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Effects of BRL38227, Salbutamol, and Aminophylline, Alone and in Combination, on Plasma Potassium and on the Heart

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ABSTRACT

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The present study was undertaken in order to examine the effects of the potassium channel activator BRL38227 and of aminophylline on hypokalaemia, cardiac stimulation (increased heart rate and contractility, dQ/dt), and electrocardiogram (ECG) changes induced by the β_2-adrenoceptor agonist, salbutamol in the anaesthetised cat. Salbutamol (1.25 $\mu g \cdot kg^{-1} min^{-1}$), but not BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1} min^{-1}$) or aminophylline (0.5 $mg \cdot kg^{-1} min^{-1}$), infusion elicited a marked hypokalaemia (plasma $K^+ < 2.5$ $mmol \cdot liter^{-1}$). This dose of salbutamol also elicited an immediate cardiac stimulation which appeared to be maximal and changes in ECG were evident as a reduction in QTc interval (20%) and T-wave height (80%). Cardiac stimulation following either BRL38227 or aminophylline was more gradual in onset, the maximum response being about 50–80% of the salbutamol effect. No ECG changes were observed in cats receiving BRL38227 whilst T-wave amplitude was reduced (50–60%) following aminophylline. Combination of salbutamol with aminophylline resulted in a greater degree of hypokalaemia, cardiac stimulation, and T-wave depression. The salbutamol-induced reduction in QTc was converted to a slight prolongation after infusion for 75 min. The ECG changes observed were not indicative of arrhythmia. Conversely, BRL38227 had no effect on salbutamol-induced hypokalaemia, cardiac stimulation, or T-wave depression, though the degree of QTc interval shortening was reduced.

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When BRL38227 was administered in conjunction with aminophylline, plasma potassium and QTc were unchanged. Depression of T-wave amplitude by aminophylline was slightly reduced in the combination group. Effects on heart rate and dQ/dt were similar to those seen for BRL38227 alone. Salbutamol-induced hypokalaemia was reversed by the non-selective β-adrenoceptor antagonist, propranolol, but not by the β_1-adrenoceptor selective antagonist, atenolol, confirming a β_2-adrenoceptor selective effect. Cardiac stimulation was reversed by both propranolol and atenolol. In conclusion, salbutamol-induced hypokalaemia, cardiac stimulation, and ECG changes, as well as the additive effect of aminophylline, were demonstrated in the anaesthetised cat, but were unchanged by BRL38227. Furthermore, combination of BRL38227 with aminophylline did not result in any adverse effects on the parameters measured. Thus, on the basis of these results in the cat, combination of BRL38227 with the bronchodilator drugs, salbutamol or aminophylline, would not be expected to exacerbate the cardiovascular effects of these drugs.

Key words: hypokalaemia, ECG, cardiac stimulation

INTRODUCTION

Cardiac side effects, in the form of chronotropic, inotropic, and electrocardiographic changes, may occur following administration of β_2-adrenoceptor agonists [Al-Hillawi et al., 1984; Flatt et al., 1990]. A further complicating factor is that, through β_2-adrenoceptor activation, salbutamol reduces plasma potassium following intravenous administration [Whyte et al., 1987] or inhalation [Rolf-Smith et al., 1984; Scheinin et al., 1987; Burgess et al., 1989]. Hypokalaemia, in addition to cardiac stimulation, has been proposed as a risk factor for cardiac arrhythmias following β_2-adrenoceptor agonist therapy in the treatment of asthma [Whyte et al., 1987]. This assumption is based on the link between ventricular arrhythmia and mortality associated with low plasma potassium [Dyckner et al., 1975; Nordrehaug and Von Der Lippe, 1983; Thomas, 1983].

Combination of β_2-adrenoceptor agonists such as salbutamol with the methylxanthine bronchodilator drug, theophylline, has been shown to be beneficial in the treatment of asthma [Smith et al., 1980; Taylor et al., 1985; Joad et al., 1987]. However, combination of salbutamol with theophylline or aminophylline also increases the degree of hypokalaemia seen in both man [Whyte et al., 1988] and animals [Joseph et al., 1981], thereby increasing the potential risk factor for cardiac side effects and possibly arrhythmia.

The potassium channel activator, BRL38227 [(−)-enantiomer of cromakalim, Buckingham et al., 1986] is both blood pressure lowering [Clapham et al., 1991] and bronchodilator [Arch et al., 1988] in animal models. The issues raised above suggested to us that the potential for any interaction between BRL38227 and salbutamol should be investigated. The aim of the present study therefore, was to investigate the effects of BRL38227 on salbutamol-induced hypokalaemia and cardiac stimulation in the anaesthetised cat. For comparison, we have included salbutamol/aminophylline and BRL38227/aminophylline combinations.

A preliminary account of this work was presented to the XIth IUPHAR congress, Amsterdam [Clapham and Hamilton, 1990].

MATERIALS AND METHODS

Cats of either sex (2.0–3.9 kg body weight; SmithKline Beecham, Welwyn, UK) were anaesthetised with halothane (2–4%; ICI, U.K.) in a 2:1 nitrous oxide/oxygen mixture. Anaesthesia was maintained by intravenous administration of α-chloralose (80 $mg \cdot kg^{-1}$). Tracheotomy was performed to allow artificial respiration using a Harvard small animal ventilator (Edenbridge, UK). Blood gas and acid/base balance, measured on a Corning 158 pH/blood gas

analyser (Haverhill, UK), were maintained within limits for normal awake cats [Herbert and Mitchell, 1971]. Both femoral arteries were cannulated: one to allow continuous blood pressure recording via an Elcomatic pressure transducer (Glasgow, UK) and the other to allow arterial blood sampling. Both femoral veins were cannulated to facilitate concurrent infusions of drugs. A pulsed Doppler flow probe was placed around the left common carotid artery and the pulsatile signal, generated by a bidirectional pulsed Doppler flowmeter (model S45C-4; University of Iowa, Iowa, USA), was integrated for heart rate and differentiated (dQ/dt, an index of cardiac contractility) to give acceleration of blood in the carotid artery. Electrocardiogram (ECG) was measured from lead II using a Cambridge ECG recorder (model VS4; Cambridge, UK).

