µmol/kg), phosphocholine (600 µmol/kg) or choline (600 µmol/kg). Blood samples were withdrawn (1 ml) from the arterial catheters slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 10 min after the second i.p. injection for plasma catecholamine measurements.

In the fourth series of experiments, effects of CDP-choline, phosphocholine and choline on plasma catecholamine release from the isolated perfused adrenal gland were studied *in vitro*. Adrenal gland was isolated and perfused with the Krebs-Ringer buffer as described above. After a 30-min equilibration period, the perfusate was collected for 5 min. The lowest concentration of the compound to be tested was then added to the perfusion medium and the perfusate was again collected for 5 min. In the next step, the adrenal gland was perfused with drug-free medium for 10 min, and a higher concentration of the drug of interest was added to the perfusion medium and the perfusate was collected for 5 min. This procedure was repeated five to seven times until the drug concentration was raised to 32 mM. Catecholamine content of the perfusate was measured directly by the fluorometric method as described previously (Ulus *et al.*, 1988). A volume of 0.25 ml of the perfusate was used for the assay. The catecholamine content of the perfusate was normalized as ng/5 min. In a related study, the effects of cholinergic receptor blockade on choline-evoked catecholamine release was tested by addition of choline (3200 µM) to perfusion medium in the absence (control) or presence of 1 µM of each hexamethonium, mecamlamine, or atropine.

In the fifth series of experiments, we determined the effect of central (i.c.v.) choline on plasma catecholamine levels in the two separate studies as follows:

Study 1. Eighty-four rats were divided equally into two groups: the first group received saline (10 µl) i.c.v., while the second group received i.c.v. choline (1.5 µmol). Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 5, 10, 20, 30, 45 and 60 min after i.p. injections

for plasma catecholamine measurements. Blood samples were obtained from six rats in each group before any treatment (time 0) to determine basal values of plasma catecholamines.

Study 2. Twenty-four rats were divided equally into four groups. The first group received i.c.v. saline (10 µl), while the second, the third, and the fourth groups received i.c.v. 0.5, 1.0, 1.5 µmol of choline, respectively. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 10 min after i.c.v. injections for plasma catecholamine measurements.

In the sixth series of experiments, we determined the effect of central cholinergic receptor blockade on plasma catecholamine responses to i.c.v. choline in 36 rats. Rats were pre-treated i.c.v. either with saline (10 µl), the muscarinic receptor antagonist, atropine (10 µg) or the nicotinic receptor antagonist, mecamlamine (50 µg), 15 min prior the i.c.v. treatment of saline (10 µl) or choline (1.5 µmol). Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 5 min after the second i.c.v. injection for plasma catecholamine measurements.

In a related study, two groups of rats (six rats in each group) were pre-treated either with i.p. hexamethonium (15 mg/kg) or i.c.v. mecamlamine (50 µg) 15 min prior to i.c.v. treatment with choline (1.5 µmol) or i.p. treatment with choline (600 µmol/kg), respectively. Blood samples (1 ml) were withdrawn from the arterial catheter slowly into ice-cold tubes containing 50 mg/ml sodium EDTA at 5 min after the second treatment for plasma catecholamine measurements.

Measurements

Blood samples were kept on ice and plasmas were obtained by centrifugation (1500 g for 10 min) at 4 °C.

Plasma noradrenaline and adrenaline were determined using a commercially available radioimmunoassay kit (Cat # DSL-BA-1500; DSL, Sinsheim, Germany) according to the instructions of the manufacturer. Briefly, noradrenaline and adrenaline were first extracted from plasma using *cis*-diol-specific affinity gel, acylated to *N*-acylnoradrenaline or *N*-acyladrenaline, and then converted enzymatically during the detection procedure to *N*-acylnormetanephrine or *N*-acylmetanephrine. The intra- and inter-assay variations coefficients were 5% or 4% and 6% or 5% for adrenaline or noradrenaline, respectively. Recoveries of adrenaline and noradrenaline from plasma were 98% and 91%, respectively. The sensitivity of assay kit was 10 pg/ml or 45 pg/ml for plasma adrenaline or noradrenaline, respectively. Plasma adrenaline and noradrenaline concentrations were expressed as pg/ml.

For CDP-choline, CMP, and cytidine assays 0.2 ml of plasma sample was deproteinized by addition of 2 ml of methanol. After centrifugation, two sets of aliquots (0.8 ml of each) from supernatant were dried under vacuum. One set of dried samples was reconstituted with 200 µl of deionized water and assayed for CDP-choline and CMP using high performance liquid chromatography (HPLC) method as described previously (Richardson *et al.*, 2003). The second set of dried samples, following purification on boronate affinity columns (Affigel-601, Bio-Rad, Hercules, CA, USA), was analysed for cytidine by HPLC on a reversed-phase column (Dynamax Microsorb C-18, 5 mm, 250 × 4.6 mm) using 4 mM potassium phosphate buffer containing 0.1% methanol at pH 5.8 as described previously (Wurtman *et al.*, 2000).

Free choline was extracted and assayed as described previously (Ilcol *et al.*, 2005). Phosphocholine was first hydrolyzed enzymatically to free choline by alkaline phosphatase, and the resulting choline was assayed, as described previously (Ilcol *et al.*, 2005).

Drugs

The following drugs were used: CDP-choline (cytidine 5'-diphosphocholine sodium), phosphocholine chloride calcium, choline chloride, CMP (cytidine 5'-monophosphate disodium), cytidine, atropine methylnitrate, atropine sulfate, mecamlamine hydrochloride, and hexamethonium hydrochloride (Sigma Chemical Co., St. Louis, MO, USA). The drugs were dissolved in saline (0.9% NaCl). All doses of drugs refer the free base.

Statistics

Statistical analyses were performed using SigmaStat®, Version 3.1 (Systat Software Inc., San Jose, CA, USA). Data were analysed by one- or two-way analysis of variance (ANOVA) followed by *post hoc* Tukey test. $P < 0.05$ was considered significant. Data are presented as mean ± SEM.

Results

Effects of CDP-choline on plasma adrenaline and noradrenaline concentrations

Baseline plasma adrenaline and noradrenaline concentrations prior to i.p. saline or CDP-choline injection were 174 ± 9 pg/ml ($n = 12$) and 153 ± 12 pg/ml ($n = 12$), respectively. In control, saline-treated, rats plasma adrenaline [$F(6,35) = 1.42$, $P = 0.2$] and noradrenaline [$F(6,35) = 0.23$, $P = 0.96$] concentrations remained unchanged during 60-min observation period (Fig. 1).

CDP-choline (600 µmol/kg; i.p.) increased plasma adrenaline [$F(1,82) = 6.54$; $P < 0.05$] and nor-

adrenaline [$F(1,82) = 5.14$; $P < 0.05$] levels in a time-dependent manner (Fig. 1). Plasma adrenaline and noradrenaline concentrations started rising within the first 5 min and peaked 10 min following i.p. administration of 600 µmol/kg of CDP-choline (Fig. 1). The increases in plasma adrenaline at 10 min after various doses of CDP-choline (200, 400 and 600 µmol/kg; i.p.) were dose-related (Fig. 1c). CDP-choline at 200 or 400 µmol/kg doses failed to alter plasma noradrenaline levels (Fig. 1d).

Plasma concentrations of CDP-choline and its metabolites

Administration of CDP-choline (600 µmol/kg; i.p.) resulted in increases in plasma concentrations of CDP-choline (Fig. 2a) and its immediate metabolites CMP (Fig. 2b), cytidine (Fig. 2c), phosphocholine (Fig. 2d), and choline (Fig. 2e).

Effects of choline and other CDP-choline metabolites on plasma adrenaline and noradrenaline concentrations

To determine whether metabolites of CDP-choline alter plasma catecholamine concentrations, rats were treated i.p. with equivalent doses of its metabolites choline, phosphocholine, CMP or cytidine.

