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Hypotensive effects of intravenously administered uridine and cytidine in conscious rats: Involvement of adenosine receptors

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Abstract

In the present study, we investigated the cardiovascular effects of intravenously injected uridine or cytidine, and the role of adenosine receptors in mediating these effects, in conscious normotensive rats. Intravenous (i.v.) administration of uridine (124, 250, 500 mg/kg) dose-dependently decreased arterial pressure and heart rate. Cytidine (124, 250, 500 mg/kg; i.v.) produced slight dose-related hypotension without changing heart rate. Plasma uridine and cytidine concentrations increased time- and dose-dependently while plasma adenosine levels did not change after injection of the respective nucleosides. Pretreatment with intravenous caffeine (20 mg/kg), 8-phenyltheophylline (8-PT) (1 mg/kg), nonselective adenosine receptor antagonists, or 8-*p*-sulfophenyltheophylline (8-SPT) (20 mg/kg), a nonselective adenosine receptor antagonist which does not cross the blood-brain barrier, abolished the cardiovascular effects of uridine (250 mg/kg; i.v.) or cytidine (250 mg/kg; i.v.). Intracerebroventricular (i.c.v.) caffeine (200 μg) or 8-SPT (50 μg) pretreatment did not change the magnitude of the cardiovascular responses induced by nucleosides. Intravenous 8-cyclopenthyl-1,3-dipropylxanthine (DPCPX) (5 mg/kg), a selective adenosine A_1 receptor antagonist, greatly attenuated the cardiovascular responses to uridine and cytidine. Pretreatment with 3,7,-dimethyl-1-propargylxanthine (DMPX) (2 mg/kg), an adenosine A_1/A_2 receptor antagonist, attenuated hypotension induced by uridine and blocked the arterial pressure decrease in response to cytidine. Uridine-induced bradycardia was blocked by DMPX. 4-(2-[7-amino-2-(2-furyl[1,2,4]-triazolo[2,3-a[1,3,5]triazin-5-yl-aminoethyl)phenol (ZM241385) (1 mg/kg; i.v.), a selective adenosine A_{2A} receptor antagonist, pretreatment produced an only very small blockade in the first minute of the hypotensive effects of uridine without affecting the bradycardia. ZM241385 pretreatment completely blocked cytidine's hypotensive effect. In Langendorff-perfused rat heart preparation, uridine (10^{-3} M), but not cytidine, decreased the heart rate. Our results show that intravenously injected uridine or cytidine is able to decrease arterial pressure by activating peripheral adenosine receptors. The data also implicates that the mainly adenosine A_1 receptor activation is involved in the uridine-induced cardiovascular effects, while both adenosine A_1 and A_{2A} receptor activations mediate the cytidine's effects.

Keywords: Uridine; Cytidine; Adenosine; A_1 receptor; A_{2A} receptor; Purinergic; Cardiovascular

1. Introduction

Cytidine 5′-diphosphocholine (CDP-choline, citicoline) is an endogenously synthesized mononucleotide composed of choline and cytidine (Weiss, 1995). It produces several physiological and pharmacological effects on body functions by altering membrane metabolism (Adibhatla et al., 2002; Adibhatla and Hatcher, 2005) and by increasing central cholinergic (Savci et al., 2002, 2003) and dopaminergic transmission (Lopez et al., 1986). In rats, exogenously given CDP-choline is rapidly hydrolyzed to its final products, choline and cytidine, resulting in increased plasma levels of these metabolites (Lopez et al., 1995; Savci et al., 2002, 2003). However, in humans, cytidine is further converted to uridine; therefore, CDP-choline elevates the plasma levels of uridine and is considered a source of

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plasma uridine (Wurtman et al., 2000). These final products—choline, cytidine and uridine—are taken up by the cells, and in the brain they mediate endogenous CDP-choline synthesis and the effects of the drug on various functions. Although choline has received the most attention by being the precursor of both phosphatidylcholine and acetylcholine, there is considerable evidence that uridine and cytidine, either alone or as their respective nucleotides, exert many significant effects on several functions (Carlezon et al., 2002, 2005; De Bruin et al., 2003; Yitzhaki et al., 2005, 2006; You et al., 1999). Recent observations imply that the individual effects of these final metabolites of CDP-choline may determine the ultimate effects of CDP-choline on various functions, including the central nervous system and the cardiovascular system. Indeed, Carlezon et al. (2002) suggested that the lack of behavioral effects of CDP-choline in the forced swim test may be due to the opposite effects of its two major metabolites; i.e. cytidine has antidepressant effects and choline has prodepressant effects. Similarly, we reported the interesting observation, that in normotensive rats the pressor effect of i.v.-administered CDP-choline on blood pressure was perturbed by the depressor effects of its final metabolites, including cytidine (Savci et al., 2003). The cardiovascular effects of choline, as one of its final metabolites, have already been described in great detail (Arslan et al., 1991; Ulus et al., 1995; Savci et al., 2002, 2003); however, there is scant information about the cardiovascular effects of uridine or cytidine, the other important metabolites of the drug. Also, CDP-choline is currently approved in Europe and Asia for the treatment of a variety of CNS disorders, including cerebral ischemia, acute ischemic stroke, and Parkinson's disease (for a recent review see Adibhatla and Hatcher, 2005). Since uridine is one of the main metabolites of CDP-choline in humans, and the individual effects of these metabolites may alter the ultimate effects of CDP-choline in the body, as observed in rats (Carlezon et al., 2002; Savci et al., 2002), it is important to determine the cardiovascular effects of both cytidine and uridine administration under *in vivo* conditions.

Both uridine and cytidine are endogenously synthesized nucleosides like adenosine, which has long been known to have prominent cardiovascular effects (Barraco et al., 1987; Evoniuk et al., 1987; reviewed by Ralevic and Burnstock, 1998). When injected exogenously, adenosine exerts negative chronotropic, dromotropic, and inotropic action via A_1 receptors located on cardiomyocytes and vascular smooth muscle cells (Shryock and Belardinelli, 1997; Windscheif, 1996), as well as muscle vasodilation via A_1 (Merkel et al., 1992; Danilaou et al., 1997; Ray and Marshall, 2006) and A_{2A} receptors found on endothelial and vascular smooth muscle cells (Tabrizchi and Bedi, 2001; Schindler et al., 2005). Peripheral vasodilation in combination with reduced cardiac output results in decreased blood pressure (Windscheif, 1996).

Adenosine receptors are one of the three classes of purinergic receptors which are preferentially stimulated by adenosine and named as P_1- or A-type receptors (Windscheif, 1996). Although the other two classes of receptors, P_{2X} and P_{2Y}, are preferentially activated by adenosine-5′-triphosphate (ATP) and its derivatives, it has been shown that several subtypes of P_{2Y} receptors are activated not only by adenine nucleotides, but also by uridine-5′-triphosphate (UTP) and its derivatives (Windscheif, 1996; You et al., 1999). Moreover, it is known that both ATP and UTP are equally effective on P_{2Y2} receptors. Thus, taking all the above findings into consideration, we hypothesized that uridine and cytidine, as nucleosides, can produce cardiovascular changes by activating adenosine receptors. The present study was designed to determine the cardiovascular effects of i.v.-administered uridine or cytidine and the involvement of adenosine A_1 and A_{2A} receptors in these effects.

2. Materials and methods

2.1. Animals

Adult male Wistar Albino rats (250–300 g) (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used in the present study. Rats were housed under a 12 h light/dark cycle with free access to food and water. The surgical and experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (http://www.oacu.od.nih.gov/regs/guide/guide1.htm).

2.2. Surgical procedures

Under sevofluorane (2–4% in 100% O_2) anesthesia, the left common carotid artery and left jugular vein of rats were cannulated with PE 50 tubing filled with heparinized saline (250 U/ml). During the arterial cannulation procedure, the vagus nerve and the cervical sympathetic trunk were separated very carefully. The catheters were exteriorized at the nape of the neck and sealed until use. At the end of the surgical procedures, 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 showed no evidence of pain.

2.3. Blood pressure recording

After the recovery period, the arterial cannula was connected to a volumetric pressure transducer (BPT 300) which was attached to a DA100B general purpose transducer amplifier (Commat Ltd., Ankara, Turkey). Blood pressures and heart rates of rats were recorded and analyzed using the MP100 system and AcqKnowledge software (BIOPAC Systems Inc., CA, U.S.A.). Blood pressure is reported as mean arterial pressure (mm Hg) and heart rate is expressed as beats per minute.

2.4. Experimental protocol

After the animals regained consciousness, the arterial line was connected to the transducer and baseline blood pressures and heart rates were recorded. Rats were allowed to stabilize for 15 min, then i.v. injections were made and monitoring of

cardiovascular parameters was continued for 60 min. In i.v. pretreatment groups, caffeine, 8-PT, 8-SPT, DPCPX, DMPX, or ZM241385 was injected intravenously 5 min before the uridine or cytidine administrations. In intracerebroventricularly (i.c.v.) pretreated groups, caffeine or 8-SPT was given i.c.v. 15 min before the uridine or cytidine injections. Respective controls of each treatment groups (saline or vehicle) were also performed accordingly.