Experimental Protocol

Salbutamol (or vehicle) or aminophylline (or vehicle) were infused at rates of 1.25 $\mu g \cdot kg^{-1}min^{-1}$ and 0.5 $mg \cdot kg^{-1}min^{-1}$ for 90 min, respectively, either alone or in combination. Infusion rates of 0.116 $ml \cdot min^{-1}$ for salbutamol and aminophylline were achieved with Harvard compact infusion pumps. Infusion of BRL38227 (or vehicle), alone or with either salbutamol or aminophylline, was divided into 3 × 30 min periods at infusion rates of 0.1, 0.3, and 1.0 $\mu g \cdot kg^{-1}min^{-1}$. These doses range from the threshold, 0.1 $\mu g \cdot kg^{-1}min^{-1}$, for hypotensive activity to that causing a maximum hypotensive response, 1.0 $\mu g \cdot kg^{-1}min^{-1}$, in anaesthetised cats [unpublished observations]. BRL38227 (or its vehicle) was infused at a rate of 0.1 $ml \cdot min^{-1}$ with a Braun infusion pump.

Thus, 6 groups of 4 cats received one of the following treatments: i) salbutamol alone; ii) aminophylline alone; iii) BRL38227 alone; iv) salbutamol + BRL38227; v) salbutamol + aminophylline; vi) BRL38227 + aminophylline.

In addition, in a further 2 groups of 2 cats, the effects of ascending bolus doses of atenolol and propranolol were examined on salbutamol-induced cardiac stimulation and hypokalaemia.

Arterial blood samples (0.5 ml) were obtained pre-dose (×3) and every 15 min from commencement (time 0) of drug infusions. Whole blood was centrifuged at 6,000 rpm for 1 min and plasma potassium, sodium, and calcium concentrations ($mmol \cdot liter^{-1}$) were determined using a Kone ion selective analyser (Labmedics, Stockport, UK). The accuracy of measurements was assessed using standard control solutions. The red cell pellet was resuspended in 0.2 ml saline to replace the volume removed for ion determination and then reinfused.

Arterial pressure (systolic/diastolic; mmHg), heart rate ($beats \cdot min^{-1}$), and dQ/dt (V = arbitrary units) were displayed continuously on Lectromed multitrace recorders (Letchworth, UK). ECG and cardiovascular data were obtained immediately before each blood sample was removed. QT interval and T-wave amplitude were measured from the ECG and QT was corrected for heart rate (QTc) using the Bazett [1920] formula:

$$QTc = \frac{QT}{\sqrt{RR}}$$

where QT and RR are measured in seconds.

Treatment of Results

Post-infusion data for individual time points were compared to pre-infusion levels by one-way analysis of variance (ANOVA) and, if statistically significant, individual time points were further compared by Dunnett's test for multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

Drugs and Solutions

Aminophylline and salbutamol were purchased from Sigma (Poole, UK) and BRL38227 was synthesised at SmithKline Beecham Pharmaceuticals, Harlow, UK. Aminophylline was

TABLE 1. Basal Values Prior to Drug Treatments*

	Salbutamol + vehicle	BRL38227 + vehicle	Aminophylline + vehicle	Salbutamol + BRL38227	Salbutamol + aminophylline	BRL38227 + aminophylline
Diastolic blood pressure (mmHg)	87 ± 4	101 ± 10	92 ± 4	92 ± 11	88 ± 7	89 ± 5
Heart rate (beats·min^{-1})	184 ± 9	178 ± 8	164 ± 9	164 ± 12	165 ± 17	180 ± 5
dQ/dt (V)	0.53 ± 0.05	0.64 ± 0.12	0.47 ± 0.04	0.67 ± 0.12	0.51 ± 0.1	0.54 ± 0.03
K^+ (mmol·liter^{-1})	3.41 ± 0.15	3.47 ± 0.04	3.56 ± 2.89	3.49 ± 0.11	3.44 ± 0.17	3.52 ± 0.17
QTc (ms)	327 ± 16	335 ± 3	307 ± 16	338 ± 5	325 ± 9	320 ± 14
T-wave (mV)	0.25 ± 0.02	0.26 ± 0.4	0.28 ± 0.03	0.29 ± 0.05	0.29 ± 0.04	0.25 ± 0.05

*Values are mean ± SEM for 4 animals per group.

dissolved in 0.9% w/v NaCl (saline) while salbutamol was initially dissolved in 1 mmol · liter^{-1} ascorbic acid (Sigma) and diluted with saline. The final concentration of ascorbic acid was 1 μmol · liter^{-1} and the pH not less than 6.8. BRL38227 was initially dissolved (0.1 mg·ml^{-1}) in 10% polyethylene glycol 200 (Sigma) in saline with further dilutions being made with saline. All doses are expressed as base.

RESULTS

In preliminary dose-ranging experiments (data not shown), we found that infusion of salbutamol at a rate of 1.25 μg·kg^{-1}min^{-1} was the lowest causing consistent hypokalaemia (plasma potassium <2.5 mmol·liter^{-1}) in the anaesthetised cat. However, at this infusion rate, effects on cardiac stimulation appeared to be near maximal. An infusion rate of 0.5 mg·kg^{-1}min^{-1} for aminophylline was chosen as the highest dose possible without compromising diastolic blood pressure; higher infusion rates were found to have variable, and often profound, hypotensive effects in the anaesthetised cat.

Basal values prior to drug treatments for all parameters are shown in Table 1.

Diastolic Blood Pressure

Infusion of salbutamol (1.25 μg·kg^{-1}min^{-1}) resulted in an immediate fall in diastolic pressure which was maximal 15 min post-infusion and returned to pre-infusion levels by 30 min despite continued infusion of salbutamol (Fig. 1a). In contrast, the blood pressure lowering profile of BRL38227 (0.1–1.0 μg·kg^{-1}min^{-1}) was more gradual in onset, reflecting the ascending dose regimen, and maintained during infusion (Fig. 1a). The effect of combined infusion of salbutamol and BRL38227 resembled the early response to salbutamol alone. The later (high dose) blood pressure effects of the combination were slightly, but not significantly, greater than the later effects of BRL38227 alone (Fig. 1a).

Aminophylline (0.5 mg·kg^{-1}min^{-1}) alone had little or no effect on diastolic blood pressure in the anaesthetised cat (Fig. 1b). In combination with salbutamol, the initial fall in pressure seen with salbutamol alone was significantly ($P < 0.05$) reduced and after approximately 45 min there was a gradual fall in diastolic pressure not observed for salbutamol or aminophylline infused individually (Fig. 1b).

