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Evidence that the positive inotropic effects of the alkylxanthines are not due to adenosine receptor blockade

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1 We investigated the possibility that the positive inotropic effects of the alkylxanthines are due to adenosine receptor blockade.

2 The potency of 8-phenyltheophylline, theophylline and enprofylline as adenosine antagonists was assessed *in vitro*, using the guinea-pig isolated atrium, and *in vivo*, using the anaesthetized dog.

3 The order of potency of the alkylxanthines as antagonists of the negative inotropic response to 2-chloroadenosine *in vitro*, and of the hypotensive response to adenosine *in vivo* was 8-phenyltheophylline > theophylline > enprofylline.

4 The order of potency of the alkylxanthines as positive inotropic and chronotropic agents in the anaesthetized dog was enprofylline > theophylline > 8-phenyltheophylline.

5 The results of this study indicate that the inotropic effects of the alkylxanthines in the anaesthetized dog are not due to adenosine receptor blockade.

Introduction

Certain xanthines exert a positive inotropic and chronotropic effect on the heart (Walton & Brodie, 1947; Nayler, 1963). This action has led to the use of alkylxanthines such as theophylline in the treatment of heart failure. At least four different mechanisms have been proposed to account for the cardiac stimulant effects of these compounds. These mechanisms are: inhibition of cyclic AMP phosphodiesterase (Hillis & Been, 1982); release of catecholamines (Atuk *et al.*, 1967); alteration in calcium handling (Blinks *et al.*, 1972); blockade of adenosine receptors (Fredholm, 1980). The aim of the present study was to investigate the role of adenosine receptor antagonism in the cardiac stimulant effects of the alkylxanthines.

There are a number of reasons why adenosine receptor blockade might be expected to exert a cardiac stimulant effect. Adenosine is continuously produced by the working heart (Thompson *et al.*, 1980) and its effects on this organ are depressant. The purine directly depresses the rate of firing of the sino-atrial pacemaker (James, 1965) and the force of atrial contraction (Collis, 1983). Adenosine has little direct effect on the contraction of the mammalian ventricle, but acts as a functional antagonist of the inotropic effect of β-adrenoceptor stimulation (Schrader, 1981). In addition, the exocytotic release of noradrenaline from the adrenergic nerves in the myocardium is attenuated by adenosine (Hedquist & Fredholm, 1979; Lokhandwala, 1979). All the cardiac depressant actions of exogenous adenosine can be blocked by the methylxanthine theophylline (Lokhandwala, 1979; Schrader, 1981; Collis, 1983). Consequently, if sufficient endogenous adenosine is produced by the heart to exert these depressant effects, an adenosine antagonist should enhance both the rate and the force of myocardial contraction.

In the present study we have examined the possibility that adenosine receptor antagonism contributes to the cardiac stimulant effects of selected alkylxanthines. The potency of three alkylxanthines (theophylline, 8-phenyltheophylline and enprofylline) have been compared as adenosine receptor antagonists and as cardiac stimulants.

Methods

(1) *Guinea-pig isolated left atrium*

Guinea-pigs of either sex (300–500 g) were killed and their hearts removed and placed in Krebs solu-

tion (37°C) (composition (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, Na-EDTA 0.026, glucose 5.5) bubbled with 95% O_2 and 5% CO_2. Left atria were mounted on punctate electrodes in 10 or 20 ml organ baths (37°C). The atria were attached to a force transducer (Pioden U.F.I.) and changes in isometric tension recorded on a Devices MX2 polygraph. An initial tension of 1 g was applied to the tissues.

Atria were stimulated by square wave electrical pulses (4 Hz,3 ms, 0.5–7 V) provided by a Grass S88 stimulator. Preliminary experiments demonstrated that the stimulus parameters used did not activate adrenergic or cholinergic nerves in the atrium as the force of contraction was not altered by β-adrenoceptor blockade (atenolol, 1μM,$n=4$) or by atropine (1μM,$n=6$).

An equilibration period of 45–60 min was allowed before experiments were started.

Acetylcholine or 2-chloroadenosine were added cumulatively to the organ bath in volumes of 0.04–0.1 ml and a dose-response curve produced. A 30 min contact time was allowed when the tissues were exposed to theophylline, 8-phenyltheophylline or enprofylline. Agonist dose-response curves were then repeated.