In separate groups of animals, adenosine was administered i.v. at a dose equimolar to 250 mg/kg cytidine or uridine as a reference nucleoside and cardiovascular parameters were monitored.

In order to determine the changes in plasma uridine and cytidine concentrations after the respective treatments, saline (1 ml/kg), uridine (124, 250 and 500 mg/kg) or cytidine (124, 250 and 500 mg/kg) was injected intravenously. Blood samples were withdrawn at 1, 3, 5, 10, 20, and 30 min after the treatments. Each set of animals was used for the two time intervals and each blood sample was replaced with a double volume of saline. In order to see the basal levels of uridine or cytidine without injections, control samples (0.5 ml) were taken ("0") just before the injections in one group of rats. The volume was replaced with 1 ml of saline as it was in the other groups.

In order to examine the changes in plasma adenosine concentrations after the uridine or cytidine injections, saline (1 ml/kg), uridine (250 mg/kg) or cytidine (250 mg/kg) was injected intravenously, in a separate group of animals. Control blood samples (0.5 ml) were taken just before injections and additional two 0.5 ml of samples were withdrawn at 1 and 3 min after injections. The volume replacement was performed as described above.

2.5. Measurement of plasma uridine, cytidine, adenosine and inosine levels

2.5.1. Sampling

For the measurement of plasma uridine and cytidine concentrations, 0.5 ml of blood was removed from the arterial catheter in ice-cold tubes containing EDTA (50 μg/ml blood) at the appropriate time points. In groups in which the four nucleosides were measured in the same run, 1.0 ml of blood samples was collected in ice-cold tubes containing 0.5 mM dipyridamole (adenosine transport inhibitor, phosphodiesterase V inhibitor) and 0.1 mM papaverine (phosphodiesterase inhibitor). Samples were centrifuged (3500 rpm; +4 °C, 10 min) and the plasma separated and deproteinized by adding formic acid (15%)/acetone (85%) solution. Samples were centrifuged again. After the centrifugation, the supernatants were separated and lyophilized and stored at −80 °C until assayed.

2.5.2. Extraction

Nucleosides were purified by boronate-affinity gel chromatography (Affi-gel 601; Bio-Rad Laboratories, Richmond, CA, U.S.A.) before analysis by high performance liquid chromatography (HPLC). To separate the nucleoside fractions, dried residues of the samples were redissolved in 0.25 M ammonium acetate (pH 8.8) and applied at +4 °C to gel columns (1.0× 1.5 cm) that had been equilibrated with 0.25 M ammonium acetate (pH 8.8). The columns were washed twice with 5 ml of the ammonium acetate solution, and the nucleosides were eluted with 5 ml of 0.1 M formic acid. Fractions containing nucleosides were dried under vacuum and redissolved in water (100 μl) just before analysis by HPLC.

2.5.3. HPLC measurements of uridine, cytidine, adenosine and inosine

In the first set of experiments intended to measure just uridine and cytidine concentrations after respective treatments, plasma uridine and cytidine levels were measured by HPLC (Jasco PU-980 Intelligent HPLC Pump; Kipp & Zonen printer) and UV detection (Jasco UV-975 Intelligent UV–VIS detector) at a wavelength of 280 nm using a C18 column (Variant; ODS 3 μm) with an isocratic system (4 mM KH_2PO_4 and 1% methanol; pH 5.8); flow rate was 1 ml/min with 15 min equilibration delay.

In the second set of experiments designed to measure four nucleosides (uridine, cytidine, adenosine and inosine) in the same run, we used a gradient system and different protocol. These measurements were performed by using a C18 column (Variant, ODS 3 μm) with a UV detector (HP1050) (264 nM) attached to an HPLC system (HP1100) and the following gradient system: solvent A, 4 mM KH_2PO_4, 1% methanol, pH 5.8; solvent B, 100% methanol; 5-min linear gradient to 100% of solvent B followed by 5 min of 100% solvent B; at 1.0 ml/min, with a 6 min equilibration delay.

2.6. Langendorff-perfused rat heart preparation

Rats were euthanized by cervical dislocation without the use of anesthetic agents and thoracotomy was performed. The hearts were removed and immediately immersed in cold (4 °C), heparinized (100 IU/l) modified Tyrode buffer solution (NaCl, 128 mM; KCl, 4.7 mM; $CaCl_2$, 1.36 mM; $NaHCO_3$, 20 mM; NaH_2PO_4, 0.36 mM; $MgCl_2$, 1 mM; glucose, 10 mM), then mounted on a stainless-steel cannula of a Langendorff perfusion apparatus. The hearts were then put in a closed heart chamber and antegradely perfused with a Minipulse 3 peristaltic pump (Gilson Medical Electronics Middleton, Wisconsin, U.S.A.) at a constant flow of 10 ml/min using an oxygenated Tyrode solution at 37 °C in a standard Langendorff fashion and allowed to beat spontaneously. A thin, saline-filled size-3 latex balloon (Hugo Sachs Electronic KG, Berlin, Germany) was inserted into the left ventricle across the mitral valve through a left atriotomy. The balloon was connected to a fluid-filled pressure transducer by metal tubing for continuous measurement of left ventricular developed pressure. We sought to reach 5 mm Hg balloon pressure at the start of experiment. Coronary perfusion pressure was also recorded using a second pressure transducer connected to the coronary perfusion line via a stopcock. Functional data were recorded using an MP 100 data-acquisition system (Biopac Systems, Santa Barbara, CA, U.S.A.). Pressure recordings were obtained by a TSD104A pressure transducer using a D_{A1} 00B general purpose transducer amplifier.

Electrocardiogram (ECG) recordings were made through the two electrodes which were placed in the aorta and apex of the heart. Real-time bipolar ECG recordings of left ventricle pressure were done on a different channel using an EL400 unipolar needle electrode via an ECG100B amplifier.

Baseline parameters were recorded for the initial 30 min equilibration period. Hearts with persistent arrhythmias or poor perfusion pressure (<70 mm Hg) during this period were excluded from the study. After a 30-min stabilization period, baseline measurements were performed and each heart was exposed to a progressively increasing concentration of a single agonist to achieve a concentration–response relationship. Each concentration of agonist was infused for 5 min, with plateau effects being achieved uniformly between 3 to 5 min. Data were sampled at the end of the 5 min infusion period.

2.7. Drugs

The following drugs were used: cytidine, uridine, adenosine, caffeine, 8-phenyltheophylline (8-PT), 8-sulfophenyltheophylline (8-SPT), 8-cyclopenthyl-1,3-dipropylxanthine (DPCPX), 3,7-dimethyl-1-propargylxanthine (DMPX) (Sigma-Aldrich, St Louis, MO, U.S.A.) and 4-(2-[7-amino-2-(2-furyl[1,2,4]-triazolo[2,3-a[1,3,5]triazin-5-yl-aminoethyl)phenol (ZM241385) (Tocris Biosci., Ellisville, MO, U.S.A.). All drugs were dissolved in NaCl (0.9%) except 8-PT, DPCPX and ZM241385. These drugs were dissolved in saline containing 10% dimethyl sulfoxide (DMSO) and 10% 1.0 M NaOH.

The doses of uridine and cytidine were chosen according to the doses of CDP-choline which had been used in our previous experiments and shown to have effects on blood pressure (Savci et al., 2002, 2003). Since the molecular weights of uridine and cytidine are nearly same (244.2 and 243.2, respectively), the doses were the same.

2.8. Data and statistical analysis

Data are represented as mean±standard error of the mean (S.E.M.). Student's *t* test was used to test the significance of differences between values from different groups of rats. Analysis of variance (one-way ANOVA) or repeated measures of analysis of variance (RM-ANOVA; one-way or two-way) was performed for appropriate groups. A *Tukey* test was performed as a posteriori test when significant interactions were found. A P value of <0.05 was considered significant.

3. Results

3.1. Cardiovascular effects of intravenously injected uridine

Intravenous administration of uridine (124, 250 and 500 mg/kg) produced prominent decreases in blood pressure. As seen in Fig. 1A, the effect was dose- and time-dependent. At all doses, the blood pressure reached its minimum within the first minute after injection. Although the magnitudes of the decrease in arterial pressure after 124 mg/kg and 250 mg/kg uridine were the same, the duration of the effect was shorter in the 124 mg/kg uridine-injected group than in the 250 mg/kg injected animals. The maximum decrease in arterial pressure in the 500 mg/kg uridine-injected group was 68±6 mm Hg and the effect disappeared within 30 min (Fig. 1A). Analysis of variance revealed a significant dose [$F(3,22)=10.70$, $P<0.001$], time [$F(10,22)=75.33$, $P<0.001$], and dose–time [$F(30,22)=14.78$, $P<0.001$] interaction of uridine on blood pressure.