Choline (200, 400 and 600 µmol/kg; i.p.) increased plasma adrenaline (Fig. 3c) and noradrenaline (Fig. 3d) concentrations in a dose- (Fig. 3c,d) and time-dependent (Fig. 3a and b) manner. Two-way ANOVA revealed a significant effect of 600 µmol/kg dose of choline [$F(1,82) = 35.30$; $P < 0.001$] or [$F(1,82) = 8.88$; $P < 0.001$], time [$F(6, 82) = 5.75$; $P < 0.001$] or [$F(6, 82) = 4.84$; $P < 0.001$] and a significant dose-time interaction [$F(6,82) = 7.95$; $P < 0.001$] or [$F(6, 82) = 4.68$; $P < 0.001$] on plasma adrenaline or noradrenaline concentrations, respectively.

Phosphocholine (200, 400 and 600 µmol/kg; i.p.) significantly increased plasma adrenaline (Fig. 4a) and noradrenaline (Fig. 4b) concentrations in a dose-dependent manner. The increases in plasma noradrenaline were relatively higher than the observed increases in plasma adrenaline (Fig. 4a and b).

Administration of CMP (200, 400 and 600 µmol/kg; i.p.) or cytidine (200, 400 and 600 µmol/kg; i.p.) failed to alter plasma concentrations of adrenaline and noradrenaline (Fig. 4c–f).

Effects of i.p. CDP-choline, phosphocholine and choline on plasma catecholamine responses to graded haemorrhage

Changes in plasma adrenaline and noradrenaline concentrations in response to graded haemorrhage

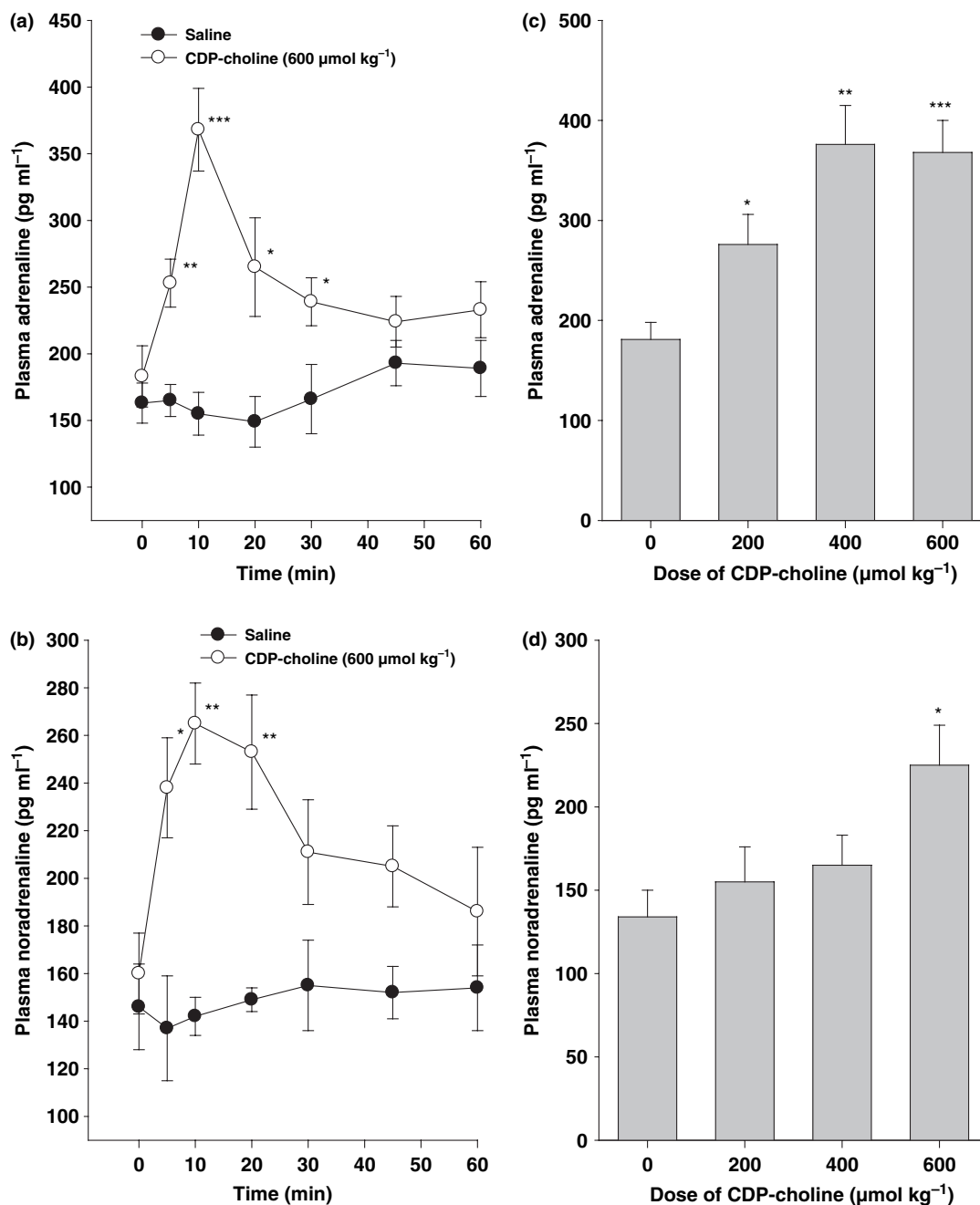


Figure 1 Time and dose relations of plasma adrenaline and noradrenaline responses to i.p. administration of CDP-choline. Time-course study (a and b): Rats were injected i.p. with either saline (1 ml/kg) or CDP-choline (600 μmol/kg). Blood samples (1 ml) were collected immediately before (0 min), and 5, 10, 20, 30, 45 and 60 min after each treatment through the catheter inserted into the left carotid artery from six rats from both groups for each time point and analysed for adrenaline (a) and noradrenaline (b). Each point represents the mean ± SEM of six measurements. Data were analysed using two-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls. Dose-course study (c and d): Rats were injected i.p. with either saline (1 ml/kg) or CDP-choline (200, 400 or 600 μmol/kg). Blood samples (1 ml) were collected at 10 min after each treatment through the catheter inserted into the left carotid artery. Each point represents the mean ± SEM of six measurements. Data were analysed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls.

are shown in Fig. 5. Sequential removal of a volume of blood equal to 0.55 ml/100 g body weight

(Fig. 5a) and noradrenaline concentrations (Fig. 5b). In control rats, plasma adrenaline and noradrenaline concentrations at the fourth blood