(2) *Anaesthetized dog*

Female beagle dogs (10–15 kg) were anaesthetized with sodium pentobarbitone (30–40 mg kg^{-1}, i.v.). The trachea was intubated and the dogs were artificially ventilated (18 cycles min^{-1}, tidal volume 13–15 ml kg^{-1}) with a mixture of room air and oxygen. Blood gases and pH were measured using a Corning 175 pH and blood gas system. These parameters were adjusted to within the normal range (when necessary) by adjustment of the ventilator and by i.v. injection of sodium bicarbonate.

Body temperature was monitored via a rectal probe and maintained at 37°C by a heating blanket.

A femoral vein was cannulated for drug administration and for the infusion of sodium pentobarbitone (0.05–0.2 mg kg^{-1} min^{-1}). The right carotid artery was cannulated and systemic blood pressure measured using a Bell and Howell L221 pressure transducer. Heart rate was derived from the blood pressure signal. The left carotid artery was exposed and a Millar Mikro-tip pressure transducer (Model pc 470) was passed down the artery, across the aortic valves and into the left ventricle. The Mikro-tip transducer was calibrated *in situ* using the pressure recorded from the saline filled lumen of the catheter for comparison. The first differential of the left ventricular pressure wave was derived electronically. Systemic blood pressure, heart rate, left ventricular pressure and left ventricular *dp/dt max* were continuously recorded on a Devices M19 chart recorder.

Experimental protocol

After a stabilization period of 15–30 min, adenosine (0.1–1.0 mg kg^{-1} i.v.) was administered. The peak decrease in diastolic blood pressure evoked by adenosine was measured. A dose of adenosine that evoked a sub-maximal response was selected and repeated at 5 min intervals until a consistent response was evoked. One of the alkylxanthines was then administered (i.v.) cumulatively every ten min. Five min after each dose of the alkylxanthine, haemodynamic parameters were measured and the adenosine injection repeated.

Drugs used

Acetylcholine chloride (Sigma), adenosine (Sigma), atenolol (Tenormin, ICI), 2-chloroadenosine (Sigma), enprofylline (3-propylxanthine, Draco), 8-phenyltheophylline (Cal-biochem) and theophylline (Sigma).

For the *in vitro* studies all compounds were made up in aqueous solution except 8-phenyltheophylline in which case a stock solution of 10 mM was made up in 80% v/v methanol containing 0.2 M NaOH, and aqueous dilutions of this used. In those experiments where 8-phenyltheophylline was used, all control responses were measured in the presence of an equivalent amount of solvent.

For the *in vivo* studies, adenosine and theophylline were prepared in 0.9% w/v NaCl solution (saline). Enprofylline (10 mg ml^{-1}) was prepared in 10% 1 M sodium hydroxide in saline and 8-phenyltheophylline (10 mg ml^{-1}) was prepared in 4% 1 M sodium hydroxide in distilled water. These vehicles had no effect on cardiovascular parameters or on the response to adenosine.

Analysis of results

Dose-ratios from the isolated tissue experiments were calculated at the EC_{50} level on the agonist dose-response curve. Results were analysed using Student's *t* test and by analysis of variance. A *P* value of < 0.05 was considered to be significant.

Results

(1) *Potency of alkylxanthines as adenosine antagonists* in vitro

The adenosine receptor agonist, 2-chloroadenosine and the muscarinic receptor agonist, acetylcholine, both evoked concentration-related decreases in the force of contraction of the guinea-pig atrium. 8-Phenyltheophylline (0.5 to 10 μM) antagonized the responses evoked by 2-chloroadenosine in a com-

Table 1 pA_2 values for theophylline and 8-phenyltheophylline as antagonists of the negative inotropic effect of 2-chloroadenosine on the guinea-pig atrium

Antagonist	pA_2	*Slope*[1]	*Correlation coefficient*	n
8-Phenyltheophylline	6.49 ± 0.17	−1.02 ± 0.18	0.87	12
Theophylline[2]	5.16 ± 0.17	−0.86 ± 0.12	0.89	16

[1] Slope of log (x − 1) against − log molar concentration of antagonist.
[2] From Collis & Pettinger (1982).

petitive manner as judged by the slope of the Arunlakshana & Schild (1959) plot (Table 1). The highest concentration of 8-phenyltheophylline used (10 μM) had no effect on responses evoked by acetylcholine (dose-ratio not significantly different from unity, $n = 4$). Enprofylline (100 μM) had no effect on the responses evoked by 2-chloroadenosine (dose-ratio not significantly different from unity, $n = 8$). Theophylline has been shown previously to be a competitive antagonist of 2-chloroadenosine in this preparation (Collis & Pettinger, 1982). The pA_2 value for theophylline is included in Table 1. However, 8-phenyltheophylline was 21 times more potent than theophylline as an adenosine receptor antagonist in the guinea-pig atrium ($P < 0.001$).