Uridine administration caused short-term bradycardia (Fig. 1B). The maximum decreases in heart rate were 181±15 beats/min and 211±15 beats/min after injection of 250 and 500 mg/kg uridine, respectively. This occurred within 1 min of the injections and lasted 5–10 min (Fig. 1B). Analysis of variance indicated significant effects of uridine treatment time [$F(10,22)=49.52$, $P<0.001$] and dose–time [$F(30,22)=7.78$, $P<0.001$] interaction on heart rate.

3.2. Cardiovascular effects of intravenously injected cytidine

In normotensive rats, intravenously injected cytidine (124, 250 and 500 mg/kg) decreased arterial pressure dose- and time-dependently (Fig. 2A). Analysis of variance confirmed that cytidine produced significant dose [$F(3,34)=7.10$, $P<0.001$],

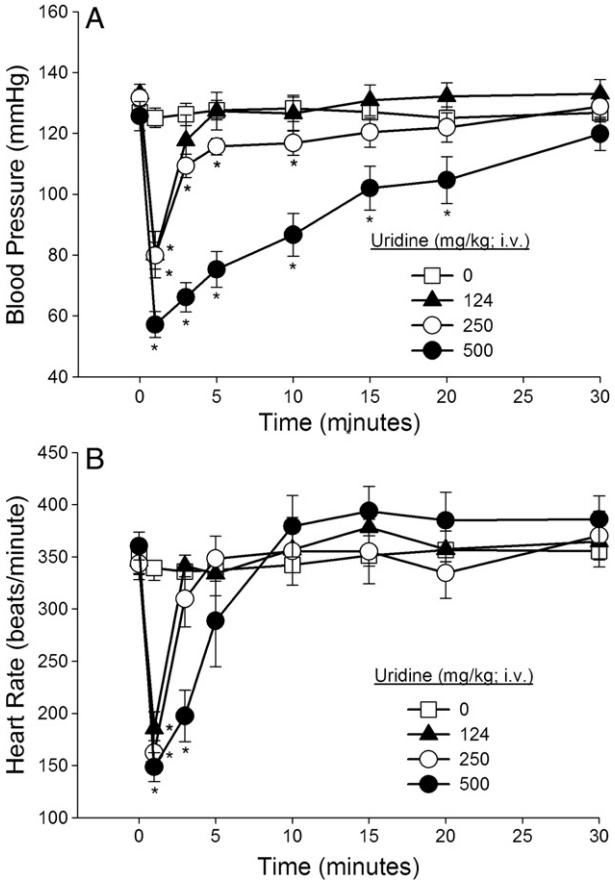

Fig. 1. Cardiovascular effects of intravenously injected uridine. Uridine (124, 250 and 500 mg/kg; i.v.) or saline (1 ml/kg; i.v.) was injected and the blood pressure (A) and heart rate (B) of rats were monitored during the 60 min period after injection. Data are given as means±S.E.M. of 6–8 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the saline group.

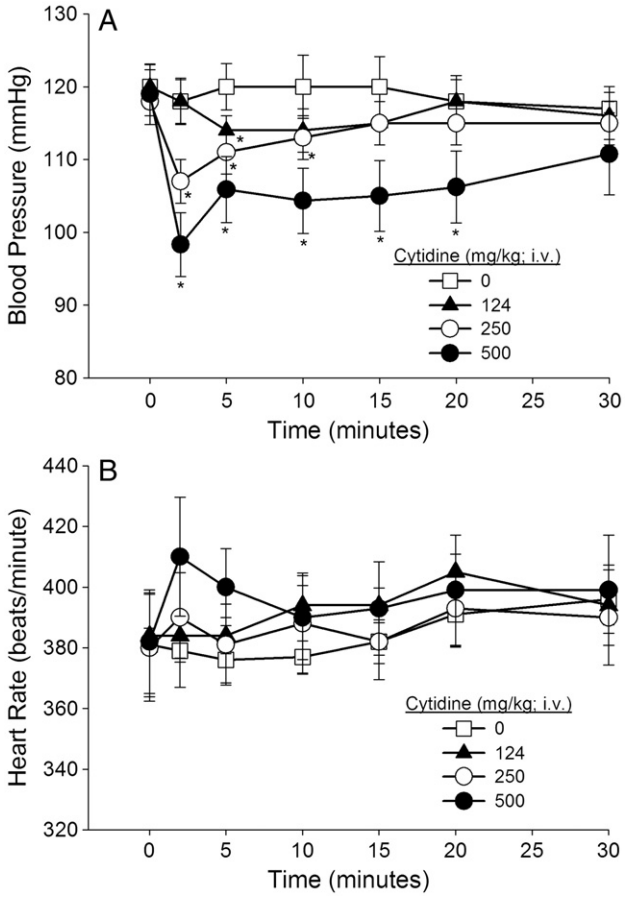

Fig. 2. Cardiovascular effects of intravenously injected cytidine. Cytidine (124, 250 and 500 mg/kg; i.v.) or saline (1 ml/kg; i.v.) was injected and blood pressure (A) and heart rate (B) of rats were monitored during the 60 min period after injection. Data are given as means±S.E.M. of 8–10 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the saline group.

time [$F(9,28)=8.2$, $P<0.001$] and dose–time [$F(27,28)=3.0$, $P<0.001$] interaction effects on blood pressure. At all injected doses, blood pressure decreased to its minimum value within the first 1–2 min and returned to control values at around 5–10 min (Fig. 2A). Maximum decreases in blood pressure were 8±2 mm Hg, 15±2 mm Hg and 21±3 mm Hg for the 124 mg/kg, 250 mg/kg and 500 mg/kg doses, respectively. Heart rate did not change significantly after i.v. cytidine injection (Fig. 2B). Although there was a tendency towards an increase in heart rate in the 500 mg/kg cytidine-injected rats (heart rate increase: 36± 16 beats/min, at first min), it failed to reach significance ($P=0.06$).

3.3. Cardiovascular effects of intravenously injected adenosine

In order to compare the cardiovascular effects of uridine and cytidine with the cardiovascular effects of adenosine, which is a nonselective adenosine receptor agonist, we injected adenosine (137 mg/kg) at the dose equimolar to 250 mg/kg uridine or cytidine. Adenosine administration decreased the blood pressure and heart rate immediately (Fig. 3A, B). The maximum decrease in blood pressure, 80±1 mm Hg, was observed within 5 min after injection and returned to control levels at 30 min (Fig. 3A). The deep bradycardia observed within first minute after adenosine injection returned to control levels within 15 min (Fig. 3B). The maximum decrease in heart rate was 268±5 beats/min (Fig. 3B).

3.4. Increase in plasma levels of cytidine and/or uridine after cytidine, uridine or CDP-choline treatments

Intravenously injected uridine (250 mg/kg) and cytidine (250 mg/kg) increased the plasma levels of uridine and cytidine, respectively (Table 1). The basal plasma concentration of each nucleoside was 0.77±0.07 μm ($n=9$) for uridine and 4.17± 0.06 μm ($n=9$) for cytidine. Uridine administration produced an immediate increase in plasma uridine levels that reached a maximum within first minute and declined gradually, as seen in Table 1. We observed the same time-course for plasma cytidine concentrations after cytidine administration (Table 1). The increases in plasma uridine and cytidine levels were dose-

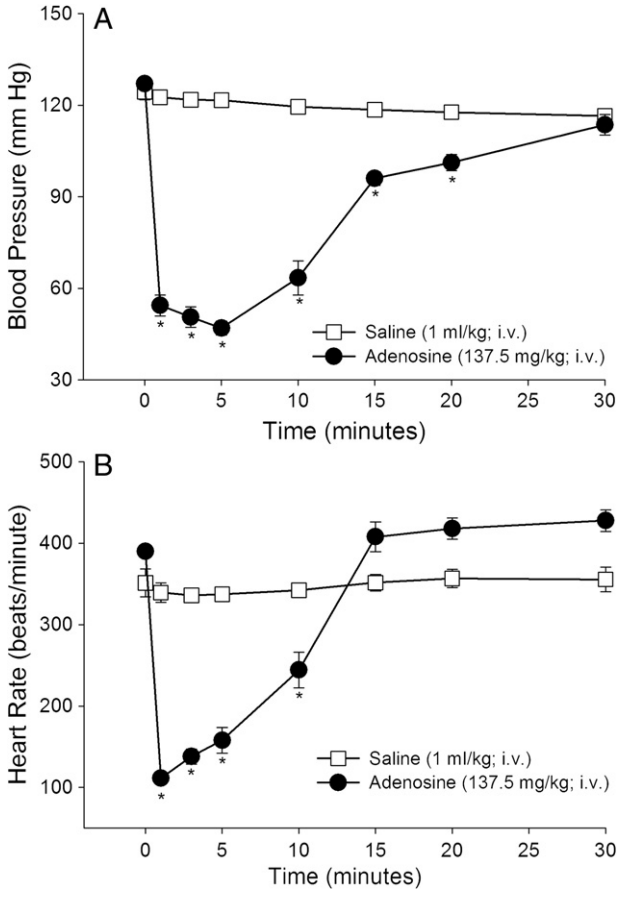

Fig. 3. Cardiovascular effects of intravenously injected adenosine. Adenosine (137 mg/kg; i.v.) or saline (1 ml/kg; i.v.) was injected at a dose equimolar to 250 mg/kg uridine or cytidine. The blood pressure (A) and heart rate (B) of rats were monitored during the 60 min period after injection. Data are given as means±S.E.M. of 5 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the saline group.