The hypotensive response to BRL38227 (0.1–1.0 μg·kg^{-1}min^{-1}) was unaffected when aminophylline (0.5 mg·kg^{-1}min^{-1}) was co-infused (Fig. 1c).

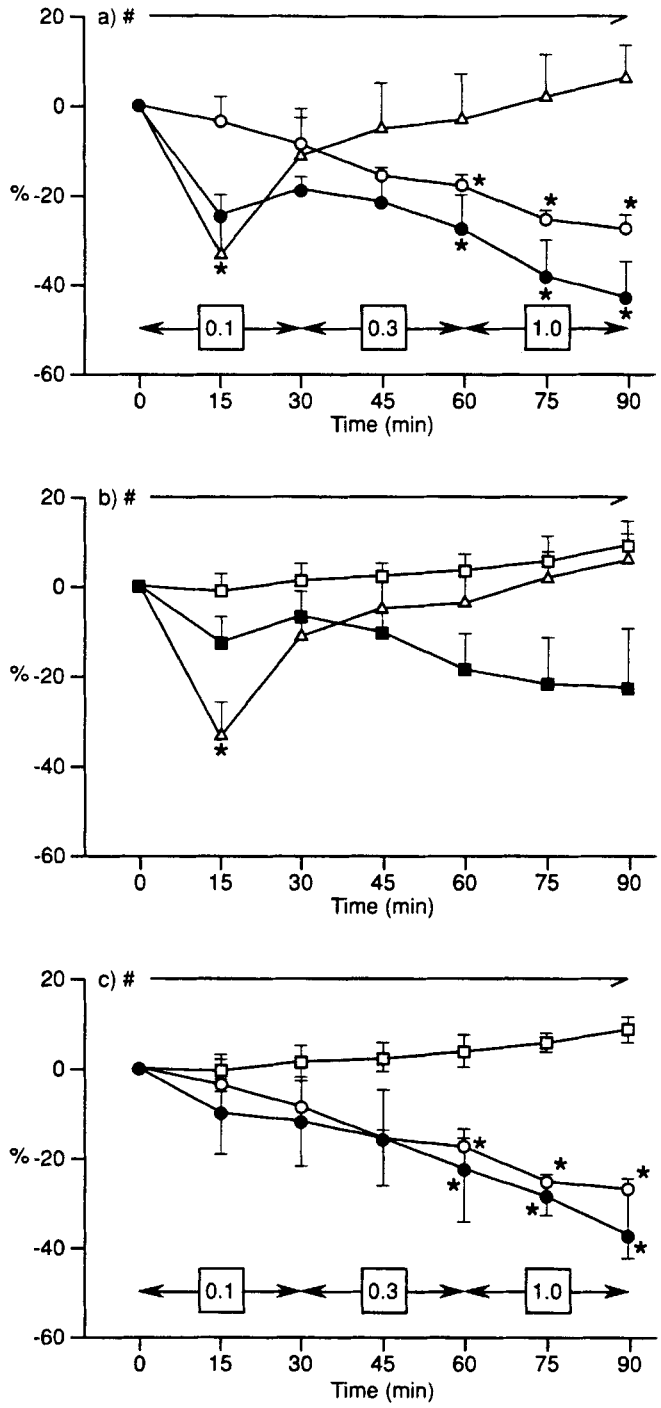

Fig. 1. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$; △), BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$; ○), aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$; □), and combination of **a:** salbutamol with BRL38227 (●); **b:** salbutamol with aminophylline (■), and **c:** BRL38227 with aminophylline (●) on % change in diastolic blood pressure in the anaesthetised cat. # indicates start of all infusions and boxes indicate the ascending infusion rates of BRL38227. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from pre-infusion levels.

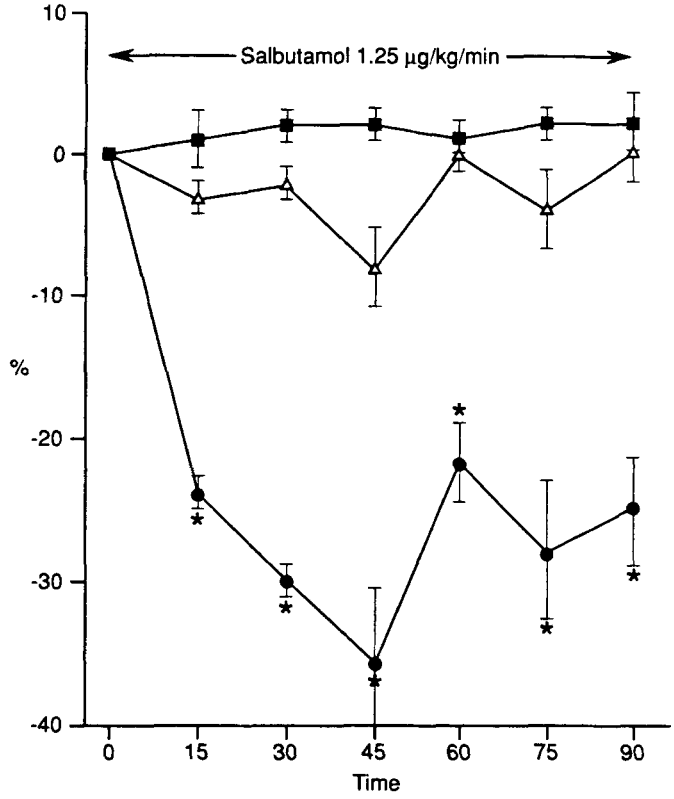

Fig. 2. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$) on % change in plasma potassium (●), sodium (■), and calcium (△) concentration in the anaesthetised cat. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from pre-infusion levels.

Plasma Electrolytes

Salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$) elicited a marked reduction (>30%) in plasma potassium concentration (to levels below 2.5 $mmol \cdot liter^{-1}$) with little or no effect on plasma sodium or calcium concentration (Fig. 2).

Neither aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$) nor BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$) affected plasma potassium (Fig. 3a–c), sodium, or calcium (not shown) levels in the anaesthetised cat.

Combination of BRL38227 with salbutamol produced a hypokalaemia that was similar in magnitude and time course to that of salbutamol alone (Fig. 3a). Combination of aminophylline with salbutamol resulted in a significantly ($P < 0.05$) greater degree of hypokalaemia over most of the infusion period (Fig. 3b). Plasma potassium was unaffected by infusion of aminophylline, alone or in combination with BRL38227 (Fig. 3c).

