inhibition theory. Further, higher concentrations of propranolol were also required to produce central nervous system effects such as anti-aggressive action in rats (Ray et al 1984) and antipsychotic actions in man (Yorkston et al 1977). In fact, the dose of propranolol (30 μmol kg^{-1}), which exhibited an anti-aggressive action (Ray et al 1984) did inhibit brain ChE enzyme activity after systemic administration, in the present study. In addition, propranolol has been shown to be accumulated in the myocardium and brain at concentrations several times higher than in plasma (Myers et al 1975: Schneck et al 1977). All this evidence strongly suggests a cholinergic mechanism (through ChE enzyme inhibition) in the mediation of some of the cardiovascular, central nervous system and ocular actions of propranolol.

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The adenosine receptor antagonist, 8-phenyltheophylline, causes diuresis and saliuresis in the rat

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The diuretic and adenosine antagonist actions of two alkylxanthines have been compared in the conscious rat. 8-Phenyltheophylline (10 mg kg^{-1}) antagonized adenosine-induced bradycardia in the rat for at least 3 h whereas enprofylline (10 mg kg^{-1}) had no effect on this response. 8-Phenyltheophylline (10 mg kg^{-1}) evoked diuresis and saliuresis in the rat whereas enprofylline (10 mg kg^{-1}) had no effect on excretory parameters. These results indicate that the diuretic action of some alkylxanthines may be related to adenosine antagonism.

The diuretic action of the alkylxanthines has been known for many years (Schmiedeberg 1905), however, the pharmacological basis for this effect has not been elucidated. One possibility is that the adenosine antagonist actions of some alkylxanthines are responsible for their renal effects, since adenosine is known to reduce urine volume and sodium excretion (Osswald 1975). In the present study we have examined this possibility by comparing the diuretic effect of an alkylxanthine with a high affinity for adenosine receptors (8-phenyltheophylline) with one which has low affinity for these receptors (enprofylline) (Collis et al 1984).

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Methods

Evaluation of adenosine antagonism in the conscious rat. Alderley Park 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 via the jugular catheter for periods of 1–2 min. The infusion was terminated when a stable heart rate response was achieved. Adenosine infusions were performed before (0 h) and 1, 3, and 5 h after i.p. injection of 8-phenyltheophylline (10 mg kg^{-1}), enprofylline (10 mg kg^{-1}) or their vehicle (0·1 M NaOH: polyethylene glycol, 50 : 50 by volume at 1 ml kg^{-1}).

Evaluation of diuretic activity (Kau et al 1984). Alderley Park Wistar rats (which had been starved overnight but had free access to water) were orally dosed with saline at 40 ml kg^{-1} and placed in metabolism cages. 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. The rats were denied access to food and water whilst in the metabolism cages. One week later, groups of animals were given either 8-phenyltheophylline (10 mg kg^{-1} i.p.), enprofylline (10 mg kg^{-1}, i.p.), their vehicle (1 ml kg^{-1}, i.p.) or saline (1 ml kg^{-1}, i.p.). The animals were placed in metabolism cages and the urine was collected and analysed as described above.

The drugs used were: adenosine (Sigma), enprofylline (3-propylxanthine, Draco), 8-phenyltheophylline (Cal-Biochem). Enprofylline and 8-phenyltheophylline were dissolved in their vehicle at 10 mg ml^{-1}.

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/100 g body weight/6 h.

Results

There was no significant difference between the control heart rates of the rats used to evaluate the adenosine antagonist effect of 8-phenyltheophylline, enprofylline or their vehicle either before or after drug administration (ANOVA, Table 1). Adenosine infusion (1 mg kg^{-1} min^{-1}) lowered heart rate to a similar extent in three groups of rats (Table 1). Enprofylline and the vehicle had no significant effect upon the bradycardia induced by adenosine. 8-Phenyltheophylline significantly attenuated the bradycardia evoked by the purine 1 h ($P < 0·02$) and 3 h ($P < 0·05$) after injection of the xanthine (Table 1). 8-Phenyltheophylline had no significant effect on the diastolic blood pressure of the rats (control = 114 ± 4 mmHg, 8-phenyltheophylline = 112 ± 5 mmHg).

Table 1. Effect of 8-phenyltheophylline (10 mg kg^{-1}, i.p.) and of enprofylline (10 mg kg^{-1}, i.p.) on adenosine-induced bradycardia in the conscious rat.

Parameter	Treatment: Vehicle	8-Phenyltheophylline	Enprofylline
Control heart rate (beats min^{-1})	504 ± 15·9	484 ± 13·5	482 ± 15·2
Heart rate 1 h after treatment	508 ± 13·8	458 ± 30·9	492 ± 24·2
Δ Heart rate evoked by adenosine (1 mg kg^{-1} min^{-1})			
0 h	−80 ± 8·3	−82 ± 10·6	−80 ± 7
1 h	−92 ± 18·2	−40 ± 7·0**	−66 ± 20
3 h	−86 ± 11·2	−24 ± 9·7*	−78 ± 11·1
5 h	−60 ± 4·4	−78 ± 8·5	−76 ± 7·4
n	5	5	5

*$P < 0·05$; **$P < 0·02$ relative to response evoked by adenosine at 0 h.

The effects of 8-phenyltheophylline, enprofylline, their vehicle and saline on excretory parameters are shown in Table 2. The control values for urine volume, Na^+, K^+ and Cl^- excretion did not differ significantly (ANOVA) between the 4 groups of rats used. When compared with the excretory parameters measured after the first oral saline load (see Methods), neither saline injection nor enprofylline had any significant effect (Table 2). The vehicle solution caused a slight reduction in urine volume and K^+ excretion, however only the latter effect was statistically significant (Table 2). 8-phenyltheophylline significantly enhanced urine volume and the excretion of Na^+ and Cl^-. The excretion of K^+ was not significantly altered by 8-phenyltheophylline (Table 2).