Table 1
Plasma uridine or cytidine levels after respective treatments: time–response profile

Treatment	Time after treatment					
	1 min	3 min	5 min	10 min	20 min	30 min
Plasma uridine levels						
Saline (1 ml/kg)	0.9±0.1 μM	0.9±0.1 μM	0.6±0.1 μM	0.6±0.1 μM	0.9±0.2 μM	0.8±0.1 μM
Uridine (250 mg/kg)	3.8±0.7[a] mM	3.2±0.9[a] mM	2.3±0.9[a] mM	1.6±0.6[a] mM	0.6±0.1[a] mM	0.4±0.2[a] mM
Plasma cytidine levels						
Saline (1 ml/kg)	3.9±0.3 μM	4.4±0.5 μM	4.1±0.2 μM	3.9±0.2 μM	3.7±0.2 μM	3.7±0.2 μM
Cytidine (250 mg/kg)	10.4±2.2[a] mM	5.9±2.3[a] mM	4.0±0.8[a] mM	4.1±1.4[a] mM	3.7±1.6[a] mM	2.5±0.8[a] mM

Saline, uridine or cytidine was injected intravenously. Blood samples were taken from the arterial line at the indicated time intervals for plasma measurement of uridine and cytidine. Data are given as means±S.E.M. of 5 measurements. Statistical analysis was performed using two-way RM-ANOVA with an a posteriori Tukey's test.
[a] $P<0.05$ significantly different from the value of the saline group.

dependent (Table 2). Uridine administration did not affect plasma cytidine levels, and cytidine administration did not change plasma uridine concentrations.

In order to compare the increases in plasma levels of uridine or cytidine observed after uridine or cytidine administration versus the increases obtained after CDP-choline injection, 500 mg/kg CDP-choline, which is equimolar to 250 mg/kg cytidine or uridine, was injected intravenously. The basal plasma uridine and cytidine levels of the rats were similar to those obtained from cytidine- or uridine-injected rats. After CDP-choline administration, plasma cytidine concentration increased from 3.1±0.7 μM ($n=5$) to 161±17 μM ($n=5$) within the first minute while uridine levels did not change from the basal levels (data not shown).

3.5. Effect of i.v. caffeine or 8-PT pretreatment on cardiovascular effects of uridine and cytidine

We next aimed to determine if adenosine receptors mediate the cardiovascular effects of uridine or cytidine. Caffeine, which is a nonselective adenosine receptor antagonist, was injected intravenously at a dose of 20 mg/kg 5 min before uridine (250 mg/kg; i.v.) or cytidine (250 mg/kg) administration. Caffeine pretreatment greatly abolished the blood pressure decrease induced by the uridine injection (Fig. 4A). Analysis of variance confirmed the blockade {the time [$F(11,16)=6.42$, $P<0.001$] and dose–time [$F(22,16)=2.55$, $P<0.001$] interaction}. The bradycardic response to uridine was also blocked by this pretreatment (Fig. 4B), which was confirmed by ANOVA {the time [$F(11,16)=6.42$, $P<0.001$] and dose–time [$F(22,16)=2.55$, $P<0.001$] interaction}.

Caffeine completely blocked the decrease in blood pressure induced by cytidine (250 mg/kg) (Fig. 5A). Analysis of variance indicated a significant dose–time interaction [$F(8,72)=61.19$, $P<0.05$] of caffeine pretreatment on the hypotensive effect of cytidine. Since caffeine is also known to be a phosphodiesterase inhibitor and this property of the drug may change the responses, we also pretreated with another nonselective adenosine receptor antagonist, 8-PT (1 mg/kg; i.v.). 8-PT pretreatment completely blocked the decrease in blood pressure induced by uridine (Fig. 4A) (dose $F(1,7)=7.58$, $P<0.05$, time $F(7,49)=17.81$, $P<0.001$, dose–time $F(7,49)=19.87$, $P<0.001$ interaction). The bradycardic response to uridine also was blocked by 8-PT pretreatment (Fig. 4B). Pretreatment of 8-PT completely abolished the decrease in blood pressure seen after cytidine treatment (Fig. 5B).

3.6. Effect of i.c.v. caffeine or 8-SPT pretreatment on the cardiovascular effects of uridine or cytidine

Since both caffeine and 8-PT cross the blood-brain barrier when given intravenously, we cannot differentiate the central or peripheral mechanisms involved in these responses. Therefore, to investigate if central adenosine receptor activation is involved in the cardiovascular effects of uridine and cytidine, rats were pretreated with caffeine (200 μg) injected intracerebroventricularly. Intracerebroventricular caffeine pretreatment did not block the hypotension induced by uridine (250 mg/kg; i.v.)

Table 2
Dose–response profile of plasma uridine or cytidine levels after uridine or cytidine injections, respectively

	Saline 1 ml/kg	Uridine 124 mg/kg	Uridine 250 mg/kg	Uridine 500 mg/kg
Plasma uridine levels	0.7±0.03 μM	0.8±0.05[a] mM	5.3±0.91[a] mM	13.1±1.01[a] mM
	Saline 1 ml/kg	Cytidine 124 mg/kg	Cytidine 250 mg/kg	Cytidine 500 mg/kg
Plasma cytidine levels	4.3±0.09 μM	0.6±0.06[a] mM	4.0±0.27[a] mM	10.4±0.49[a] mM

Saline, uridine or cytidine was injected intravenously. 5 min after injections, blood samples were collected for uridine and cytidine measurements. Data are given as means±S.E.M. of 5 measurements. Statistical analysis was performed using two-way RM-ANOVA with an a posteriori Tukey's test.
[a] $P<0.05$ significantly different from the value of the saline group.

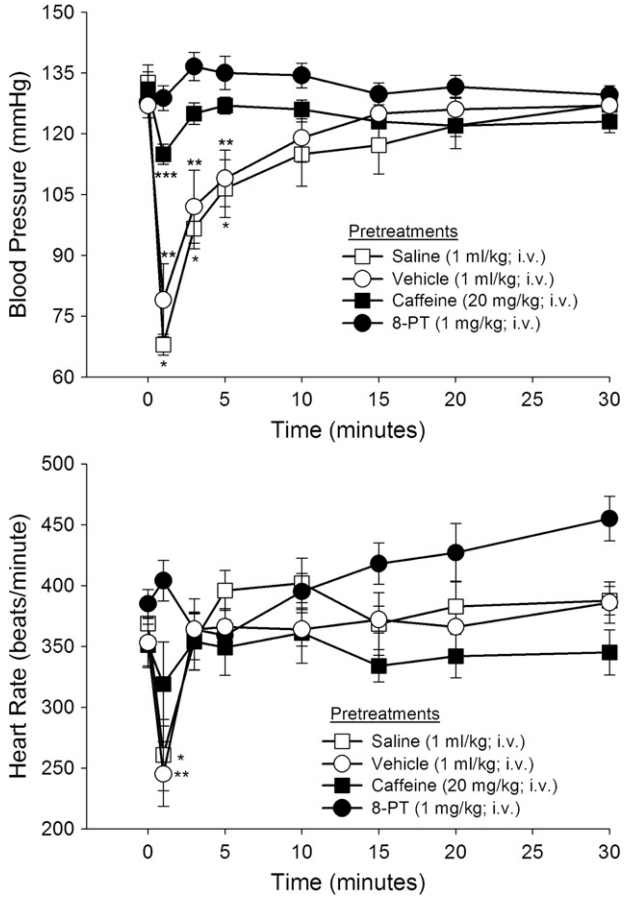

Fig. 4. Effect of i.v. caffeine or 8-PT pretreatment on uridine-induced cardiovascular effects. Caffeine (20 mg/kg; i.v.), 8-PT (1 mg/kg; i.v.), saline (1 ml/kg; i.v.) or vehicle (saline containing 10% DMSO and 10% 1.0 M NaOH) was administered 5 min before uridine (250 mg/kg; i.v.) injection. Blood pressure (A) and heart rate (B) of rats were monitored throughout the study. Monitoring was stopped 60 min after uridine injection. Since the effect was seen in the first 30 min, we did not show the other time points on the figure. Data are given as means±S.E.M. of 5–6 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the caffeine group and the value of the before uridine treatment ("0" time point). **$P<0.05$ significantly different from the value of the 8-PT group and the value of the before uridine treatment ("0" time point). ***$P<0.05$ significantly different from the value of the before uridine treatment ("0" time point).