Heart Rate

Infusion of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$) elicited an immediate rise in heart rate which was sustained throughout the infusion period (Fig. 4a). The tachycardia evoked by BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$) was more gradual in onset and related to dose (Fig. 4a). The rise in heart rate in animals receiving both salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$) and BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$) was equivalent to that of salbutamol alone (Fig. 4a).

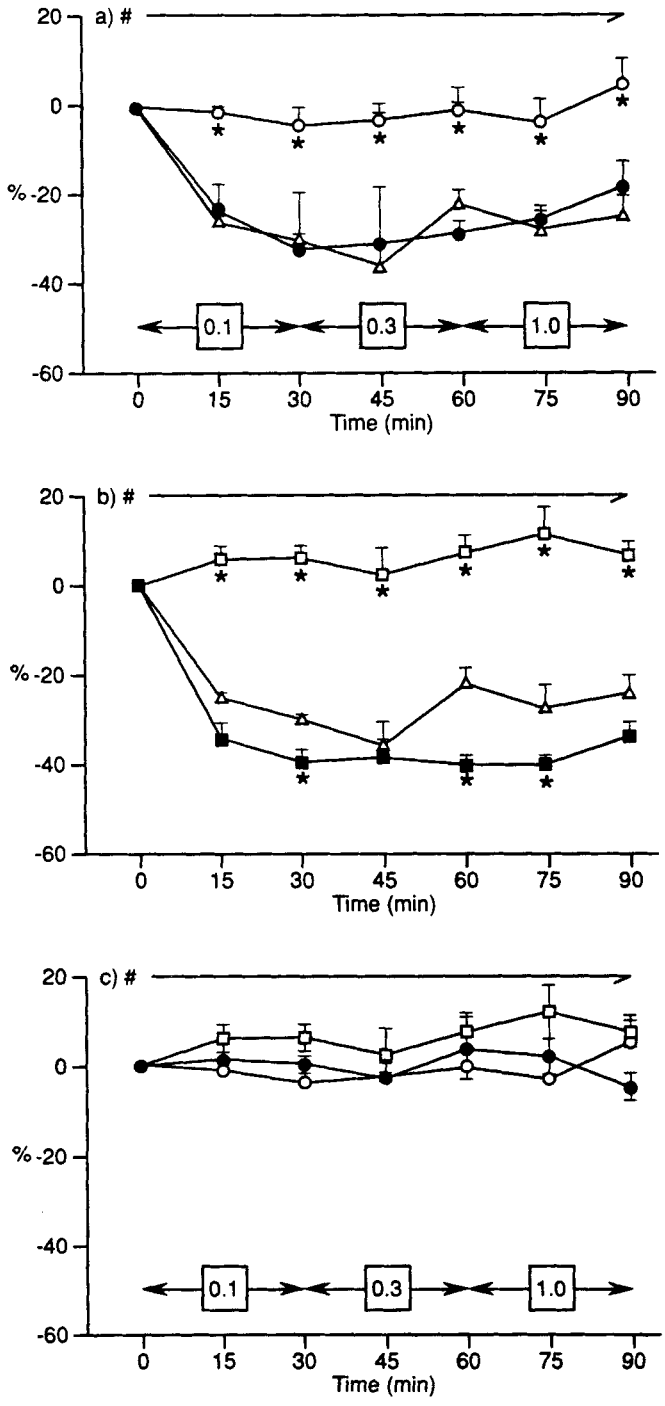

Fig. 3. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$; △), BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$; ○), aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$; □), and combination of **a:** salbutamol with BRL38227 (●); **b:** salbutamol with aminophylline (■); and **c:** BRL38227 with aminophylline (●) on % change in plasma potassium concentration in the anaesthetised cat. # indicates start of all infusions and boxes indicate the ascending infusion rates of BRL38227. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from salbutamol (a,b) or aminophylline (c) alone.

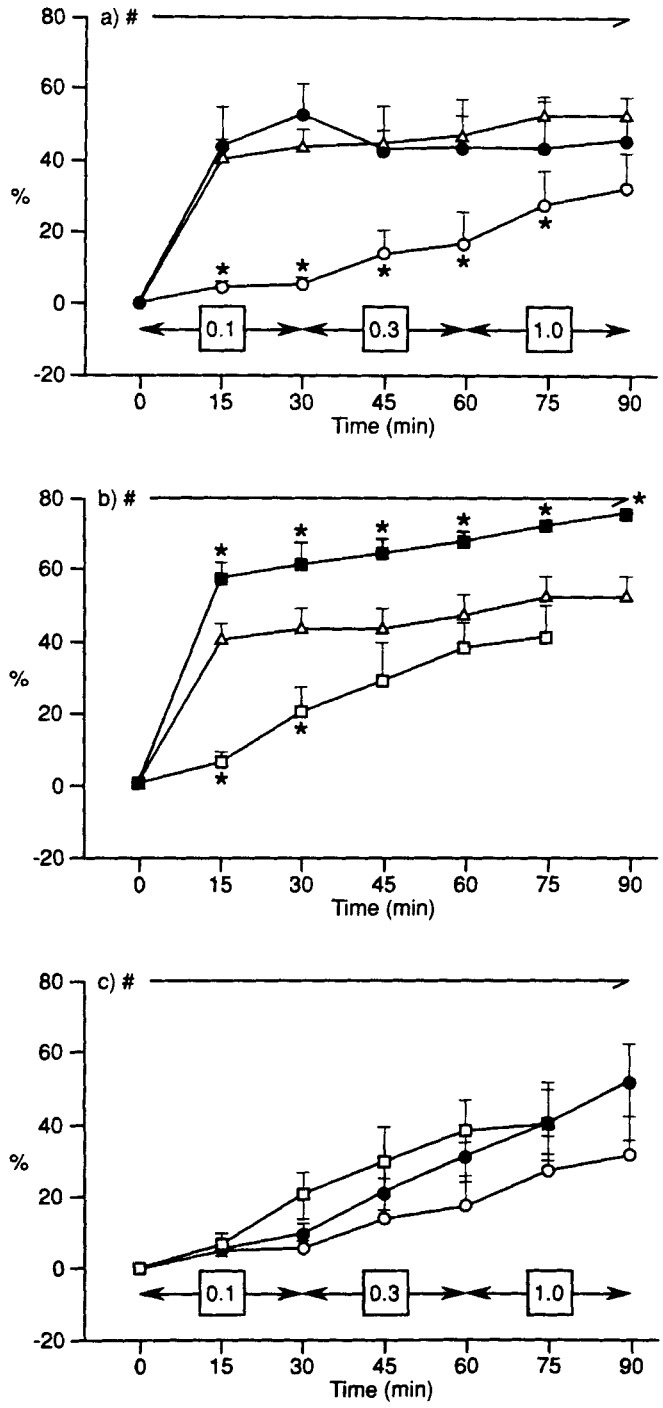

Fig. 4. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$; △), BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$; ○), aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$; □), and combination of **a:** salbutamol with BRL38227 (●); **b:** salbutamol with aminophylline (■); and **c:** BRL38227 with aminophylline (●) on % change in heart rate in the anaesthetised cat. # indicates start of all infusions and boxes indicate the ascending infusion rates of BRL38227. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from salbutamol (a,b) or aminophylline (c) alone.