(2) *Potency of alkylxanthines as adenosine antagonists* in vivo

Intravenous administration of adenosine (0.1–1.0 mg kg^{-1}) to the anaesthetized dog evoked a transient decrease in diastolic blood pressure and heart rate and a small increase in *dp/dt max*. The most pronounced effect of the purine was on diastolic blood pressure, a maximum decrease of 90–100 mmHg occurring at high doses. The effects of the three alkylxanthines were evaluated against a submaximal hypotensive response evoked by adenosine (0.41 ± 0.08 mg kg^{-1}). The amplitude of this control response was not significantly different between the three groups of dogs used (Table 2).

Theophylline and 8-phenyltheophylline antagonized the hypotensive response evoked by adenosine in a dose-related manner (Figure 1). 8-Phenyltheophylline was significantly more potent ($P < 0.05$) than theophylline as an adenosine antagonist ($-\log IC_{50} = 5.89 \pm 0.15$; 5.17 ± 0.22 mol kg^{-1} respectively). Enprofylline (0.1 to 5 mg kg^{-1}) had no significant effect on the decrease in diastolic blood pressure evoked by adenosine (Figure 1).

The small bradycardia evoked by adenosine was antagonized by 8-phenyltheophylline, however the increase in *dp/dt* evoked by the purine was not altered significantly (Figure 2).

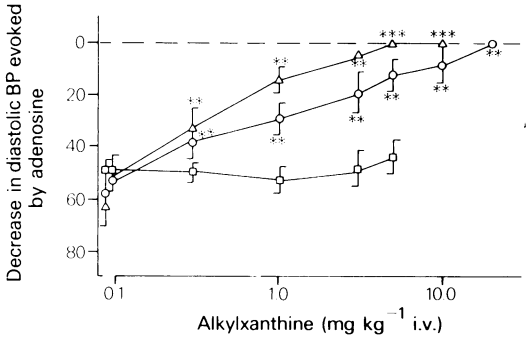

Figure 1 Effect of theophylline (○), 8-phenyltheophylline (△) and enprofylline (□) on the decrease in diastolic blood pressure evoked by adenosine (i.v.) in the anaesthetized dog. Each point represents the mean of 4 experiments, vertical lines show s.e.mean. $^{**}P < 0.01$ and $^{***}P < 0.001$ denotes a significant reduction in the response to adenosine.

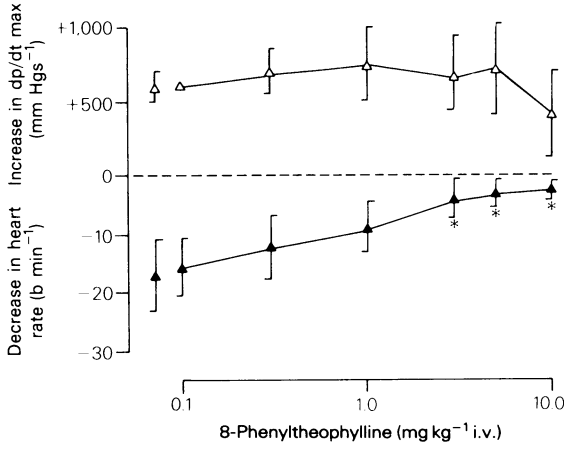

Figure 2 Effect of 8-phenyltheophylline on the increase in *dp/dt max* (△) and the decrease in heart rate (▲) evoked by adenosine in the anaesthetized dog. Each point represents the mean of 4 experiments; vertical lines show s.e.mean. $^{*}P < 0.05$ denotes a significant reduction in the response to adenosine.

Table 2 Control cardiovascular parameters and response to adenosine in the anaesthetized dog

	Control parameters			*Response to adenosine (mean dose 0.41 ± 0.08 mg kg^{-1})*			
Group	*Heart rate* (b min^{-1})	*dp/dt max* (mmHg s^{-1})	*Diastolic blood pressure* (mmHg)	*Change in heart rate* (b min^{-1})	*Change in dp/dt max* (mmHg s^{-1})	*Change in diastolic blood pressure* (mmHg)	n
Enprofylline	157 ± 10	3725 ± 377	115 ± 12	− 13 ± 3*	+ 354 ± 98*	− 50 ± 3*	4
Theophylline	152 ± 5	3425 ± 226	130 ± 7	− 8 ± 4	+ 1134 ± 153*	− 58 ± 5*	4
8-Phenyltheophylline	145 ± 7	3025 ± 165	122 ± 4	− 17 ± 6*	+ 590 ± 95*	− 63 ± 6*	4

There was no significant difference between the three groups of dogs in their control parameters and in their hypotensive and bradycardic response to adenosine.
The positive inotropic effect of adenosine was significantly different between the groups of dogs ($P < 0.01$, analysis of variance).
Significant difference from control, *$P < 0.05$.