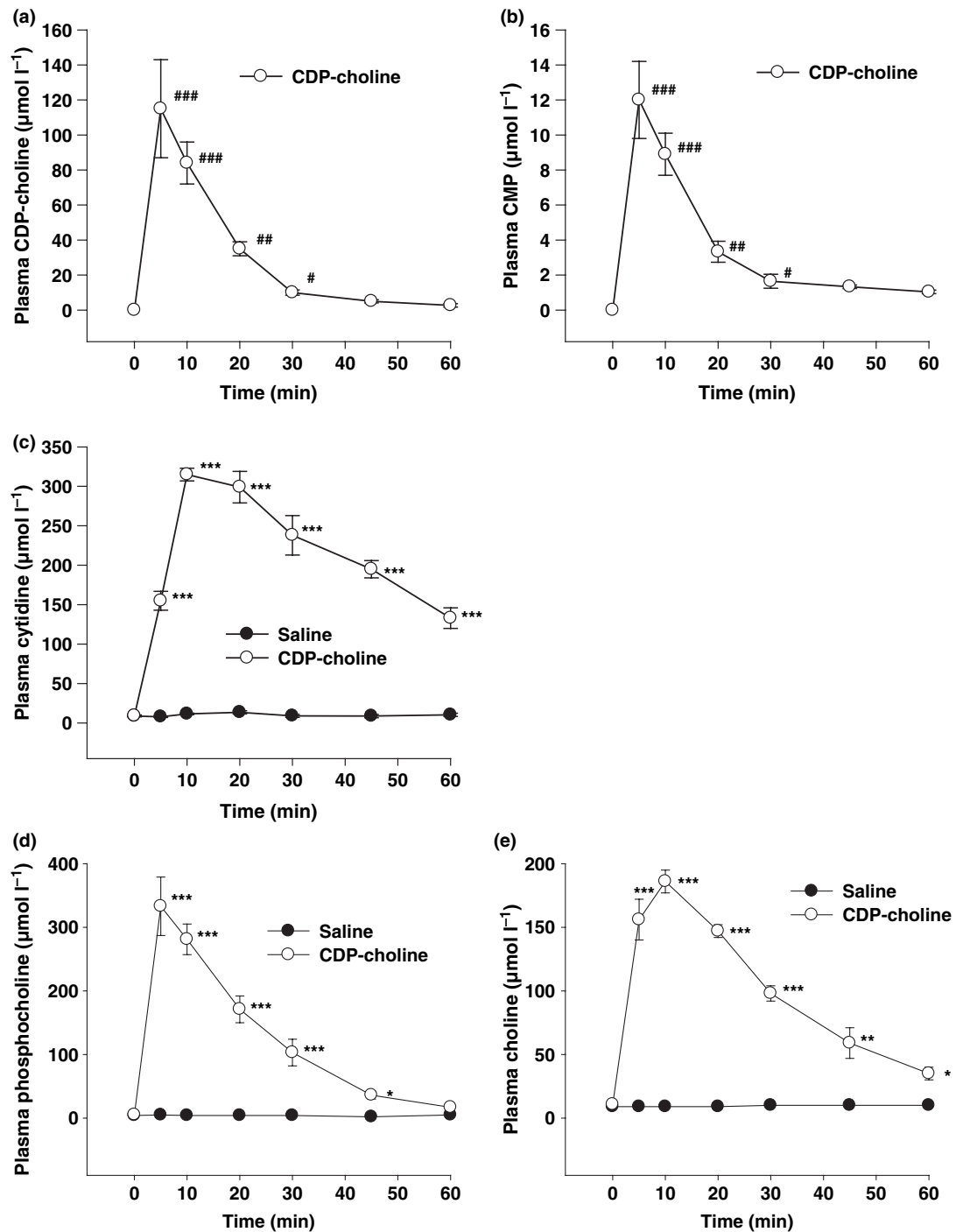


Figure 2 Increases in plasma concentrations of CDP-choline, CMP, cytidine, phosphocholine and choline after i.p. administration of CDP-choline. Aliquots of plasma from blood samples obtained from rats in 'time-course study' described in Figure 1 legend were analysed for CDP-choline (a), CMP (b), cytidine (c), phosphocholine (d) and choline (e). Data were analysed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls (for cytidine, phosphocholine and choline). Since basal plasma levels of CDP-choline and CMP were undetectable, we assume that basal levels of these compounds were equal to zero (0). # $P < 0.05$; ## $P < 0.01$ and ### $P < 0.001$ compared with basal values (for CDP-choline and CMP).

samples (S4) were, 1574 ± 106 pg/ml ($n = 6$) and 398 ± 39 pg/ml ($n = 6$), about 8.8- and 2.4-fold higher than those found in the first blood samples (S1), 180 ± 24 pg/ml ($n = 6$) and 168 ± 17 pg/ml

($n = 6$), respectively. Plasma adrenaline and nor-adrenaline responses were enhanced significantly by i.p. administration of $600 \mu\text{mol/kg}$ of CDP-choline, phosphocholine and choline (Fig. 5).

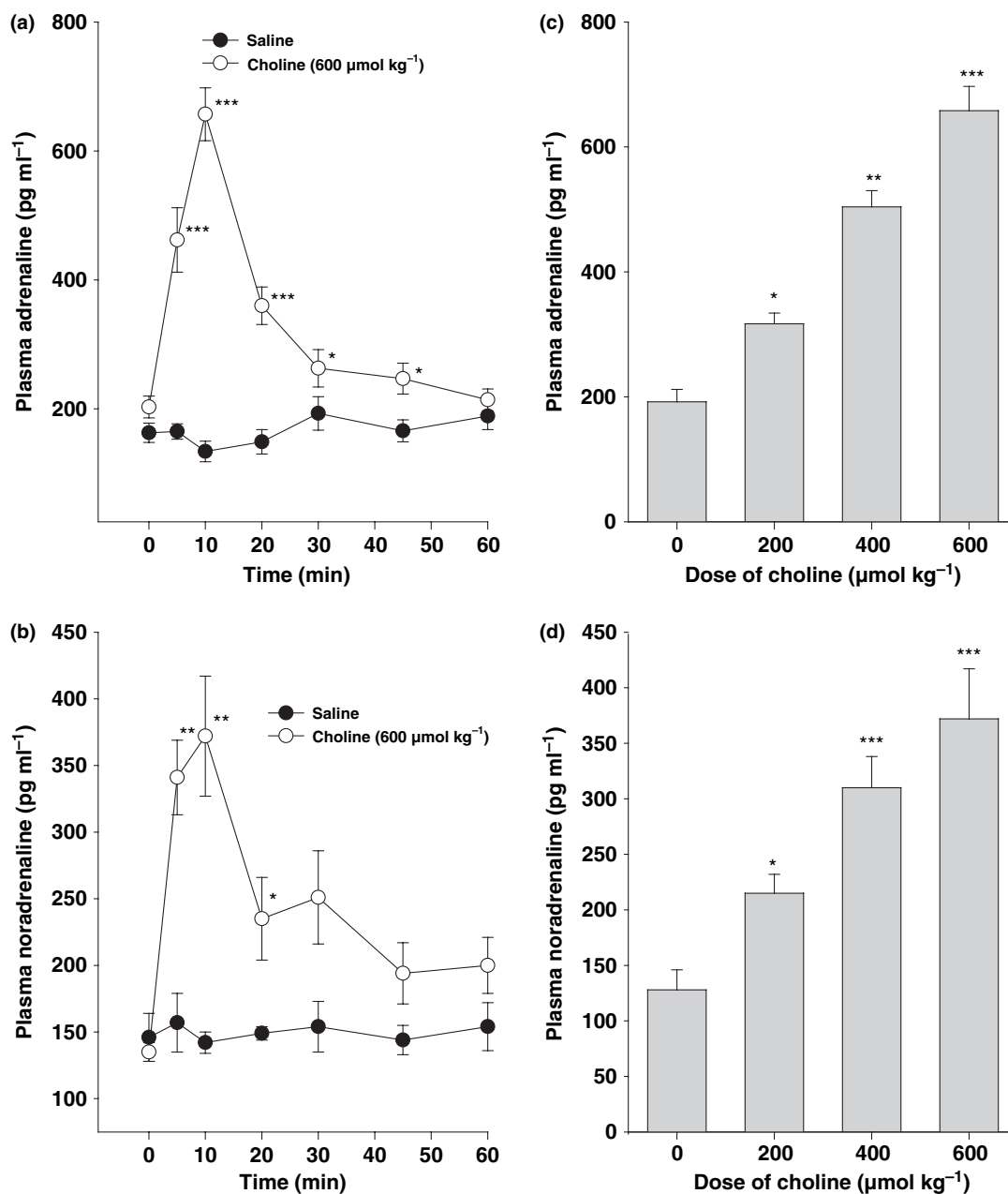


Figure 3 Time and dose relations of plasma adrenaline and noradrenaline responses to i.p. administration of choline. Time-course study (a and b): Rats were injected i.p. with either saline (1 ml/kg) or choline (600 μmol/kg). Blood samples (1 ml) were collected immediately before (0 min), and 5, 10, 20, 30, 45 and 60 min after each treatment from six rats from both groups for each time point and analysed for adrenaline (a) and noradrenaline (b). Each point represents the mean ± SEM of six measurements. Data were analysed using two-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls. Dose-course study (c and d): Rats were injected i.p. with either saline (1 ml/kg) or choline (200, 400 or 600 μmol/kg). Blood samples (1 ml) were collected at 10 min after each treatment. Each point represents the mean ± SEM of six measurements. Data were analysed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls.