Aminophylline (0.5 mg·kg^{-1}min^{-1}) produced a gradual rise in heart rate that was not significantly different from that of salbutamol (1.25 μg·kg^{-1}min^{-1}) 45 min post-infusion (Fig. 4b). The rise in heart rate in the combination group was significantly ($P < 0.05$) greater than that for salbutamol or aminophylline alone (Fig. 4b).

The rise in heart rate following infusion of BRL38227 (0.1–1.0 μg·kg^{-1}min^{-1}), aminophylline (0.5 mg·kg^{-1}min^{-1}), or the combination was similar in onset and magnitude (Fig. 4c).

Contractility Index

As with heart rate, salbutamol (1.25 μg·kg^{-1}min^{-1}) elicited an immediate rise in dQ/dt which was maximal 15 min post-infusion. Thereafter the response declined by approximately 50% despite continuing infusion of salbutamol (Fig. 5a). An increase in dQ/dt was observed only after the highest infusion rate of BRL38227 (1.0 μg·kg^{-1}min^{-1}) but combination of BRL38227 (0.1–1.0 μg·kg^{-1}min^{-1}) did not modify the response to salbutamol (1.25 μg·kg^{-1}min^{-1}) alone (Fig. 5a).

Aminophylline (0.5 mg·kg^{-1}min^{-1}) exerted only a marginal effect (20%) on contractility up to 75 min post-infusion with an increase of 60% seen only at the 90 min time point (Fig. 5b). On combination with aminophylline (0.5 mg·kg^{-1}min^{-1}), the initial increase in dQ/dt elicited by salbutamol (1.25 μg·kg^{-1}min^{-1}) was equivalent to that of salbutamol alone (Fig. 5b). However, the increase was sustained at between 100 to 120% for the duration of the experiment (Fig. 5b).

BRL38227 (1.0 μg·kg^{-1}min^{-1}) alone, or in combination with aminophylline (0.5 mg·kg^{-1}min^{-1}), produced a slightly greater increase in dQ/dt than aminophylline (0.5 mg·kg^{-1}min^{-1}) alone (Fig. 5c).

ECG

Continuous infusion of salbutamol (1.25 μg·kg^{-1}min^{-1}) caused a progressive, but not significant, shortening of QTc in the anaesthetised cat (Fig. 6a), whilst BRL38227 (0.1–1.0 μg·kg^{-1}min^{-1}) and aminophylline (0.5 mg·kg^{-1}min^{-1}) were without effect (Fig. 6a,b). Combination of salbutamol with BRL38227 left QTc unchanged when compared to pre-infusion levels (Fig. 6a). Combination of salbutamol with aminophylline on the other hand, resulted in an initial shortening of QTc, of a similar degree to salbutamol alone (Fig. 6b). This was converted to a significant ($P < 0.05$) prolongation, with respect to pre-infusion levels, after 75 min of continuous infusion (Fig. 6b). Aminophylline and BRL38227 combined infusion was without effect on QTc (Fig. 6c).

T-wave amplitude was progressively flattened by continuous infusion of salbutamol (1.25 μg·kg^{-1}min^{-1}) and aminophylline (0.5 mg·kg^{-1}min^{-1}) by 80% and 50–60%, respectively (Fig. 6d,e). BRL38227, up to 1.0 μg·kg^{-1}min^{-1}, caused a slight, but not significant reduction in T-wave height (Fig. 6d,e). Combined infusion of salbutamol and BRL38227 resulted in a profile which was similar to that of salbutamol alone (Fig. 6d). When aminophylline was co-infused with salbutamol, T-wave amplitude was reduced by 80% ($P < 0.05$) after only 15 min compared to 45 min for salbutamol alone (Fig. 6e). Combined infusion of aminophylline and BRL38227 produced an initial reduction in T-wave amplitude similar to that of aminophylline alone, but continued infusion of both drugs did not cause any further reduction in T-wave (Fig. 6f) even though the dose of BRL38227 was increased.

Arrhythmogenic activity was not observed following any treatment. Actual ECG traces obtained from individual animals receiving the combinations salbutamol + BRL38227, salbutamol + aminophylline, and aminophylline + BRL38227 are shown in Figure 7.

Effect of β-Adrenoceptor Blockade

The β_1-adrenoceptor antagonist, atenolol, caused a dose related reversal of salbutamol (1.25 μg·kg^{-1}min^{-1})-induced tachycardia and an increase in cardiac contractility with no