(Fig. 6A). The decreases in blood pressure in the saline- and caffeine-pretreated groups were 42±7 mm Hg and 34±7 mm Hg, respectively. However, blood pressure returned to control levels faster in the caffeine-pretreated animals than in the saline-pretreated group (Fig. 6A). The bradycardic effect of uridine was not changed by this pretreatment (Fig. 6B).

Intracerebroventricular caffeine pretreatment did not significantly alter the blood pressure decrease induced by cytidine (250 mg/kg; i.v.) (Fig. 5B). Since 8-SPT is different from caffeine by being a purely peripherally acting nonselective adenosine receptor antagonist, which does not cross blood-brain barrier, we tested the effect of central or peripheral 8-SPT pretreatment on the cardiovascular effects of uridine or cytidine. Intracerebroventricularly injected 8-SPT (50 μg) failed to change the effects of uridine (250 mg/kg; i.v.) on blood pressure (Fig. 6A) and heart rate (Fig. 6B). The hypotensive effect of cytidine was not affected by this pretreatment (Fig. 5B). Intravenous administration of 8-SPT (20 mg/kg) completely blocked the cardiovascular effects of uridine (Fig. 7A, B) and the hypotensive response to cytidine (Fig. 5A).

3.7. Effect of DPCPX, DMPX or ZM241385 pretreatment on cardiovascular effects of uridine and cytidine

In order to examine whether adenosine A_1 and/or A_{2A} receptors are involved in the cardiodepressor effects of uridine or cytidine, we first pretreated rats with DPCPX (5 mg/kg; i.v.), a selective adenosine A_1 receptor antagonist, 5 min before the uridine (250 mg/kg; i.v.) or cytidine (250 mg/kg; i.v.) injections. DPCPX pretreatment greatly abolishes the hypotensive and bradycardic responses to uridine (Fig. 8A, B). Uridine increased heart rate significantly ($P<0.05$) in DPCPX-pretreated rats (294±11 beats/min vs 368±21; before vs 1 min after uridine, $n=8$) (Fig. 8B). Adenosine A_1 receptor antagonist pretreatment abolished the hypotensive effect of cytidine at 250 mg/kg (Fig. 5A) and 500 mg/kg doses (126±3 mm Hg vs 130±4 mm Hg; before vs after cytidine, $n=6$) and evoked the increase in heart rate ($P<0.05$) observed in cytidine-injected rats at the

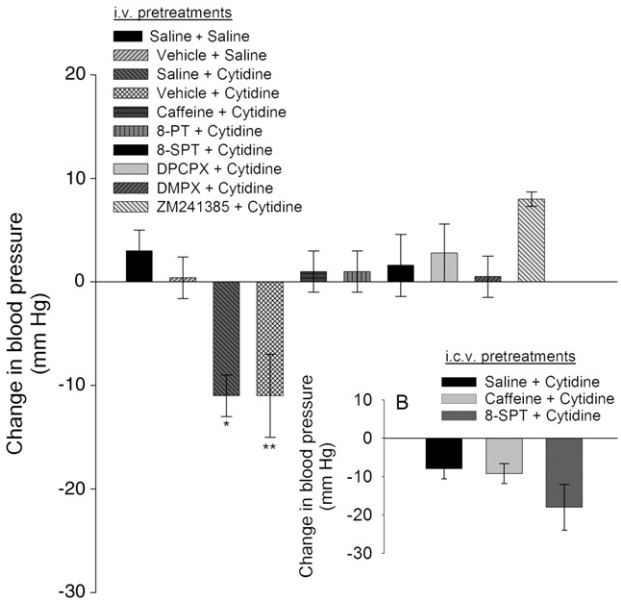

Fig. 5. Effect of adenosine antagonist pretreatments on the decrease in blood pressure induced by cytidine. A) Saline (1 ml/kg; i.v.), vehicle (saline containing 10% DMSO and 10% 1.0 M NaOH) (1 ml/kg; i.v.), caffeine (20 mg/kg; i.v.), 8-PT (1 mg/kg; i.v.), 8-SPT (20 mg/kg; i.v.), DPCPX (5 mg/kg; i.v.), DMPX (2 mg/kg; i.v.), or ZM241385 (1 mg/kg; i.v.) was administered 5 min before cytidine (250 mg/kg; i.v.) injection. B) Caffeine (200 μg; i.c.v.), 8-SPT (50 μg; i.c.v.) or saline (10 μl; i.c.v.) was given 5 min before cytidine injection (250 mg/kg; i.v.). Blood pressure of rats was monitored throughout the study. Monitoring was stopped 60 min after cytidine injection. Each bar represents the delta change of blood pressure 1 or 3 min after cytidine administration. Data are given as means±S.E.M. of 5–6 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the saline group. **$P<0.05$ significantly different from the value of the vehicle group.

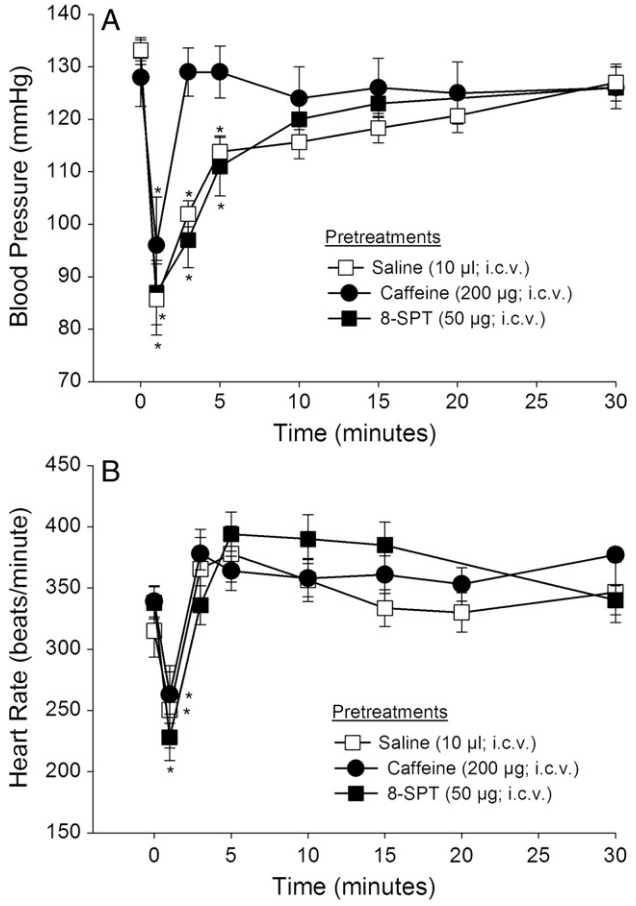

Fig. 6. Effect of i.c.v. injected caffeine or 8-SPT pretreatment on uridine-induced cardiovascular effects. Caffeine (200 µg; i.c.v.), 8-SPT (50 µg; i.c.v.) or saline (10 µl; i.c.v.) was injected 5 min before uridine injection (250 mg/kg; i.v.). Blood pressure (A) and heart rate (B) of rats were monitored for the next 60 min. Data are given as means±S.E.M. of 4–6 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the control ("0" time point) values.

dose of 500 mg/kg (328±12 beats/min vs 366±20 beats/min; before vs after cytidine, $n=6$, $P<0.05$). On the other hand, DMPX (2 mg/kg; i.v.), an adenosine A_{2A} receptor antagonist, pretreatment partially attenuated the hypotensive effect of uridine observed at the first minute after injection and then greatly attenuated the blood pressure response to uridine (Fig. 8C). This pretreatment also completely blocked the bradycardic effect of uridine (Fig. 8D) and the blood pressure response to cytidine (Fig. 5A). Since DMPX is considered a mixed A_1/A_{2A} antagonist with more selectivity for A_{2A} (Muller et al., 1997), and because it is possible that A_1 receptors together with A_{2A} can be blocked by DMPX at the dose used in the present study, we used another more selective A_{2A} antagonist, ZM241385, was used to define the A_{2A}-mediated effects. Pretreatment of rats with ZM241385 (1 mg/kg; i.v.) partially attenuated the hypotensive response to uridine (250 mg/kg; i.v.) (dose $F(2,14)=6.10$, $P<0.05$, time $F(7,98)=13.96$, $P<0.001$, dose–time $F(7,98)=4.89$, $P<0.001$ interaction) without influencing bradycardia (Fig. 8E, F). ZM241385 completely blocked the blood pressure effect of cytidine (250 mg/kg; i.v.) (Fig. 5A).

3.8. Effects of uridine and cytidine administration on plasma adenosine levels

In order to examine if uridine or cytidine exerts these cardiovascular effects by causing an increase in plasma adenosine concentration, we injected uridine (250 mg/kg; i.v.) or cytidine (250 mg/kg; i.v.) into rats and took blood samples 1 and 3 min after the injections. Uridine or cytidine administration increased plasma uridine (0.3±0.06 µm before vs 1.3±0.2 mM after uridine injection) and cytidine (4.6±0.9 µm before vs 3.7±0.4 mM after cytidine injection) levels respectively. Plasma cytidine levels (3.9±0.9 µm before vs 2.6±0.3 µM after uridine injection) did not change after uridine administration, and plasma uridine levels (0.4±0.04 µm before vs 0.5±0.02 µm after cytidine injection) stayed the same after cytidine administration. Neither uridine nor cytidine changed the plasma adenosine levels (Table 3) under these conditions, while plasma inosine levels increased almost twofold after uridine administration (Table 3).