Effects of blockade of peripheral cholinergic receptors on plasma adrenaline and noradrenaline responses to CDP-choline, choline and phosphocholine

To determine the involvement of peripheral nicotinic and/or muscarinic receptors in plasma cate-

cholamine responses to CDP-choline and its cholinergic metabolites, rats were pre-treated i.p. with saline (1 ml/kg), hexamethonium (15 mg/kg), or atropine methylnitrate (2 mg/kg). Plasma catecholamine concentrations were similar 10 min after saline treatment in rats pre-treated with saline and atropine methylnitrate, while they were lower in

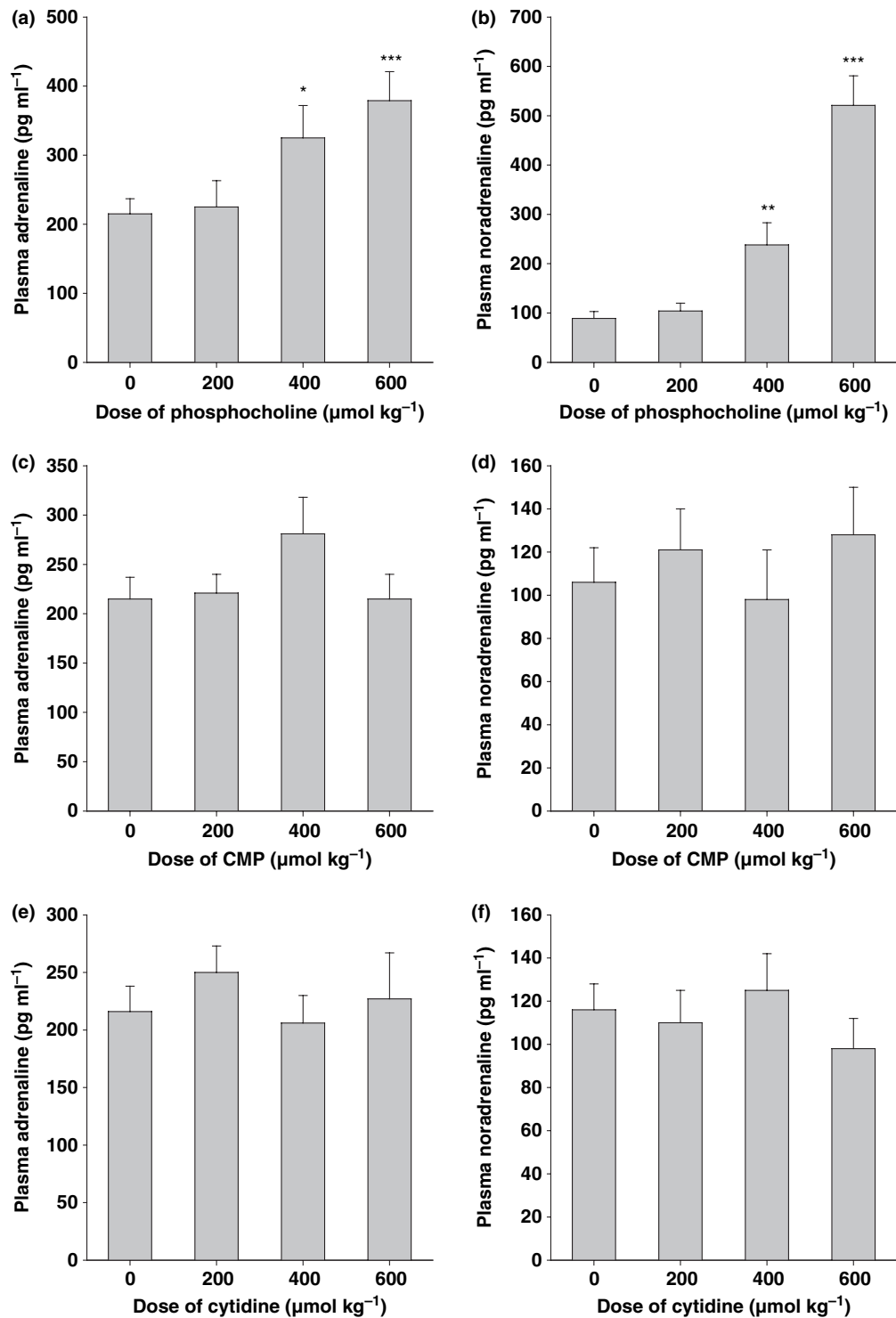


Figure 4 Plasma adrenaline and noradrenaline responses to i.p. phosphocholine, CMP and cytidine. Rats were injected i.p. with either saline (1 ml/kg), phosphocholine (200, 400 or 600 μmol/kg), CMP (200, 400 or 600 μmol/kg), or cytidine (200, 400 or 600 μmol/kg) and blood samples (1 ml) were collected at 10 min after each treatment. Each point represents the mean ± SEM of six measurements. Data were analysed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls.

hexamethonium- pre-treated rats (Table 1). Atropine methylnitrate failed to alter the increases in plasma adrenaline and noradrenaline in response to

i.p. administration of 600 μmol/kg of CDP-choline, phosphocholine or choline (Table 1). On the other hand, hexamethonium, the ganglionic nicotinic

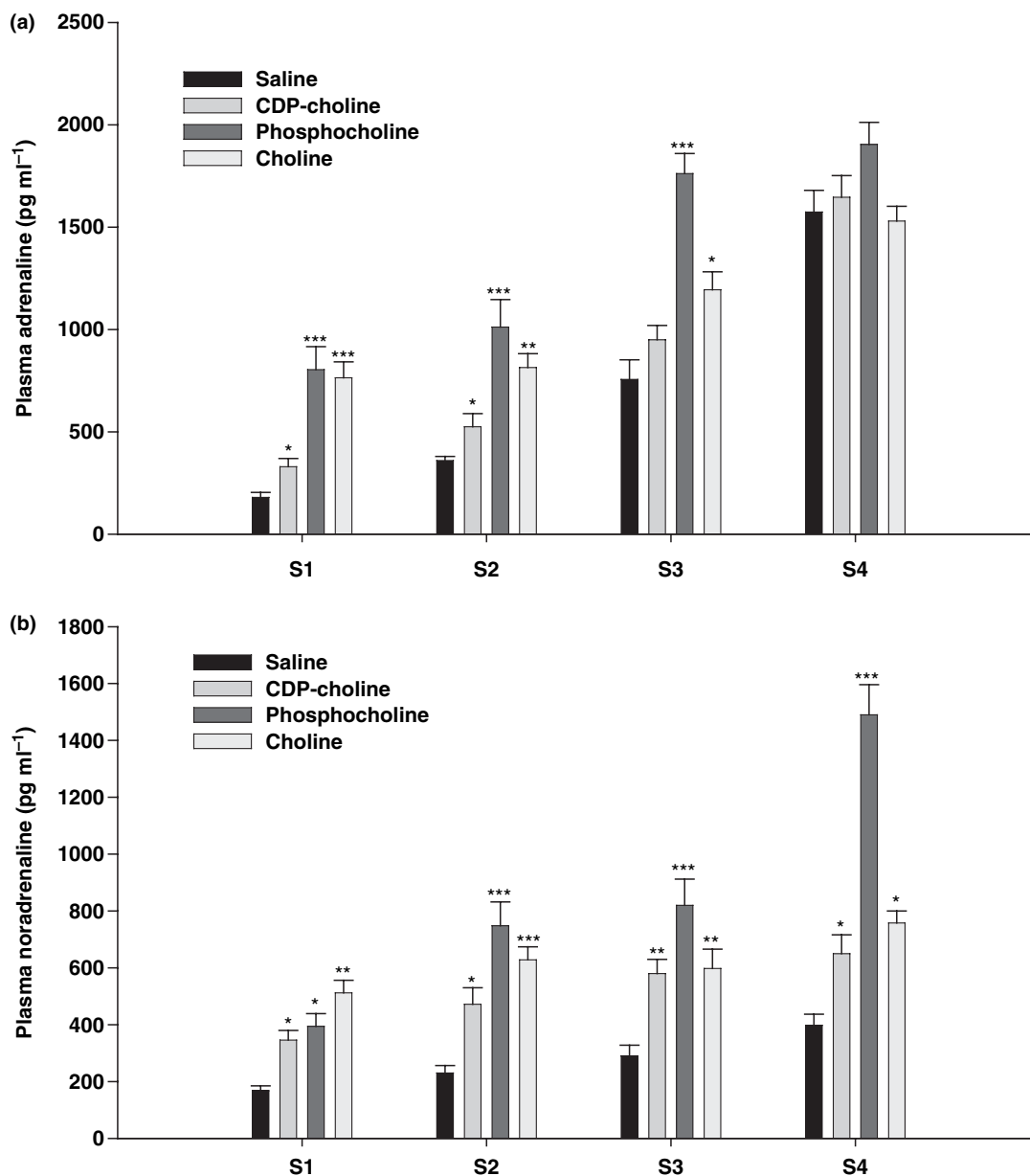


Figure 5 Enhancements of plasma adrenaline and noradrenaline responses to graded haemorrhage by i.p. CDP-choline, phosphocholine and choline. Rats were subjected to graded haemorrhage by removal of 0.55 ml blood per 100 g of body weight from arterial catheter four times with 5-min intervals starting 5 min after i.p. injection of saline (1 ml/kg), CDP-choline (600 μ mol/kg), phosphocholine (600 μ mol/kg), or choline (600 μ mol/kg). Blood samples were obtained at 5, 10, 15 and 20 min after the treatments (designated S1, S2, S3, and S4, respectively) and assayed for adrenaline (a) and noradrenaline (b). Data are expressed as mean \pm SEM of six measurements. Data were analysed using repeated measures two-way ANOVA followed by Tukey's test in each groups of rats. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared to respective values for saline treatment.