Since the vehicle for 8-phenyltheophylline and enprofylline had a slight antidiuretic and antikaliuretic effect, the actions of the two xanthines were studied in a further group of rats. These rats were given the vehicle or the xanthine on separate occasions in a randomized fashion. When compared with the excretory parameters in the same rats treated with vehicle alone, 8-phenyltheophylline (10 mg kg^{-1}, i.p., n = 10) increased urine volume, Na^+, K^+ and Cl^- excretion by 66·1 ± 18·4% ($P < 0·01$), 75 ± 25·9% ($P < 0·02$), 79·5 ± 40·1% ($P > 0·05$) and 54·2 ± 16·7% ($P = 0·01$), respectively. Enprofylline (10 mg kg^{-1}, i.p. n = 9) had no effect on urine volume (−2·6 ± 12·0%), or on Na^+ (−0·4 ± 15·9%), K^+ (+13·6 ± 12·8%) or Cl^- (−1·0 ± 14·0%) excretion when compared with the effect of the vehicle in the same animals.

Discussion

This study is the first to demonstrate that 8-phenyltheophylline has a diuretic and saliuretic effect. This effect is probably related to the potent antagonist action of the xanthine at adenosine receptors, since enprofylline which did not block adenosine-evoked responses, was not a diuretic. The low affinity of enprofylline for adenosine receptors and its lack of diuretic activity at 10 mg kg^{-1} are consistent with previous reports on the effects of this compound (Persson et al 1982; Baer et al 1983).

The ability of 8-phenyltheophylline to block adenosine receptors was assessed from its effects on the bradycardic response evoked by adenosine. The attenuation of this cardiac response by 10 mg kg^{-1} of 8-phenyltheophylline does not prove that this dose also attenuates the renal effects of adenosine. Since a similar dose of the less potent adenosine antagonist, theophylline (Collis et al 1984) has been shown to block the intra-renal effects of

Table 2. Effect of 8-phenyltheophylline, enprofylline, their vehicle and saline on urine volume and electrolyte excretion.

Treatment	Urine volume (ml/100 g/6 h) Control	Test	Na^+ (mequiv/100 g/6 h) Control	Test	K^+ (mequiv/100 g/6 h) Control	Test	Cl^- (mequiv/100 g/6 h) Control	Test	n
8-Phenyl-theophylline 10 mg kg^{-1} (i.p.)	3·39 ± 0·05	4·15 ± 0·23*	0·51 ± 0·01	0·55 ± 0·02*	0·137 ± 0·006	0·148 ± 0·019	0·500 ± 0·006	0·570 ± 0·014**	6
Enprofylline 10 mg kg^{-1} (i.p.)	3·01 ± 0·06	2·85 ± 0·30	0·44 ± 0·01	0·41 ± 0·06	0·15 ± 0·01	0·13 ± 0·02	0·44 ± 0·01	0·42 ± 0·05	8
Vehicle 1 ml kg^{-1} (i.p.)	3·35 ± 0·17	2·90 ± 0·17	0·46 ± 0·01	0·43 ± 0·02	0·17 ± 0·01	0·12 ± 0·01**	0·47 ± 0·02	0·45 ± 0·03	9
Saline 1 ml kg^{-1} (i.p.)	2·98 ± 0·25	2·83 ± 0·03	0·44 ± 0·04	0·42 ± 0·05	0·14 ± 0·03	0·13 ± 0·02	0·44 ± 0·02	0·45 ± 0·03	6

*$P < 0·05$, **$P < 0·02$ relative to control data.

adenosine (Osswald et al 1982), it is highly likely that 8-phenyltheophylline had a similar or greater effect in the present study.

In the preliminary evaluation of diuretic activity, the effects of the xanthines on excretory parameters were compared with the values measured one week previously. The injection of saline (1 ml kg^{-1} i.p.) in place of drug had no effect on excretory parameters, confirming the previous report that these are highly reproducible using this protocol (Kau et al 1984). The vehicle for 8-phenyltheophylline and enprofylline appeared to have a slight antidiuretic and antikaliuretic effect. This was probably due to the presence of hyperoncotic polyethylene glycol (Bennett & Gardiner 1984). Despite this vehicle effect, 8-phenyltheophylline still evoked a significant saliuresis and diuresis. The apparent lack of effect of 8-phenyltheophylline on potassium excretion could have been a consequence of the antikaliuretic effect of the vehicle. When the effects of 8-phenyltheophylline were compared with those of the vehicle in the same group of rats, it became apparent that it caused similar increases in Na^+ and K^+ excretion, although the latter was more variable.

The results of this study cannot be used to define the site of the diuretic action of 8-phenyltheophylline, however, a number of possibilities can be considered. A gross haemodynamic change can be discounted as the xanthine did not alter heart rate or systemic blood pressure. Osswald et al (1982) have proposed that intra-renal adenosine may mediate tubulo-glomerular feedback. According to this hypothesis, adenosine generated from the consumption of ATP by tubular Na-K ATPase causes decreased glomerular filtration via afferent arteriolar vasoconstriction. Blockade of this effect would increase glomerular filtration rate and might then account for the diuretic action of 8-phenyltheophylline. This suggestion is not supported, however, by the recent observation that 8-phenyltheophylline does not enhance inulin clearance in the normal rat (Bowmer et al 1986). A tubular site of action of 8-phenyltheophylline may be relevant to its diuretic effects since adenosine receptors have been identified in the renal papillae (Woodcock et al 1984) and the adenosine antagonist aminophylline has been reported to inhibit tubular solute reabsorption in man (Brater et al 1983).

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