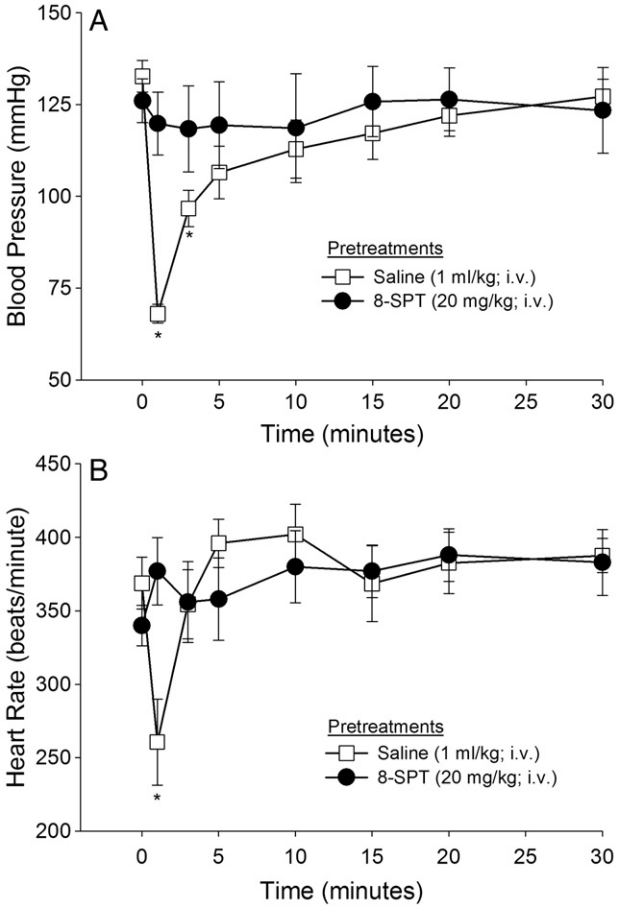

Fig. 7. Effect of intravenously administered 8-SPT pretreatment on uridine-induced blood pressure (A) and heart rate (B) changes. 8-SPT (20 mg/kg; i.v.) or saline (1 ml/kg i.v.) was injected 5 min before uridine administration (250 mg/kg; i.v.). Cardiovascular measurements were recorded for the next 60 min. Data are given as means±S.E.M. of 5–6 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the value of the saline group.

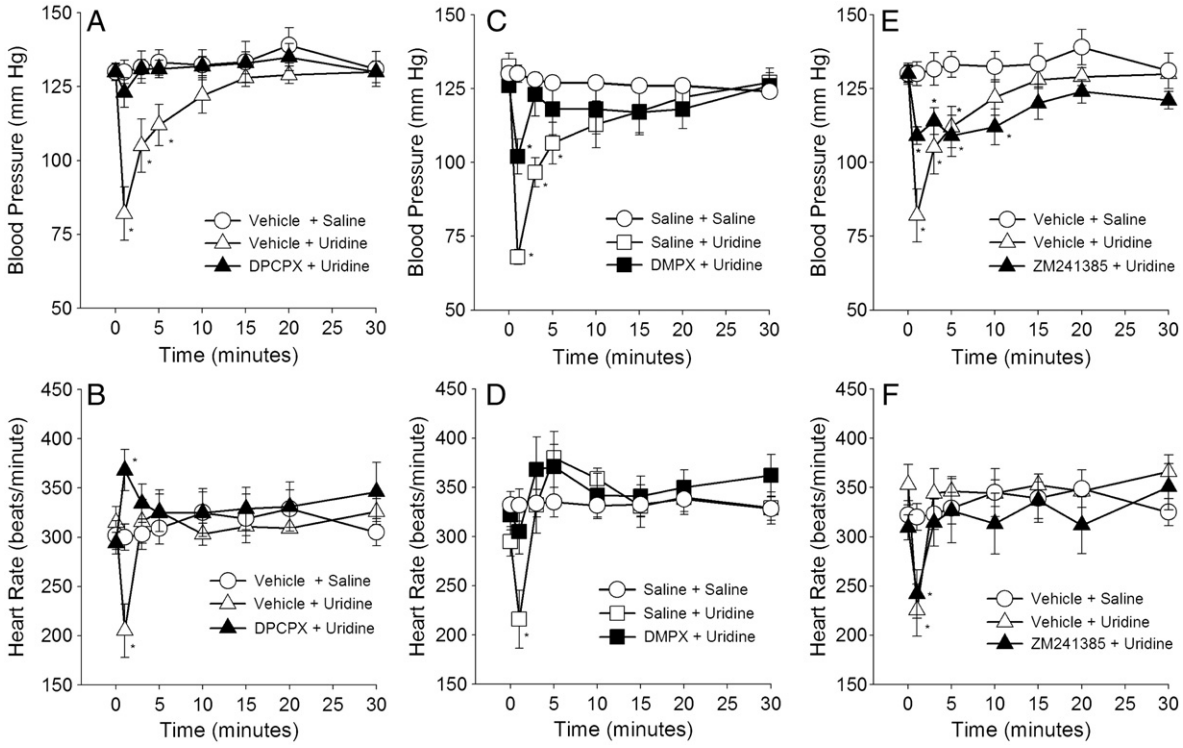

Fig. 8. Effect of i.v. injected DPCPX, DMPX and ZM241385 pretreatments on uridine-induced cardiovascular effects. DPCPX (5 mg/kg; i.v.), DMPX (2 mg/kg; i.v.), ZM241385 (1 mg/kg; i.v.), saline (1 ml/kg; i.v.) or vehicle (saline containing 10% DMSO and 10% 1.0 M NaOH) was injected 5 min before uridine (250 mg/kg; i.v.) or saline (1 ml/kg; iv.) administration. Blood pressure (A, C, E) and heart rate (B, D, F) of rats were monitored for the next 60 min. Data are given as means±S.E.M. of 4–8 measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Tukey's test. *$P<0.05$ significantly different from the corresponding value of the "saline+saline" or "vehicle+saline" group.

3.9. Effect of uridine or cytidine on cardiac parameters in Langendorff-perfused rat heart preparation

In order to determine if uridine or cytidine can affect cardiac parameters under *in vitro* conditions, we used Langendorff-perfused rat heart preparations and calculated the changes in heart rate, dP/dT values, and left ventricular developed pressure values, while controlling the perfusion pressure. Heart weights of rats in the uridine and cytidine perfused groups were 0.81±0.1 g and 0.89±0.2 g, respectively. Uridine at a 10^{-3} M concentration decreased the heart rate significantly ($P<0.05$) while cytidine did not influence the heart rate (Fig. 9). Neither uridine nor cytidine affected the dP/dT_{max}, dP/dT_{min}, left ventricular developed pressure values in isolated rat hearts (Table 4).

4. Discussion

Our results show that intravenously injected uridine or cytidine decreases blood pressure. Uridine also causes bradycardia while cytidine does not change the heart rate.

The doses of uridine or cytidine used in the present study were chosen from previous CDP-choline experiments as equimolar to CDP-choline (Carlezon et al., 2002, 2005; Savci et al., 2003). Uridine produced a more profound decrease in blood pressure (maximum decreases in blood pressure: 68±6 mm Hg vs 21±3 mm Hg; uridine vs cytidine) and heart rate (maximum decrease in heart rate: 181±12 beats/min) while cytidine at the 250 mg/kg dose had no effect on the heart rate of rats and exerted a tendency to increase the heart rate (395±17 beats/min before vs 431±16 after cytidine, administration; $n=9$, $P=0.06$) at the 500 mg/kg dose. Uridine's cardiovascular effects were also time- and dose-related. The hypotensive effects of the nucleosides lasted longer in the uridine-injected group than in animals given cytidine. In our positive control

Table 3
Plasma adenosine and inosine levels in rats treated with uridine or cytidine

Plasma nucleoside levels (μM)	Before treatment	After treatment	
		1 min	3 min
Uridine treatment (250 mg/kg; i.v.)			
Adenosine	0.48±0.13	0.49±0.17	0.39±0.18
Inosine	0.29±0.01	0.54±0.09[a]	0.42±0.06
Cytidine treatment (250 mg/kg; i.v.)			
Adenosine	0.49±0.08	0.50±0.08	0.48±0.08
Inosine	0.31±0.06	0.22±0.04	0.16±0.02

Uridine or cytidine injections were done intravenously. "Before treatment" values represent the levels measured from the samples that were taken just before the injections. "After treatment" values were measurements from blood samples that were collected 1 and 3 min after uridine (250 mg/kg; i.v.) or cytidine (250 mg/kg; i.v.) injections. Data are given as means±S.E.M. of 6 measurements. Statistical analysis was performed using one-way RM-ANOVA with an a posteriori Tukey's test.