receptor antagonist, blocked the plasma catecholamine responses to CDP-choline, and its cholinergic metabolites phosphocholine and choline (Table 1). In hexamethonium- pre-treated rats, plasma adrenaline and noradrenaline concentrations following CDP-choline, phosphocholine or choline were slightly, but not significantly, higher than the values observed following saline treatment (Table 1).

Effects of CDP-choline, phosphocholine and choline on catecholamine secretion from the perfused adrenal gland

As seen in Fig. 6, the perfusion of adrenal gland with increasing concentrations of choline caused a concentration-dependent secretion of catecholamines. The EC₅₀ of choline was $2450 \pm 240 \mu$ M

Table 1 Effects of atropine methylnitrate and hexamethonium chloride on the increases in plasma catecholamines elicited by CDP-choline, phosphocholine and choline

Pre-treatment + treatment	Adrenaline (pg/ml)	Noradrenaline (pg/ml)
Saline + saline	200 ± 13	109 ± 16
Saline + CDP-choline	368 ± 32***	205 ± 21*
Saline + phosphocholine	316 ± 23***	415 ± 40***
Saline + choline	437 ± 16***	345 ± 33***
Atropine methylnitrate + saline	226 ± 16	128 ± 26
Atropine methylnitrate + CDP-choline	392 ± 32†††	288 ± 21†
Atropine Methylnitrate + phosphocholine	415 ± 28†††	603 ± 22†††
Atropine methylnitrate + choline	554 ± 44†††	378 ± 30††
Hexamethonium + saline	123 ± 18	63 ± 16
Hexamethonium + CDP-choline	168 ± 15	83 ± 14
Hexamethonium + phosphocholine	175 ± 23	95 ± 18
Hexamethonium + choline	195 ± 34	97 ± 14

Rats were pre-treated i.p. with saline (1 ml/kg), atropine methylnitrate (2 mg/kg) or hexamethonium chloride (15 mg/kg) 15 min prior to i.p. administration of saline (1 ml/kg), CDP-choline (600 µmol/kg), phosphocholine (600 µmol/kg) or choline (600 µmol/kg). Blood samples were obtained from the arterial catheter 10 min after the second i.p. injection; plasmas were separated and assayed for adrenaline and noradrenaline. Data are expressed as the mean ± SEM ($n = 7$). Data were analysed by two-way ANOVA followed by Tukey's test. * $P < 0.05$ and *** $P < 0.001$ compared with the values from 'saline + saline'. † $P < 0.05$; †† $P < 0.01$ or ††† $P < 0.001$ compared with the respective values from 'atropine methylnitrate + saline' group.

($n = 5$ separate experiments). At 10–10 000 µM concentrations range neither CDP-choline nor phosphocholine increased catecholamine content significantly in the perfusate (Fig. 6). Perfusion of adrenal gland with the broad-spectrum nicotinic receptor antagonist hexamethonium (1 µM) or a relatively $\alpha 3\beta 4$ subtype selective nicotinic antagonist mecamlamine (1 µM) attenuated choline (3200 µM)-induced catecholamine secretion by 75% or 85%, respectively (Fig. 6b). Atropine at 1 µM concentration failed to alter the effect of choline (3200 µM) on catecholamine release (Fig. 6b).

Effects of i.c.v. CDP-choline and choline administration on plasma adrenaline and noradrenaline concentrations

Peripheral administration of CDP-choline results in a rise in extracellular choline concentrations in rat brain (Savci *et al.*, 2003). Moreover, i.c.v. administration of both CDP-choline (Savci *et al.*, 2002) and choline (Arslan *et al.*, 1991; Ulus *et al.*, 1995) increases plasma adrenaline and noradrenaline concentrations. To confirm these previous observations and to characterize further the dose and time relations of central choline effects on plasma catecholamines, rats were treated i.c.v. with choline (0.5, 1 and 1.5 µmol). Choline increased plasma adrenaline dose- and time-dependently (Fig. 7a and c). Choline at 1.5 µmol dose also increased plasma noradrenaline for at least 60 min, but its lower doses (0.5 and 1 µmol) were ineffective (Fig. 7b and d).

Interaction of i.c.v. choline with mecamlamine and atropine

To determine if central nicotinic and/or muscarinic cholinergic receptors mediate the effects of choline on plasma catecholamines, rats were pre-treated with mecamlamine (50 µg; i.c.v.) or atropine (10 µg; i.c.v.) 15 min prior to i.c.v. injection of choline (1.5 µmol). Mecamlamine, but not atropine, blocked the effect of choline on plasma adrenaline and noradrenaline (Table 2). Given alone, these compounds did not produce any significant changes in plasma adrenaline and noradrenaline concentrations (Table 2).

Pre-treatment with hexamethonium (15 mg/kg, i.p.) also blocked the increases in plasma adrenaline and noradrenaline evoked by i.c.v. administration of 1.5 µmol of choline (Table 2).

In a related study, i.c.v. mecamlamine pre-treatment (50 µg) failed to alter the effects of i.p. injection of CDP-choline or choline (each 600 µmol/kg) on plasma adrenaline and noradrenaline responses (data not shown).

Discussion

These data show that intraperitoneal administration of CDP-choline increases plasma adrenaline and noradrenaline concentrations in a dose- and time-dependent manner. CDP-choline administration also elevates plasma concentrations of itself and its immediate metabolites phosphocholine, CMP, choline and cytidine. Intraperitoneal administration of equivalent doses of CDP-choline's

