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Diuretic and saliuretic effects of 1,3-dipropyl-8-cyclopentylxanthine, a selective A_1-adenosine receptor antagonist

M. G. COLLIS, G. SHAW, J. R. KEDDIE, *ICI Pharmaceuticals, Mereside, Alderley Park Macclesfield, Cheshire SK10 4TG, UK*

Abstract—We have previously shown that 8-phenyltheophylline (8-PT), a non-selective antagonist at adenosine A_1- and A_2-receptors, has a diuretic effect. In this study, the diuretic and adenosine antagonist effects of the A_1-receptor selective compound 1,3-dipropyl-8-cyclopentylxanthine (CPX) have been examined in the conscious rat. CPX (0·1 and 0·3 mg kg^{-1} i.v.) significantly attenuated bradycardic but not hypotensive responses evoked by adenosine. In contrast, 8-PT (3 mg kg^{-1} i.v.) significantly antagonized both adenosine-induced bradycardia and hypotension. CPX (0·1 and 0·3 mg kg^{-1} i.v.) evoked a dose-related diuretic and saliuretic response in the conscious rat. These results indicate that the diuretic effects of adenosine antagonists are associated with blockade of the A_1-receptor sub-type.

The diuretic action of the alkylxanthines has been known for many years, but it is only recently that the pharmacological basis for this has been shown to be adenosine receptor antagonism. This conclusion has been reached because xanthines which have a high affinity for adenosine receptors are diuretic whilst those with a low affinity for these receptors are not (Persson et al 1982; Collis et al 1986). Adenosine receptors have been divided into two types, A_1 and A_2, on the basis of agonist structure-activity relationships. This sub-division has also been supported by the discovery of antagonists with selectivity for the A_1-adenosine receptor, such as 1,3-dipropyl-8-cyclopentylxanthine (CPX, Haleen et al 1987; Collis et al 1989). Previous studies of the diuretic effects of xanthines have used compounds such as 8-phenyltheophylline (8PT) which do not distinguish between A_1- and A_2-adenosine receptors (Collis et al 1986). In the current study we have examined the effects of A_1-receptor selective doses of CPX on urine volume and electrolyte excretion in the conscious rat in order to determine which adenosine receptor sub-type is responsible for the diuretic effects of the xanthines.

Materials and methods

Evaluation of adenosine antagonism in the conscious rat. Spontaneously hypertensive Wistar rats were anaesthetized with halothane and vinyl catheters were surgically implanted in the right jugular vein and thoracic aorta (via the left carotid artery). After a recovery period of 24 h, the aortic blood pressure was recorded directly via a pressure transducer (Bell and Howell L221) and displayed on a chart recorder (Devices MX2). Heart rate was derived from the blood pressure trace. Adenosine, dissolved in saline (0·9% NaCl) was infused (1 mg kg^{-1} min^{-1}) via the jugular catheter for periods of 1–2 min. The infusion was terminated when the heart rate and blood pressure response had stabilized. Adenosine infusions were performed before and 90 min after intravenous injection of 0·1 and 0·3 mg kg^{-1} of CPX or its vehicle (DMSO, 3·3% 1 M NaOH, 0·25% in saline) or 1 and 3 mg kg^{-1} of 8-phenyltheophylline (8-PT) or vehicle (0·1 M NaOH, 50%, PEG 400, 50%).

Evaluation of diuretic activity (Kau et al 1984). Fasted rats were given an oral saline load of 40 mL kg^{-1}, and urine was collected for 6 h. Rats shown to be in the normal range for the excretion of this saline load were paired so that the rat with the highest urine volume was paired with the one with the lowest urine volume; the second highest with the second lowest etc. The rats were fasted overnight with free access to water. The rats were then given CPX (0·1 and 0·3 mg kg^{-1} i.v.) or its vehicle (i.v.) plus 40 mL kg^{-1} of saline (p.o.). Pairs of rats were placed in metabolism cages and urine was collected for 6 h and its volume and the concentration of Na^+, K^+ and Cl^- were measured using flame photometry and a chloride meter, respectively. The rats were denied access to food and water whilst in the metabolism cages. The compounds used were: adenosine (Sigma), 8-phenyltheophylline (Sigma) and 1,3-dipropyl-8-cyclopentylxanthine (synthesized by Dr R. James, Chemistry Department, ICI).

Data are expressed as the mean ± s.e.m. Significant differences ($P < 0·05$) between means were evaluated using Student's paired *t*-test and analysis of variance (ANOVA). Urine volume and electrolyte excretion are expressed as mL or mequiv/100g body weight/6 h.

Results

Adenosine infusion evoked decreases in blood pressure and in heart rate (Table 1). CPX at 0·1 and 0·3 mg kg^{-1} i.v. significantly attenuated the decrease in heart rate and at the higher dose converted an adenosine induced bradycardia into a tachycardia. CPX also caused a small reduction in the hypotensive response to adenosine but this did not achieve statistical significance. 8-PT (3 mg kg^{-1} i.v.) caused a significant attenuation of both the bradycardic and the hypotensive response to adenosine. The lower dose of (1 mg kg^{-1} i.v.) 8-PT did not significantly attenuate adenosine-induced bradycardia or hypotension.