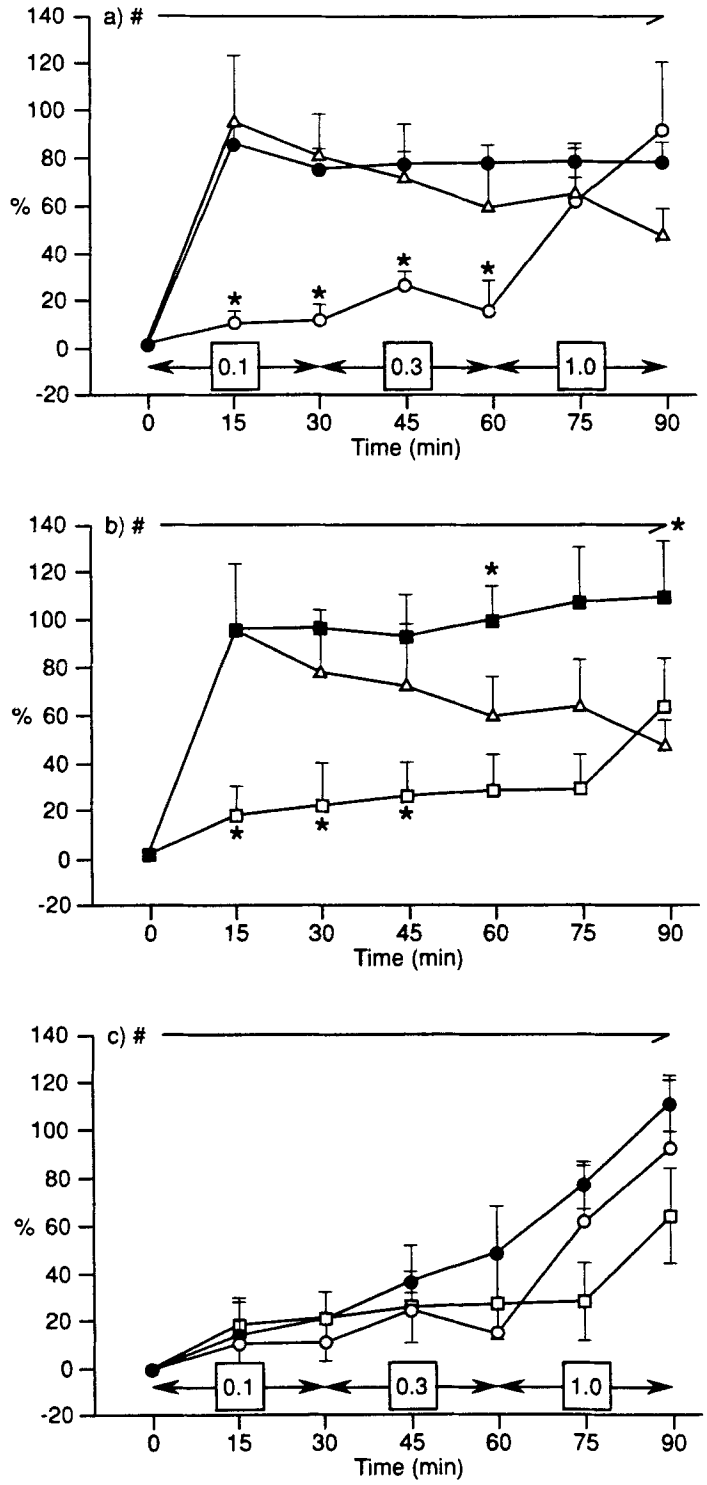

Fig. 5. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$; △), BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$; ○), aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$; □), and combination of **a:** salbutamol with BRL38227 (●); **b:** salbutamol with aminophylline (■); and **c:** BRL38227 with aminophylline (□) on % change in carotid dQ/dt in the anaesthetised cat. # indicates start of all infusions and boxes indicate the ascending infusion rates of BRL38227. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from salbutamol (a,b) alone.