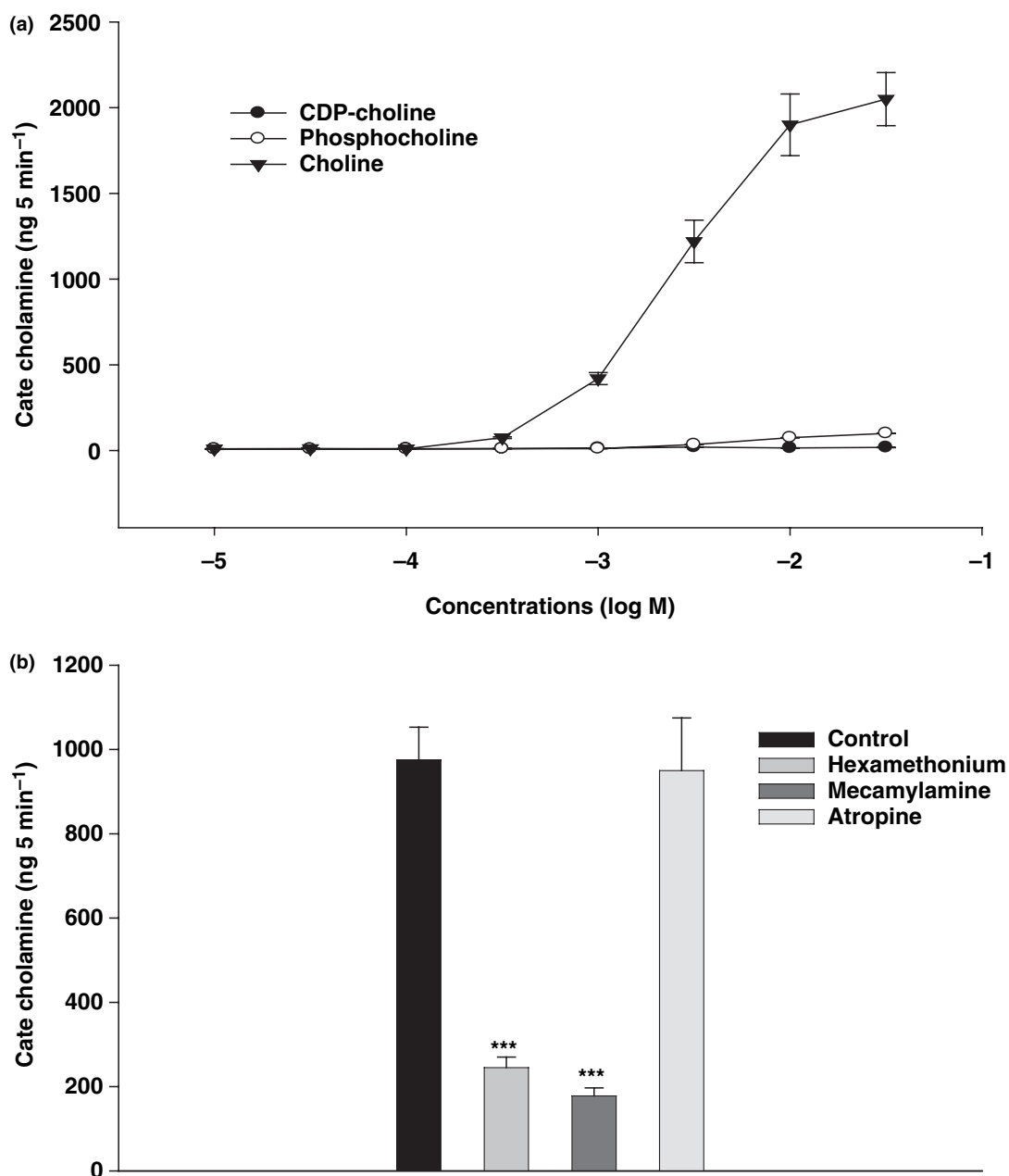


Figure 6 Effects of CDP-choline, phosphocholine and choline on catecholamine secretion from isolated perfused rat adrenal gland (a) and inhibition of choline-evoked catecholamine release by hexamethonium and mecaylamine (b). a: The adrenal gland was isolated vascularly-perfused with the Krebs-Ringer buffer at rest for 30 min. After a 30-min equilibration period, the perfusate was collected for 5 min and then the lowest concentration of compounds to be tested (CDP-choline, phosphocholine or choline) was added to the perfusion medium and the perfusate was again collected for 5 min. The adrenal gland was then perfused with drug-free medium for 10 min, and a higher concentration of drugs was added to the perfusion medium and the perfusate was collected for another 5 min. This procedure was repeated five to seven times until the drug concentration was raised to 3200 μM . Catecholamine contents of the perfusate were assayed by fluorometric method. Catecholamine release is expressed as ng/5 min and the basal release rate was 9 ± 1 ng/5 min. Data are expressed as mean \pm SEM of 4–5 measurements. Data were analysed using repeated measures one-way ANOVA followed by Tukey's test. b: After a 30-min equilibration period, choline (3200 μM) was added to perfusion medium in the absence (control) or presence of 1 μM of hexamethonium, mecaylamine or atropine and the perfusate was collected for 5 min. Data are expressed as mean \pm SEM of 4–5 measurements. Data were analysed one-way ANOVA followed by Tukey's test. *** $P < 0.001$ compared with the control value.

cholinergic metabolites phosphocholine or choline, but not pyrimidineric metabolites CMP or cytidine, also enhances plasma adrenaline and

noradrenaline. CDP-choline, phosphocholine or choline likewise enhances plasma adrenaline and noradrenaline responses to graded haemorrhage.

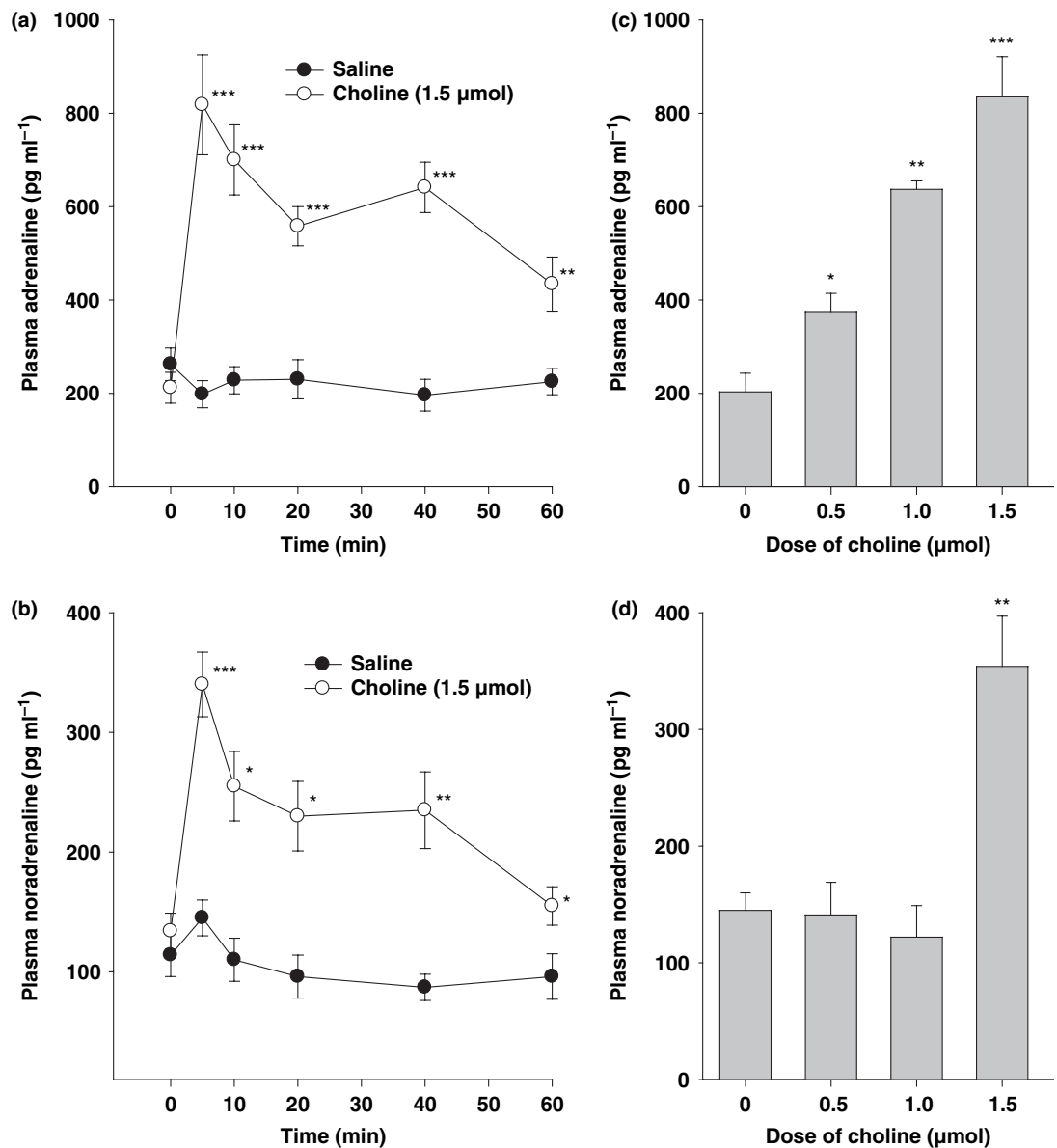


Figure 7 Time and dose relations of plasma adrenaline and noradrenaline responses to i.c.v. administration of choline. Time-course study (a and b): Rats were injected i.c.v. with either saline (10 μl) or choline (1.5 μmol). Blood samples (1 ml) were collected immediately before (0 min), and 5, 10, 20, 30, 45 and 60 min after each treatment from six rats from both groups for each time point and analysed for adrenaline (a) and noradrenaline (b). Each point represents the mean ± SEM of six measurements. Data were analysed using two-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls. Dose-course study (c and d): Rats were injected i.c.v. with either saline (10 μl) or choline (0.5, 1.0 or 1.5 μmol). Blood samples (1 ml) were collected at 5 min after each treatment and analysed for adrenaline (c) and noradrenaline (d). Each point represents the mean ± SEM of six measurements. Data were analysed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls.