Table 1. Effect of 1,3-dipropyl-8-cyclopentylxanthine (CPX) and 8-phenyltheophylline (8-PT) on hypotensive and bradycardic responses to adenosine (1 mg kg^{-1} min^{-1} i.v.) in the conscious rat.

Group (Dose mg kg^{-1})	Change in diastolic blood pressure (mm Hg)	Change in heart rate (beats min^{-1})
Control	−53·6 ± 7·1	−89·6 ± 22·2
CPX (0·1)	−38·2 ± 7·0	+29·2 ± 29·7*
Control	−65·2 ± 5·6	−98·6 ± 20·6
CPX (0·3)	−49·8 ± 4·4	+58·0 ± 14·9*
Control	−53·6 ± 5·5	−130·8 ± 22·4
8-PT(3)	−22·6 ± 6·6*	+38·2 ± 17·4*

* $P < 0·01$. n = 5 for all groups.

CPX at 0·1 and 0·3 mg kg^{-1} i.v. evoked increases in urine volume and in sodium, potassium and chloride excretion when compared with vehicle treated control animals (Table 2). These increases were significant ($P < 0·05$, ANOVA) for all parameters at CPX doses of 0·3 mg kg^{-1} i.v. and for sodium and chloride excretion at CPX doses of 0·1 mg kg^{-1} i.v.

Discussion

The results of this study demonstrate that CPX exerts a diuretic, saliuretic and kaliuretic effect in the conscious rat. A quantitatively similar effect to increase urine volume and electrolyte excretion has been previously reported using another adenosine

Correspondence to: M. G. Collis, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK.

Table 2. Effect of 1,3-dipropyl-8-cyclopentylxanthine (CPX) on urine volume and electrolyte excretion in the conscious rat.

		CPX (mg kg^{-1} i.v.)	
	Vehicle	0·1	0·3
Urine volume (mL/100 g/6 h)	3·51 ± 0·15	4·47 ± 0·17	5·14 ± 0·23*
Urine sodium (mequiv/100 g/6 h)	0·44 ± 0·01	0·67 ± 0·03*	0·71 ± 0·03*
Urine potassium (mequiv/100 g/6 h)	0·095 ± 0·009	0·119 ± 0·005	0·127 ± 0·004*
Urine chloride (mequiv/100 g/6 h)	0·47 ± 0·01	0·65 ± 0·02*	0·69 ± 0·03*

* $P < 0·05$ compared with vehicle control (ANOVA) (n = 5 for all groups).

receptor antagonist, 8-PT (10 mg kg^{-1} i.p.) in this species (Collis et al 1986). The current results do not allow a direct comparison of the diuretic potency of 8-PT and CPX; this would require controlled administration of the two xanthines so that their potencies and durations of action were matched during the 6 h period of urine collection.

An important difference between CPX and 8-PT lies in their relative selectivities for the A_1 and A_2 sub-types of the adenosine receptor. Studies using isolated tissue preparations have demonstrated that 8-PT is non-selective between A_1- and A_2-receptors whereas CPX exhibits a 30–50-fold higher affinity for the A_1 subtype but is equi-effective with 8-PT as an A_2-receptor antagonist (Collis et al 1989). In the present study, this difference in selectivity has been investigated in conscious rats. In this preparation the bradycardic response to adenosine is mediated via A_1-receptors in the sino atrial node and perhaps also via inhibitory pre-synaptic A_1-receptors on the adrenergic nerves innervating the node (Hedquist & Fredholm 1979; Brown & Collis 1983; Collis & Saville 1984). In contrast, the hypotensive response to adenosine is mainly due to dilation of arterial smooth muscle mediated by an A_2-receptor (Collis & Brown 1983). A small A_1-receptor mediated component of the hypotensive response is also a possibility as adenosine could potentially reduce cardiac output and inhibit adrenergic vasoconstrictor neurotransmitter release and both these effects are via A_1-receptor activation (Brown & Collis 1983; Collis & Saville 1984). 8-PT significantly antagonized both the hypotensive and the bradycardic response to adenosine, which illustrates its non-selective nature. The observation that 8-PT (3 mg kg^{-1} i.v.) abolished adenosine-induced bradycardia whereas in the previous study (Collis et al 1986) 10 mg kg^{-1} i.p., reduced, but did not abolish, this response may be due to the limited bioavailability of 8-PT via the i.p. route (Wormald et al 1989). CPX significantly antagonized the bradycardic actions of adenosine but had a much smaller non-significant and non-dose related effect on the hypotensive response. These results support a previous study in the anaesthetized rat which suggested that CPX is an A_1 selective antagonist at low doses (0·1 mg kg^{-1} i.v.) (Kellet et al 1989).

Since CPX exhibits characteristics both in-vitro and in-vivo which are consistent with selective blockade of A_1-receptors it is reasonable to conclude that the diuretic effects evoked by low doses of the compound are also mediated via this mechanism. A_1-receptor activation in the rat kidney is known to evoke preglomerular arteriolar and mesangial cell constriction (Murray & Churchill 1984; Lopez-Novoa et al 1987) which would reduce GFR and could be antidiuretic. A_1-adenosine receptors have also been identified on renal cortical collecting tubule cells (LeVier et al 1990). Further studies are required to determine the location of the A_1-receptors that are associated with the diuretic action of CPX.

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