Figure 3 Effect of theophylline (○), 8-phenyltheophylline (△) and enprofylline (□) on diastolic blood pressure of the anaesthetized dog. Each point represents the mean of 4 experiments, vertical lines show s.e.mean.

Figure 4 Effect of theophylline (○), 8-phenyltheophylline (△) and enprofylline (□) on heart rate in the anaesthetized dog. Each point represents the mean of 4 experiments; vertical lines show s.e.mean. Significantly different from control *$P < 0.05$, **$P < 0.01$.

(3) *Cardiovascular effects of alkylxanthines in the anaesthetized dog*

The control haemodynamic parameters in the three groups of dogs used were not significantly different (Table 2). The three alkylxanthines had no significant effect on diastolic blood pressure (Figure 3). Enprofylline evoked a significant increase in heart rate and in *dp/dt max* at doses from 0.1 to 5 mg kg^{-1} (Figures 4 and 5). A 50% increase in *dp/dt max.* was evoked by a mean dose (−log) of 5.49 ± 0.12 mol kg^{-1}. Theophylline (5–20 mg kg^{-1}) also exerted a cardiac stimulant effect (Figures 4 and 5). Theophylline evoked a 50% increase in *dp/dt max*, at

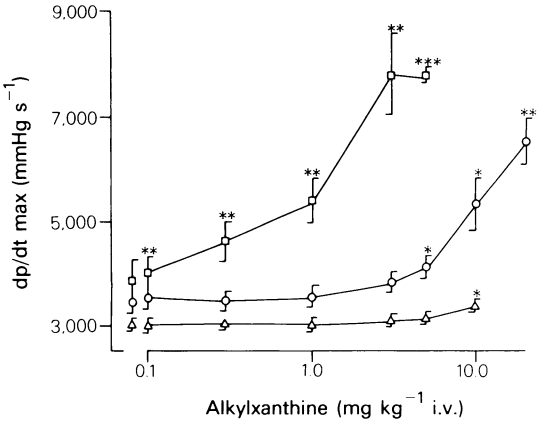

Figure 5 Effect of theophylline (○), 8-phenyltheophylline (△) and enprofylline (□) on left ventricular *dp/dt max* in the anaesthetized dog. Each point represents the mean of 4 experiments; vertical lines show s.e.mean. Significantly different from control $*P<0.05$), $**P<0.01$, $***P<0.001$.

a mean dose (−log) of 4.22 ± 0.18 mol kg^{-1} ($P<0.001$ compared with enprofylline). By contrast, 8-phenyltheophylline had minimal effects on cardiac parameters, evoking a small (+11%) increase in *dp/dt max* at the highest dose of 10 mg kg^{-1} (Figures 4 and 5).

(4) *Effect of atenolol on* dp/dt max *in the anaesthetized dog.*

In six anaesthetized dogs, atenolol (5 mg kg^{-1} i.v.) reduced left ventricular *dp/dt max* from 4533 ± 769 mmHg s^{-1} to 2050 ± 62 mmHg s^{-1} ($P<0.05$).

Discussion

The results of this study indicate that blockade of adenosine receptors does not contribute to the cardiac stimulant effect of the alkylxanthines in the anaesthetized dog. This conclusion is based on the different order of potency of the three compounds under study as adenosine antagonists (8-phenyltheophylline > theophylline > enprofylline) and as cardiac stimulants (enprofylline > theophylline > 8-phenyltheophylline). The most potent cardiac stimulant, enprofylline, was without effect at the adenosine receptor.

The potency of the three alkylxanthines as adenosine antagonists was examined both *in vitro* and *in vivo*. In the isolated atrial preparation the stable adenosine analogue, 2-chloroadenosine was used to evoke a decrease in the force of contraction. In the anaesthetized dog, adenosine was used as an agonist and the decrease in diastolic blood pressure was measured. The negative chronotropic effect of the purine was judged to be too small and too easily influenced by reflex cardiac stimulation (consequent upon hypotension) to be used to assess the antagonist potency of the alkylxanthines. The small positive inotropic response evoked by adenosine was an unexpected observation. The origin of this response is discussed below.