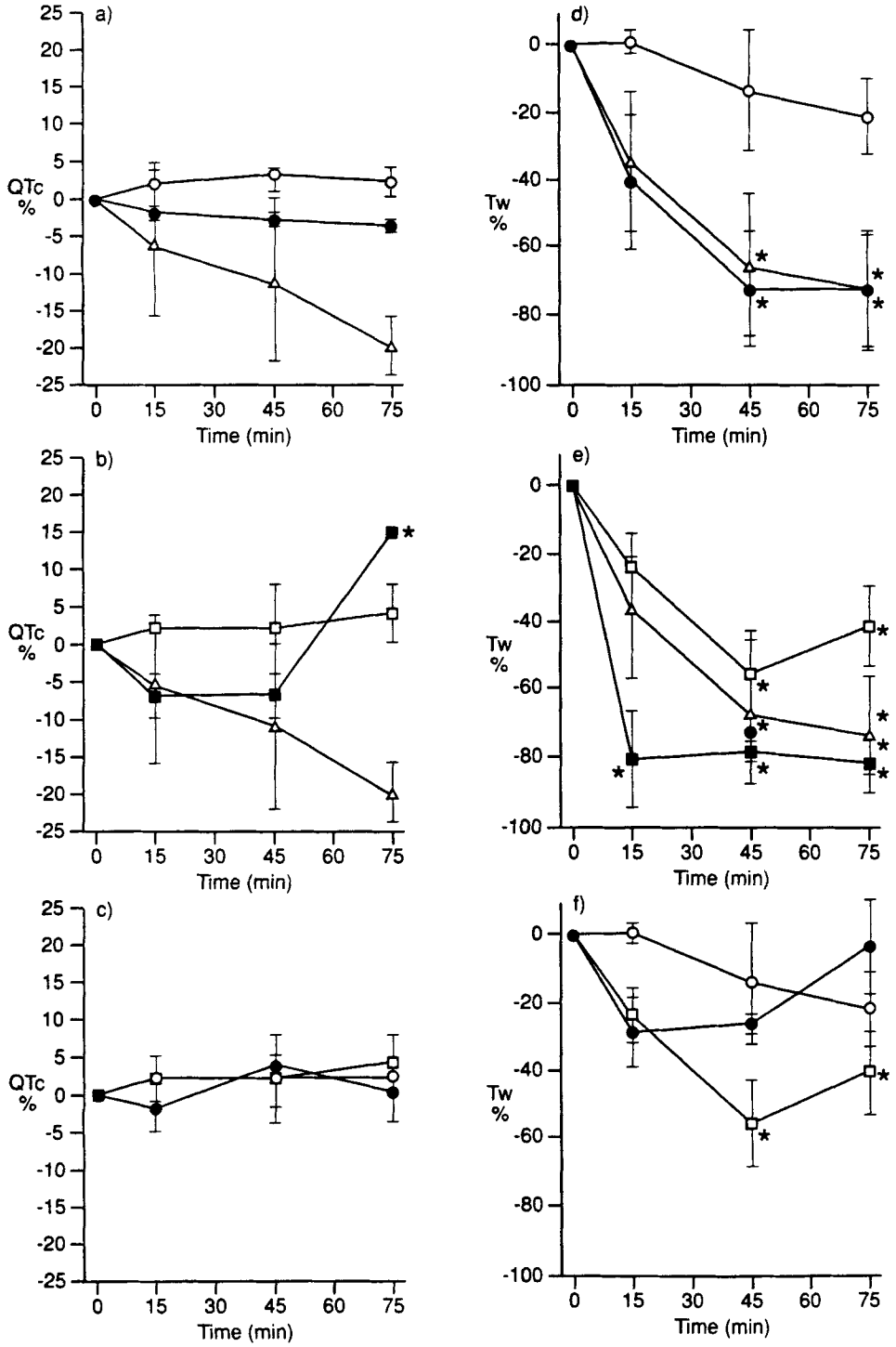

Fig. 6. Effect of salbutamol (1.25 $\mu g \cdot kg^{-1}min^{-1}$; △), BRL38227 (0.1–1.0 $\mu g \cdot kg^{-1}min^{-1}$; ○), aminophylline (0.5 $mg \cdot kg^{-1}min^{-1}$; □), and combination of **a,d:** salbutamol with BRL38227 (●); **b,e:** salbutamol with aminophylline (■); **c,f:** BRL38227 with aminophylline (●), on % change in QTc interval (a–c) and T-wave amplitude (d–f) in the anaesthetised cat. All infusions start at time 0 and BRL38227 infusion rates are shown in parentheses. Values for BRL38227 are 15 min from the start of each rate. Values are mean ± SEM for 4 cats per group. *Indicates a significant ($P < 0.05$) difference from pre-infusion levels.

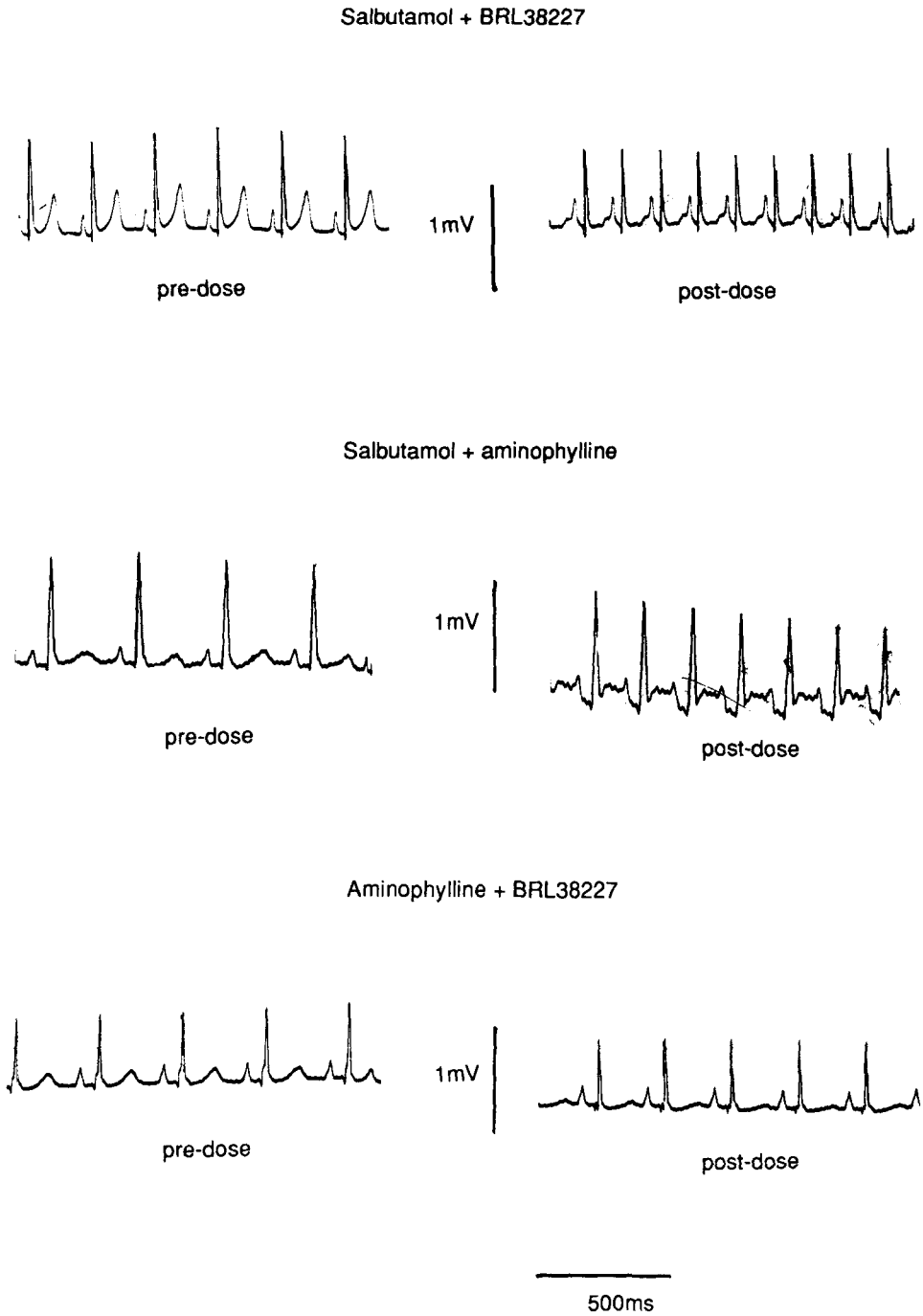

Fig. 7. Example ECG records from cats receiving salbutamol + BRL38227 **(top trace)** salbutamol + aminophylline **(middle trace)**, and aminophylline + BRL38227 **(bottom trace)** combination. Examples were taken pre-dose and 90 min from the start of infusions (post-dose). This corresponds to the final infusion rate for BRL38227 of 1.0 $\mu g \cdot kg^{-1} min^{-1}$. Salbutamol and aminophylline were infused at rates of 1.25 $\mu g \cdot kg^{-1} min^{-1}$ and 0.5 $mg \cdot kg^{-1} min^{-1}$, respectively.