Choline, but not CDP-choline or phosphocholine, stimulates catecholamine release from isolated perfused adrenal gland *in vitro* in a concentration-dependent manner. Pre-treatment with hexamethonium, a peripheral nicotinic receptor antagonist, blocks the increase in plasma adrenaline and noradrenaline induced by i.p. CDP-choline, phosphocholine and choline, while

pre-treatment with atropine methylnitrate, a peripheral muscarinic receptor antagonist, is without effect. Central administration of choline, a CDP-choline metabolite and a precursor for the neurotransmitter acetylcholine, also increases plasma adrenaline and noradrenaline concentrations. Pre-treatment with central administration of mecamylamine, but not atropine, blocks the effects of

Table 2 Effects of atropine or mecamlamine on the increases in plasma catecholamines elicited by i.c.v. choline

Pre-treatment + treatment	Adrenaline (pg/ml)	Noradrenaline (pg/ml)
Saline + saline	221 ± 28	141 ± 19
Saline + choline	647 ± 61**	255 ± 29*
Atropine + saline	215 ± 21	148 ± 18
Atropine + choline	612 ± 71**	245 ± 28*
Mecamylamine + saline	193 ± 31	158 ± 16
Mecamylamine + choline	279 ± 32	197 ± 15
Hexamethonium + saline	135 ± 24	91 ± 14
Hexamethonium + choline	195 ± 29	121 ± 22

Rats were pre-treated with saline (10 µl; i.c.v.), atropine (10 µg; i.c.v.), mecamlamine (50 µg, i.c.v.) or hexamethonium (15 mg/kg; i.p.) 15 min prior to i.c.v. administration of saline (10 µl) or choline (1.5 µmol). Blood samples were collected from the arterial catheter at 5 min after the second i.c.v. injection for plasma adrenaline and noradrenaline measurements. Data are expressed as the mean ± SEM ($n = 6$). Data were analysed by two-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.001$ compared with the values from 'saline + saline' group.

central choline on plasma adrenaline and noradrenaline.

Previous studies from our laboratory have shown that plasma adrenaline and noradrenaline increase following intraperitoneal administration of 120 mg/kg of choline (Ilcol *et al.*, 2002) or intravenous injection of 250 mg/kg of CDP-choline (Savci *et al.*, 2003). Data from the present study confirm and extend these previous observations by examining the dose- and time-relations of choline and CDP-choline actions on plasma adrenaline and noradrenaline following i.p. administration of their various doses. Apparently, the increases in plasma adrenaline in response to 200–600 µmol/kg of CDP-choline or choline were dose-related (Figs 1c and 3c). The increases in plasma adrenaline in response to a given dose of CDP-choline are smaller in magnitude and slightly shorter in duration compared with the observed response after the equivalent doses of choline. Indeed, i.p. injection of 600 µmol/kg of CDP-choline increased plasma adrenaline significantly for 30 min with a maximum 1.6-fold increase at 10 min (Fig. 1a), while the same dose of choline increased plasma adrenaline for 45 min with about three-fold increase at 10 min after the treatment (Fig. 3a). Intraperitoneal administration of choline (200–600 µmol/kg) also increased plasma noradrenaline concentrations in a dose-related manner (Fig. 3d), while CDP-choline increased plasma noradrenaline only after its highest dose, 600 µmol/kg (Fig. 1b and d). In the present study, we also show for the first time, that phosphocholine, a choline-containing metabolite of CDP-choline, increases plasma adrenaline and noradrenaline concentrations in a dose-related manner (Fig. 4a and b). However, there were considerable variations in plasma adrenaline and noradrenaline levels

in response to saline or drugs treatments. These variations in plasma catecholamine levels could be explained by unavoidable stress actions of injection and/or blood sampling in our conscious animals.

When administered to rats, CDP-choline is rapidly metabolized to CMP and phosphocholine which subsequently are dephosphorylated to cytidine and choline, respectively (Lopez G-Coviella *et al.*, 1987, 1995). In the present study, we observe that i.p. administration of CDP-choline enhances serum concentrations of CDP-choline itself and of its metabolites CMP, phosphocholine, choline and cytidine (Fig. 2). In theory, plasma adrenaline and noradrenaline responses to i.p. CDP-choline result from increased blood concentrations of CDP-choline and/or its metabolites. As discussed above, this suggestion is true for phosphocholine and choline, but not for CMP and cytidine. Inasmuch as the increases in plasma adrenaline and noradrenaline in response to i.p. injection of equivalent doses (200–600 µmol/kg) of CDP-choline, phosphocholine or choline were accompanied by several-fold increases in circulating free choline concentrations which have long been known to augment cholinergic neurotransmission in the sympatho-adrenal system (Ulus *et al.*, 1977a,b, 1978; Scally *et al.*, 1978), it is reasonable to assume that the increases in plasma adrenaline and noradrenaline by choline itself, and by the two choline precursors CDP-choline and phosphocholine, result from elevations in serum free choline concentrations. This assumption is supported by the observation that choline, but not CDP-choline or phosphocholine, induces catecholamine secretion, under *in vitro* conditions, from the isolated perfused adrenal gland (Fig. 6a, see below). The absence of significant changes in plasma adrenaline and noradrenaline concentrations following i.p. injection of equivalent doses (200–600 µmol/kg) of CMP or cytidine (Fig. 4) indicates that these pyrimidineric intermediates are not involved in the observed rise in plasma catecholamines in response to the parent compound, CDP-choline. This finding can be considered as another indication of the cholinergic effect of CDP-choline on plasma catecholamine concentrations.

The observed nicotinic receptor-mediated elevations in plasma adrenaline and noradrenaline following CDP-choline, phosphocholine or choline were associated with several-fold increase in plasma free choline concentrations. Elevated circulating choline enhances nicotinic cholinergic neurotransmission in the sympatho-adrenal system and evokes catecholamine release by activating ganglionic neuronal type acetylcholine receptors (nAChR) indirectly by increasing acetylcholine synthesis and release by acting indirectly as acetylcholine's precursor (Ulus *et al.*, 1977a; Ulus *et al.*, 1977b; Ulus *et al.*, 1989; Ulus *et al.*, 2006; Ilcol *et al.*, 2003) and/or by directly acting as an agonist