[a] $P<0.05$ significantly different from the value of the "before treatment" group.

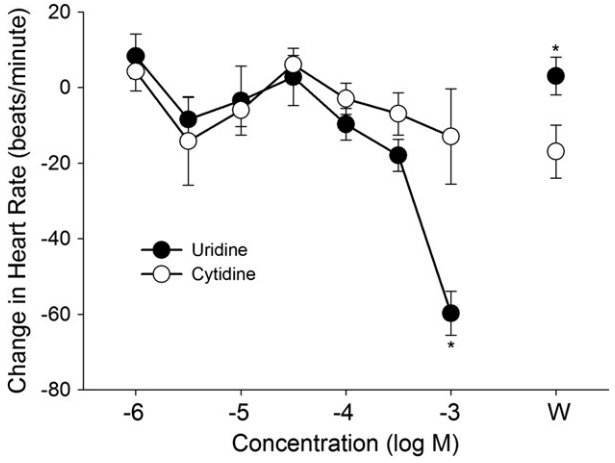

Fig. 9. Heart rate effects of uridine or cytidine in Langendorff-perfused heart preparation. After a 30-min stabilization period, uridine or cytidine was perfused in increasing concentrations. Each perfusion period lasted no more than 5 min and the last 30 s of each perfusion period was used for calculation of heart rate. A 10-min washout period was performed at the end of the perfusion period at the highest concentration of agonist. Changes in heart rate values were calculated by substracting the heart rate values from the values obtained with the perfusion of the just previous concentration, except the washout period. Washout data were calculated by substracting the heart rate values from control heart rates. Data are given as means±S.E.M. of 4 measurements. Statistical analysis was performed using one-way ANOVA. *$P<0.05$ significantly different from other groups. Abbreviations: W; washout.

experiments, we also investigated the blood pressure effect of adenosine injected at doses equimolar to uridine (250 mg/kg) or cytidine (250 mg/kg). Adenosine (137 mg/kg; i.v.) decreased both the blood pressure and heart rate by 80±1 mm Hg ($n=5$) and 268±5 beats/min ($n=5$). Although the cardiovascular effects of uridine, but not cytidine, resemble those of adenosine, its effects were shorter and smaller than those observed in rats given adenosine.

Recently, in separate studies, similar doses of cytidine (238 mg/kg) (Carlezon et al., 2002) and uridine (239 mg/kg) (Carlezon et al., 2005) injected intraperitoneally to test their behavioral effects in rats were reported to produce an antidepressant-like effect. These studies support our findings by demonstrating the individual effects of these nucleosides; however, our study is the first to show the pharmacological effects of these nucleosides on cardiovascular parameters at similar doses. Moreover, our data suggest that adenosine receptors mediate the cardiovascular effects of uridine and cytidine, since the i.v. caffeine or 8-PT pretreatment completely blocked the responses. Peripheral mechanisms appear to be involved in these effects because the purely peripherally effective nonselective antagonist 8-SPT completely blocked the responses to uridine or cytidine while centrally injected caffeine or 8-SPT did not abolish the effects. Although adenosine has long been known to play important roles in cardiovascular functions, including vasodilatation and negative chronotropic and inotropic effects in several species (Baracco et al., 1987; Ravelic and Burnstock, 1998, Schindler et al., 2005), our results are the first to show that the cardiovascular effects of uridine or cytidine are mediated by activation of peripheral adenosine receptors.

At least four types of adenosine receptors are found in the central and peripheral nervous systems, namely A_1, A_{2A}, A_{2B} and A_3. The widely accepted primary mechanism by which adenosine exerts its cardiac effects is by directly activating the inwardly rectifying potassium current ($I_{K\text{-}Ado}$) in the atrial and nodal tissues through stimulation of A_1 receptors (Dhalla et al., 2003). Activation of A_{2A} receptors produces peripheral and coronary vasodilation (Hinschen et al., 2003; Schindler et al., 2005). One or the combination of these effects causes hypotension, as observed in adenosine-injected species. Our results obtained from uridine-injected rats indicate that mainly A_1, but also A_{2A} adenosine receptor activations are involved in the cardiovascular effects of uridine. This conclusion is based on the data that the pretreatment with DPCPX, an A_1 adenosine receptor antagonist, greatly attenuated both the hypotensive and bradycardic effects of uridine while pretreatment with ZM241385, an A_{2A} adenosine receptor antagonist, caused only a small blockade of the hypotensive effects of uridine

Table 4
Effect of uridine or cytidine perfusion on cardiac parameters in Langendorff-perfused heart preparation

Parameters	Uridine concentration (log *M*)								
	Control	−0.6	−5.5	−5.0	−4.5	−4.0	−3.5	−3.0	Washout
Perfusion pressure	111±9	117±12	113±11	119±12	122±14	121±14	114±12	108±10	117±15
dP/dT_{max} (mmHg s^{-1})	1557±171	1496±180	1556±214	1781±300	1569±215	1943±467	1696±221	2076±527	1259±240
$-dP/dT_{max}$ (mmHg s^{-1})	−1022±171	−1021±180	−1003±214	−1079±300	−1003±215	−1083±467	−1032±221	−1128±527	−943±240
Left ventricular developed pressure	65±6	61±6	61±7	68±9	59±7	69±13	66±8	75±14	51±10
Parameters	Cytidine concentration (log *M*)								
	Control	−0.6	−5.5	−5.0	−4.5	−4.0	−3.5	−3.0	Washout
Perfusion pressure	137±10	131±11	134±10	143±11	142±11	143±11	139±10	131±13	144±15
dP/dT_{max} (mmHg s^{-1})	1636±127	1381±81	1967±292	1890±351	1467±42	1347±37	1414±44	1487±122	1202±55
$-dP/dT_{max}$ (mmHg s^{-1})	−947±133	−800±13	−1106±181	−1007±180	−871±56	−810±50	−832±51	−863±91	−697±61
Left ventricular developed pressure	69±7	55±3	75±9	74±11	57±3	52±4	56±4	60±8	46±3

After stabilization period, hearts were perfused with increasing concentrations of uridine or cytidine for 5-min periods. The last 30 s of each perfusion period was calculated for each dose effects and the last 30 s of stabilization period was used as a control measurements. Data are given as means±S.E.M. of 4 measurements. Statistical analysis was performed using one-way ANOVA.

without affecting bradycardia. Those findings also suggest that uridine-induced bradycardia is mediated by A_1 receptor activation. Results obtained from DMPX pretreated group in which the great attenuation of the hypotension together with the complete blockade of the bradycardia induced by uridine was observed support this hypothesis. Because DMPX is considered an antagonist for both A_1 and A_{2A} receptors (Muller et al., 1997). Although its selectivity for A_1 receptors is lower than for A_{2A} receptors, in the dose ranges we used in the present study it is quite possible that DMPX blocks A_1 receptors as well as A_{2A} receptors. The great abolition of cardiovascular effects of uridine by pretreatment with the DPCPX implies a mainly A_1-mediated and bradycardia-dependent hypotension without A_{2A} involvement. However, the observation of ~25 mm Hg decrease in blood pressure in the first minute after uridine injection, despite no fall in heart rate in rats pretreated with DMPX implicates an A_2-mediated hypotension which occurs independently of bradycardia. In this case, the failure of DMPX pretreatment, as an A_1/A_{2A} antagonist, to reduce the A_{2A}-mediated hypotension as well can be explained by its low affinity for adenosine A_{2A} receptors. Indeed, the findings obtained with the more potent and selective adenosine A_{2A} receptor antagonist, ZM241385, suggest that A_{2A}-mediated dilatation is only a minor and early component of uridine-induced hypotension. This pretreatment did not block the hypotensive effect of uridine as effectively as DMPX pretreatment and the only a very small component of the initial hypotension, in the first minute of treatment, was influenced by adenosine A_{2A} receptor blockade. Altogether, these findings imply that A_1-mediated bradycardia is the primary response to uridine which results in secondary hypotension that is likely facilitated by some A_{2A}-mediated dilation in the early minutes of stimulation. This profound decrease in blood pressure, in turn, leads to the secondary baroreceptor-mediated tachycardia that supersedes A_1-mediated bradycardia and reverses hypotension, which explains the observed temporal shifts in the time profile showing the sustained hypotension after the bradycardia abates.