Theophylline and 8-phenyltheophylline antagonized responses evoked by adenosine receptor agonists both *in vivo* and *in vitro*. Enprofylline had no effect on responses evoked by the purines in either preparation, confirming the results of previous studies on this alkylxanthine (Persson *et al.*, 1981). Both theophylline and 8-phenyltheophylline appeared to be competitive antagonists *in vitro*. 8-Phenyltheophylline was about 20 times more potent than theophylline as an adenosine receptor antagonist in the atrium. The difference in potency between these two compounds as adenosine antagonists *in vivo* was about 5 fold. This quantitative discrepancy between results obtained *in vitro* and *in vivo* may be due to at least two factors. Firstly, the use of a single dose of agonist *in vivo* in order to assess the potency of an antagonist is inferior to the full agonist dose-response curves that were produced *in vitro*. Secondly, the distribution and metabolism of theophylline and of 8-phenyltheophylline may differ *in vivo*.

The small positive inotropic effect evoked by a bolus injection of adenosine in the dog was an unexpected observation. Other studies have generally demonstrated that adenosine infusion has a negative inotropic effect *in vitro* and *in vivo* in the dog (Chiba *et al.*, 1981; Devous, 1983). Lammerant *et al.* (1970) and Lammerant & Becsei (1973), however, have demonstrated that intracoronary infusion of adenosine has no effect on *dp/dt* whereas an intravenous dose has a positive effect in the anaesthetized dog. The positive inotropic effect observed in the present study could not have been a reflex evoked by hypotension as 8-phenyltheophylline blocked the latter but not the former. The resistance of the positive inotropic response, to doses of 8-phenyltheophylline that abolished both the hypotensive and the negative chronotropic effect of adenosine, implies that it is not mediated via an adenosine receptor. One explanation of this response is that it is due to the adenosine metabolite inosine. Adenosine injected directly into the coronary circulation is rapidly converted to inosine and hypoxanthine (Hopkins & Goldie, 1971). Inosine is known to have a positive inotropic effect in the dog (Chiba *et al.*, 1981; Smiseth, 1983).

The absence of a major cardiac stimulant effect of the potent adenosine antagonist 8-

phenyltheophylline in the anaesthetized dog implies that either endogenous adenosine has no negative inotropic effect or that insufficient amounts are produced to have a negative inotropic effect. The positive inotropic effect of a bolus injection of adenosine observed in the present study supports the former explanation. However, negative inotropic effects of adenosine in the dog have been demonstrated (Chiba *et al.*, 1981; Devous, 1983) when the purine is continuously infused. Consequently, the positive inotropic effect we observed cannot be regarded as typical. In addition, the actions of high concentrations of adenosine continuously generated by the myocardial cell may be different from those of a bolus injection of the purine circulating in the bloodstream. Since the major action of adenosine on the ventricle is to act as a functional antagonist of β-adrenergic tone (Schrader, 1981), it follows that the effects of endogenous adenosine will be critically dependant on the level of that tone. β-Adrenergic tone in our dogs was high, since the β-adrenoceptor antagonist atenolol evoked a significant reduction in dp/dt *max*. Consequently the failure of 8-phenyltheophylline to increase dp/dt cannot have been due to a low level of adrenergic drive to the ventricle. The most likely explanation for the lack of an inotropic effect of 8-phenyltheophylline is that the cardiac tissue of the anaesthetized dog produces insufficient adenosine to effect myocardial contractility. The possibility that adenosine levels in the ischaemic heart might be sufficient to alter contractility is worthy of further study.

The mechanism of the cardiac stimulant effects of enprofylline and of theophylline in the anaesthetized dog cannot be positively identified from the results of the present study. However, they cannot be due to reflex activation of the heart since neither compound lowered diastolic blood pressure. Both of these alkylxanthines are inhibitors of cyclic AMP phosphodiesterase, which could explain their cardiac stimulant effect. This suggestion is supported by the greater potency of enprofylline than theophylline as a cardiac stimulant and as a phosphodiesterase inhibitor (Persson *et al.*, 1981). The lack of an effect of 8-phenyltheophylline on cardiac contractility and on phosphodiesterase (Smellie *et al.*, 1979) is also consistent with this idea. However, further studies are necessary to confirm or refute this suggestion.

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