on nAChRs (Ulus *et al.*, 1988; Alkondon *et al.*, 1997; Alkondon & Albuquerque, 2006). Recent studies have shown that the $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ subunits of nAChRs (Rust *et al.*, 1994; Zhou *et al.*, 1998; Zhou *et al.*, 2001; Di Angelantonio *et al.*, 2003; Voitenko *et al.*, 2001) and the nicotinic receptors containing $\alpha 3\beta 4$ subunits are mainly involved in the release of catecholamines from rat adrenal chromaffin cells (Tachikawa *et al.*, 2001; Tani *et al.*, 2002; Tani *et al.*, 2002; Park *et al.*, 2006). Choline evokes inward Ca^{2+} currents in sympathetic neurons (Cuevas *et al.*, 2000; Seddik *et al.*, 2003) and chromaffin cells (Fuentelba *et al.*, 2004; Gonzalez-Rubio *et al.*, 2006), displaces L-[3H]-nicotine from membrane preparations of adrenal medulla and sympathetic ganglia (Ulus *et al.*, 1988) and stimulates catecholamine release from the vascularly perfused rat adrenal glands (Ulus *et al.*, 1988) and bovine adrenal chromaffin cells (Holz & Senter, 1981; Fuentelba *et al.*, 2004). In accordance with these previous observations, here we show that choline evokes catecholamine release mainly via a nicotinic receptor-mediated mechanism (i.e. 3200 μM choline-induced catecholamine release was attenuated by the presence of 1 μM of hexamethonium, a non-selective antagonist of nicotinic acetylcholine receptors, or mecamylamine, an antagonist of neuronal nicotinic acetylcholine receptors with relative selectivity for $\alpha 3\beta 4$ nAChR (Cachelin & Rust, 1995; Wong *et al.*, 1995; Papke *et al.*, 2001), from the perfused adrenals in a concentration-dependent manner at 320–10 000 μM concentrations ranges. Choline's EC_{50} for releasing catecholamines ($2450 \pm 240 \mu\text{M}$) was comparable with values reported in the previous studies for catecholamine secretion (Ulus *et al.*, 1988; Holz & Senter, 1981) and with its EC_{50} for displacing L-[3H] nicotine binding to membrane preparations of the adrenal or sympathetic ganglia (Ulus *et al.*, 1988), but was considerably higher than the circulating choline concentrations attained maximally at about 200 μM following i.p. administration of the highest dose (600 $\mu\text{mol/kg}$) of CDP-choline and phosphocholine, or at about 400 μM after 600 $\mu\text{mol/kg}$ of choline. Previous studies have clearly shown that choline at a concentration within 10–130 μM range, which is attainable in the circulation following administration of CDP-choline, phosphocholine or choline itself, enhances acetylcholine synthesis and levels in the autonomic cholinergic neurons (Dieterich *et al.*, 1978; Meyer & Baker, 1986; Ulus *et al.*, 1977a,b; Ilcol *et al.*, 2003) and increases cholinergic neurotransmission in the sympatho-adrenal system (Ulus *et al.*, 1977a; b). Also, CDP-choline or phosphocholine failed to stimulate catecholamine release at 10–10 000 μM concentrations. Taken together, it is reasonable to assume that the observed increases in plasma adrenaline and noradrenaline following

choline, phosphocholine or CDP-choline administration mainly, but not necessarily solely, resulted from the increased cholinergic transmission in the sympatho-adrenal system because of the precursor action of elevated plasma choline (Ulus *et al.*, 1977a,b, 1978, 1979). In addition, elevated plasma choline can modulate nicotinic cholinergic neurotransmission in the splanchnic nerve-chromaffin cell synapses and sympathetic ganglion synapses because choline desensitizes $\alpha 7$ nAChRs at 10–100 μM concentrations (Mandelzys *et al.*, 1995; Papke *et al.*, 1996, 2000, 2002; Albuquerque *et al.*, 1997; Alkondon *et al.*, 1997), inhibits $\alpha 3\beta 4^*$ and $\alpha 4\beta 2^*$ nAChRs at 10–1000 μM concentrations (Alkondon & Albuquerque, 2006), and potentiates $\alpha 4\beta 4$ nAChR-mediated acetylcholine currents at 10–300 μM (Zwart & Vijverberg, 2000).

The observed increases in plasma adrenaline and noradrenaline concentrations in rats subjected to graded haemorrhage agree well with previous studies which showed that catecholamine secretion was stimulated by haemorrhage (Fejes-Toth *et al.*, 1988; Ulus *et al.*, 1995). The graded haemorrhage-induced increases in plasma adrenaline and noradrenaline were greater in CDP-choline-, phosphocholine- and choline-treated rats than those observed in saline-treated control rats (Fig. 5). Previous studies have shown that the effect of choline on acetylcholine synthesis and release was enhanced significantly when cholinergic neurons were stimulated (Ulus *et al.*, 1989, 2006; Wecker, 1991). Similarly, choline-induced increases in cholinergic neurotransmission in sympatho-adrenal system were enhanced considerably by treatments that are known to increase the firing rates of pre-ganglionic cholinergic nerves (Ulus *et al.*, 1977b, 1978; Ulus & Wurtman, 1979). The observed enhancement in haemorrhage-induced elevations in plasma adrenaline and noradrenaline by CDP-choline, phosphocholine and choline could be explained by an augmentation of cholinergic neurotransmission within the catecholamine-secreting adrenomedullary cells and sympathetic neurons because of increased availability of circulating free choline.

It is well known from early studies (Brown, 1967; Tsujimoto & Nishikawa, 1975; Wakade & Wakade, 1983; Critchley *et al.*, 1986) that acetylcholine-mediated ganglionic transmission and catecholamine secretion in the sympatho-adrenal system is mainly nicotinic, while there is also a hexamethonium-insensitive and atropine-sensitive component. Our finding that plasma adrenaline and noradrenaline responses to CDP-choline, phosphocholine and choline were prevented by ganglionic nicotinic acetylcholine receptor blockade with hexamethonium indicates that the actions of these choline-containing compounds on plasma catecholamines are mediated by the ganglionic nicotinic acetylcholine receptors. Failure of atropine

methylnitrate to alter plasma adrenaline and noradrenaline responses to CDP-choline itself or to any of its cholinergic metabolites suggests that peripheral muscarinic receptors have no major role in the observed plasma adrenaline and noradrenaline responses. However, in hexamethonium-pretreated rats, the observed tendency to increase in plasma adrenaline and noradrenaline concentrations by CDP-choline, phosphocholine, or choline treatment compared with saline treatment may be due to activation of sympatho-adrenal system by the muscarinic receptor-mediated mechanism (Brown, 1967; Tsujimoto & Nishikawa, 1975; Wakade & Wakade, 1983; Critchley *et al.*, 1986), as discussed above for CDP-choline and phosphocholine.

The observed increase in plasma adrenaline and noradrenaline concentrations in response to i.c.v. choline (0.5, 1.0 and 1.5 μmol) were in good accordance with previous observations following central administration of choline (Arslan *et al.*, 1991; Ulus *et al.*, 1995; Gurun *et al.*, 2002; Savci *et al.*, 2002b). In the present study, we show that central choline evokes increases in plasma adrenaline and noradrenaline concentrations that are blocked by central pre-treatment with a neuronal type nicotinic receptor antagonist, mecamlamine (Table 2), but not with the muscarinic receptor antagonist, atropine. In earlier studies, atropine (10 μg , i.c.v.), abolished the decrease in body temperature (Unal *et al.*, 1998) and the increase in plasma oxytocin (Savci *et al.*, 1996) and prolactin levels (Gurun *et al.*, 1997) induced by the same dose of i.c.v. choline. Thus, the atropine dose used in the present study is sufficient to block central muscarinic effects of choline. The increases in plasma adrenaline and noradrenaline evoked by central choline were also prevented by peripheral pre-treatment with the ganglionic blocker hexamethonium (15 mg/kg, i.p.). Taken together, it is suggested that central choline increases plasma adrenaline and noradrenaline concentrations by activating nicotinic receptors in the central nervous system (as evidenced by blockade by central mecamlamine), which activates peripheral sympatho-adrenal system through the autonomic ganglia (as evidenced by blockade by peripheral hexamethonium) and stimulates secretion of adrenaline and noradrenaline from adrenal glands. Since, pre-treatment with the same dose of mecamlamine which completely blocked the effects on plasma catecholamines of i.c.v. choline (Table 2) failed to alter plasma adrenaline or noradrenaline responses to i.p. choline and CDP-choline, it is clear that central nicotinic receptors are not involved in plasma catecholamine responses to peripheral administration of choline or CDP-choline.

In summary, the present study shows that central or peripheral administration of CDP-choline and choline increases plasma adrenaline and noradrenaline concentrations in a dose- and time-

related manner. The activation of nicotinic receptors at the peripheral sympatho-adrenal system is involved in plasma adrenaline and noradrenaline responses to both central and peripheral administration of CDP-choline or choline. The activation of central nicotinic receptors is also involved in plasma catecholamine response to central, but not peripheral, choline or CDP-choline injection. Since catecholamines have important cardiovascular and metabolic actions in the periphery, it is likely that CDP-choline and choline can alter various body functions in which catecholamines have regulatory roles, and, also, elevated plasma adrenaline and noradrenaline can mediate some actions of choline and CDP-choline, as demonstrated previously (Arslan *et al.*, 1991; Ulus *et al.*, 1995; Savci *et al.*, 2002a,b, 2003; Gurun *et al.*, 2002; Ilcol *et al.*, 2002).

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