On the other hand, as can be seen in Figs. 1 and 2, the cardiovascular effects of cytidine were quite different from those of uridine, mainly in two ways: i) the cytidine-induced decrease in blood pressure was smaller and shorter than those observed in uridine-injected rats; and ii) cytidine generally did not significantly alter the heart rates of rats whereas uridine treatment was always associated with bradycardia. These differences imply differing receptor involvement of the cardiovascular effects of cytidine and uridine. As we discussed above, uridine may primarily activate A_1 receptors with minor A_{2A} receptor activation, whereas cytidine more likely behaves as an A_{2A} agonist. The A_{2A}-mediated dilatation produces the modest hypotension observed after cytidine administration with adequate baroreceptor control of heart rate. The complete blockade of the cytidine-induced drop in blood pressure by pretreatment with the potent and selective A_{2A} antagonist ZM241385 confirms this hypothesis. On the other hand, DPCPX pretreatment abolished the blood pressure effect of cytidine at both doses while evoking a tachycardic response to cytidine (500 mg/kg). Although we cannot yet explain the mechanism of the complete blockade of the blood pressure effect of cytidine by A_1 antagonist, we suppose that cytidine, differently from uridine, can activate the A_1 adenosine receptors localized in various non-cardiac tissues (i.e. vascular smooth muscle) resulting in different responses from those observed with uridine. This can be related to cytidine's bioavailability.

Although the above data strongly suggest that these pyrimidine nucleosides exert their cardiovascular effects by activating adenosine receptors, it was still possible that the increase in plasma adenosine concentration after uridine or cytidine administration caused the effects, because it has been reported that these nucleosides can block the uptake of adenosine by competing for the same nucleoside transporter molecules (Wang and Giacomini, 1997). However, our measurements of plasma adenosine concentrations after uridine or cytidine administration ruled out this possibility, since the plasma adenosine levels of the rats did not change while the uridine or cytidine concentrations increased dramatically. Interestingly, plasma inosine levels increased nearly twofold in uridine-injected rats. Since inosine is the deamination product of adenosine and we did not use a adenosine deaminase inhibitor during the blood collection, we suspect that in animals given uridine, adenosine may have been rapidly metabolized to inosine. Therefore, we investigated the cardiac effects of uridine or cytidine in isolated Langendorff-perfused rat heart preparation in which there is no exogenous adenosine. Results from these experiments clearly show that uridine decreases heart rate under *in vitro* conditions when its concentration in the perfusion fluid increased to the range seen in plasma after uridine administration. However cytidine did not influence the cardiac parameters in these preparations.

The basal plasma levels of uridine and cytidine are different between rats and humans and also within each species. In rats, the plasma concentration of cytidine is 4 times higher than that of uridine (4.17 ± 0.06 μm and 0.77 ± 0.07 μm) while in humans the plasma level of cytidine (<100 nM) is reported to be sixty times lower than that of uridine (~6 μm) (Wurtman et al., 2000). Our data show that the observed increases in plasma levels of uridine or cytidine were different even though they were injected at the same dose. Although their levels both reached peak values within the first minute, the observed increases in plasma uridine levels after uridine injection were almost 2 times higher than the plasma cytidine levels after cytidine injection. Approximately 4.2- and 2.6-fold increases were observed after uridine or cytidine injection, respectively (at 250 mg/kg doses). The differences in the magnitudes of the pressor and cardiac effects between uridine and cytidine can be explained by these relative increases. The cytidine levels resulting from injection of CDP-choline at the dose equimolar to 250 mg/kg cytidine (500 mg/kg; i.v.) are sixty times lower than those obtained from cytidine injection (250 mg/kg; i.v.). This can be expected because of the differences in bioavailabilities of the drugs. In the CDP-choline form, cytidine will be released to the blood after the parent drug has been metabolized by phosphodiesterases. However, when administered as cytidine or uridine it can be absorbed directly. The observation of no

change in plasma uridine levels after CDP-choline injection in rats is consistent with previous reports (Lopez et al., 1987, 1995).

Very interestingly, the substantial increases in plasma levels of the nucleosides after intravenous injection were tolerated very well and did not cause any serious behavioral side effects. In conclusion, our data show that both uridine and cytidine can affect cardiovascular parameters and reduce blood pressure. These effects appear to be mediated by adenosine receptors in the periphery. The almost entirely adenosine $_{A1}$ receptor activation seems to be responsible from uridine-induced cardiovascular responses, however both A_1 and A_{2A} receptor activations are involved in the cytidine's effects.

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References

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

Adibhatla, R.M., Hatcher, J.F., 2005. Cytidine 5′-diphosphocholine (CDP-choline) in stroke and other CNS disorders. Neurochem. Res. 30, 15–23.

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

Barraco, R.A., Marcantonio, D.R., Phillis, J.W., Campbell, W.R., 1987. The effects of peripheral injections of adenosine and its analogs on blood pressure and heart rate in the rat. Gen. Pharmacol. 18, 405–416.

Carlezon, W.A., Pliakas, A.M., Parow, A.M., Dekte, 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.

Carlezon, W.A., Mague, S.D., Parow, A.M., Stoll, A.L., Cohen, B.M., Renshaw, P.F., 2005. Antidepressant-like effects of uridine and omega-3 fatty acids are potentiated by combined treatment in rats. Biol. Psychiatry 57, 343–350.

Danilaou, G., Vicaut, E., Same, A., Aubier, M., Boczkowski, J., 1997. Predominant role of A_1 adenosine receptors in mediating adenosine induced vasodilatation of rat diaphragmatic arterioles: involvement of nitric oxide and the ATP-dependent K^+ channels. Br. J. Pharmacol. 121, 1355–1363.

De Bruin, N.M.W.J., Kiliaan, A.J., De Wilde, M.C., Broersen, L.M., 2003. Combined uridine and choline administration improves cognitive deficits in spontaneously hypertensive rats. Neurobiol. Learn. Mem. 80, 63–79.

Dhalla, A.K., Shryock, J.C., Shreeniwas, R., Belardinelli, L., 2003. Pharmacology and therapeutic applications of A_1 adenosine receptor ligands. Curr. Top. Med. Chem. 3, 369–385.

Evoniuk, G., Von Borstel, R.W., Wurtman, R.J., 1987. Antagonism of cardiovascular effects of adenosine by caffeine or 8-(*p*-sulfophenyl) theophylline. J. Pharmacol. Exp. Ther. 240, 428–432.

Hinschen, A.K., Rose'Meyer, R.B., Headrick, J.P., 2003. Adenosine receptor subtypes mediating coronary vasodilation in rat hearts. J. Cardiovascular Pharmacol. 41, 73–80.

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.

Merkel, L.A., Lappe, R.W., Rivera, L.M., Cox, B.F., Perrone, M.H., 1992. Demonstration of vasorelaxant activity with an A_1-selective adenosine agonist in porcine coronary artery: involvement of potassium channels. J. Pharmacol. Exp. Ther. 260, 437–443.

Muller, C.E., Geis, U., Hipp, J., Schobert, U., Frobenius, W., Pawlowski, M., Suzuki, F., Sandoval-Ramirez, J., 1997. Synthesis and structure–activity relationship of 3,7-dimethyl-1-prppargylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. J. Med. Chem. 40, 4396–4405.

Ralevic, V., Burnstock, G., 1998. Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.

Ray, C.J., Marshall, J.M., 2006. The cellular mechanisms by which adenosine evokes release of nitric oxide from rat aortic endothelium. J. Physiol. 570, 85–96.

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.

Savci, V., Goktalay, G., Cansev, M., Cavun, S., Yilmaz, M.S., Ulus, I.H., 2003. Intravenously injected CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock: effect is mediated by central cholinergic activation. Eur. J. Pharmacol. 468, 129–139.

Schindler, C.W., Karcz-Kubicha, M., Thorndike, E.B., Muller, C.E., Tella, S.R., Ferre, S., Goldberg, S.R., 2005. Role of central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine A_1 and A_{2A} subtype receptor agonists. Br. J. Pharmacol. 144, 642–650.

Shryock, J.C., Belardinelli, L., 1997. Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology, and pharmacology. Am. J. Cardiol. 79, 2–10.

Tabrizchi, R., Bedi, S., 2001. Pharmacology of adenosine receptors in the vasculature. Pharmacol. Ther. 91, 133–147.

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.

Wang, J., Giacomini, K.M., 1997. Molecular determinants of substrate selectivity Na^+-dependent nucleoside transporters. J. Biol. Chem. 272, 28845–28848.

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

Windscheif, U., 1996. Purinoceptors: from history to recent progress. A review. J. Pharm. Pharmacol. 48, 993–1011.

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.

Yitzhaki, S., Shneyvays, V., Jacobson, K.A., Shainberg, A., 2005. Involvement of uracil nucleotides in protection of cardiomyocytes from hypoxic stress. Biochem. Pharmacol. 69, 1215–1223.

Yitzhaki, S., Shainberg, A., Cheporko, Y., Vidne, B.A., Sagie, A., Jacobson, K.A., Hochhauser, E., 2006. Uridine-5-triphosphate (UTP) reduces infarct size and improves rat heart function after myocardial infarct. Biochem. Pharmacol. 72, 949–955.

You, J., Johnson, T.D., Marrelli, S.P., Mombouli, J.V., Bryan Jr., R.M., 1999. P2u receptor-mediated release of endothelium-derived relaxing factor/nitric oxide and endothelium-derived hyperpolarizing factor from cerebrovascular endothelium in rats. Stroke 30, 1124–1133.