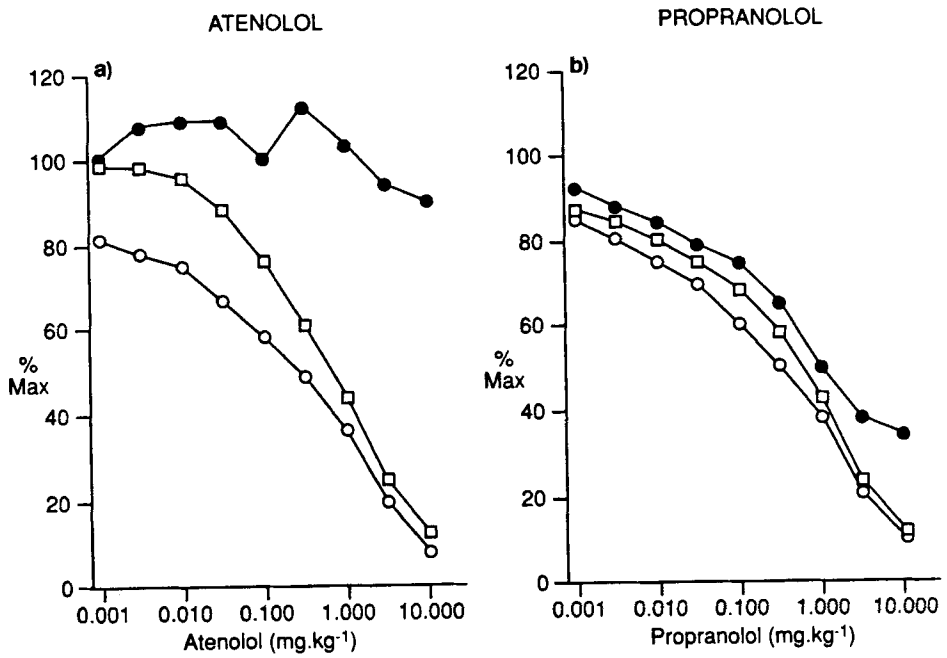

Fig. 8. Effect of atenolol (**a**) and propranolol (**b**) on the maximum change in plasma potassium (●), heart rate (□), and carotid dQ/dt (○) evoked by salbutamol (1.25 $\mu g \cdot kg^{-1} min^{-1}$) in the anaesthetised cat. Values are means of 2 cats per group.

effect on salbutamol-induced hypokalaemia (Fig. 8a). In contrast, the non-selective β-adrenoceptor antagonist, propranolol, elicited dose related reversal of salbutamol-induced tachycardia, increased cardiac contractility, and hypokalaemia (Fig. 8b).

DISCUSSION

In the present study we have demonstrated that salbutamol-induced hypokalaemia occurs in the anaesthetised cat, the effect being specific to potassium since neither plasma sodium nor calcium was affected. We have also confirmed that this hypokalaemia is a consequence of β_2-adrenoceptor activation, since the non-specific β-adrenoceptor antagonist, propranolol, but not the β_1-adrenoceptor selective antagonist, atenolol, reversed salbutamol-induced hypokalaemia. Both drugs antagonised the cardiac stimulation evoked by salbutamol. These data are in agreement with findings in the anaesthetised dog [Castro-Tavares, 1976; Klein et al., 1989]. Furthermore, exacerbation of salbutamol-induced hypokalaemia and cardiac stimulation by aminophylline, as demonstrated in the present study, are also in agreement with other findings in animals and man [Joseph et al., 1981; Whyte et al., 1988]. Clinically, salbutamol is usually administered orally or by inhalation. In the present study salbutamol was administered by intravenous infusion. Nevertheless, the effects seen after intravenous infusion of salbutamol are still representative of the perceived effects in the clinical situation.

Although long-term potassium homeostasis is mainly controlled by the kidney, acute hypokalaemia such as that elicited by salbutamol, is due to stimulation of β_2-adrenoceptors in skeletal muscle [Brown, 1985; Vincent et al., 1985]. Activation of these receptors stimulates Na^+/K^+ adenosine triphosphatase (ATPase), via an increase in cyclic adenosine monophosphate (cAMP), leading to potassium influx [Tashiro, 1973; Buur et al., 1982]. Salbutamol-

induced hypokalaemia has been demonstrated when salbutamol is given by intravenous administration [Whyte et al., 1987] or by inhalation [Rolf-Smith et al., 1984; Scheinin et al., 1987; Burgess et al., 1989]. It is in this latter category when overdose is most likely to occur. However, in comparisons between inhaled β_2-adrenoceptor agonists at standard therapeutic doses, fenoterol and terbutaline cause more prolonged and greater degrees of hypokalaemia than salbutamol [Scheinin et al., 1987; Burgess et al., 1989].

At present, the mechanism for the exacerbation by aminophylline of salbutamol-induced hypokalaemia is not fully understood. Aminophylline's action, on the basis of phosphodiesterase inhibition, has been previously doubted [Rall, 1982], since therapeutic levels of theophylline elicited only 5–10% inhibition of phosphodiesterase from human lung extracts [Polson et al., 1978]. However, it is now recognised that mammalian phosphodiesterases exist in multiple molecular forms that differ in substrate specificity, intracellular modulation, pharmacological inhibition, and immunoreactivity [Beavo and Reifsnyder, 1990]. Thus, when phosphodiesterase enzymes were examined individually it was found that theophylline, albeit at slightly higher than therapeutic levels, was able to produce 50% inhibition of two, a cAMP and a cyclic guanosine monophosphate (cGMP) specific, phosphodiesterase isoenzymes [Polson et al., 1982, 1985]. Although these isoenzymes may represent only a low proportion of total phosphodiesterase activity, their inhibition may have a marked effect on β_2-adrenoceptor-mediated increases in intracellular cAMP. Antagonism of A_2 receptors on skeletal muscle cells or enhancement of noradrenaline release from sympathetic nerve terminals via antagonism of P_1-purinoceptors has also been suggested as mechanisms for enhancement of salbutamol-induced effects by theophylline [Whyte et al., 1988].

Unlike aminophylline, BRL38227, up to 1.0 $\mu g \cdot kg^{-1} min^{-1}$, which is a maximal blood pressure lowering dose in the anaesthetised cat, did not alter the level of salbutamol-induced hypokalaemia and cardiac stimulation. The increases in heart rate and dQ/dt (by the highest infusion rate) following BRL38227 were completely masked by those of salbutamol and there was no evidence of an additive effect. This is an important finding, since salbutamol is the leading first-line therapy in the treatment of reversible obstructive airways disease [Price and Clissold, 1989]. As such, patients receiving BRL38227 may also be receiving salbutamol (or another β_2-adrenoceptor agonist). Therefore, a drug combination that can be shown to be beneficial in terms of therapeutic effects whilst not increasing risk liability would have obvious advantages.

Exacerbation of salbutamol-induced hypokalaemia and cardiac stimulation by aminophylline were reflected by changes in QTc interval and T-wave amplitude. Salbutamol elicited a marked reduction in QTc interval which, in itself, is without serious consequences [Attwell and Lee, 1988]. Although aminophylline had no effect on QTc interval per se, it converted the shortening of QTc interval by salbutamol to a prolongation, though only when infused for over an hour. Where prolongation of QTc interval occurs in a situation of increased heart rate there is an increased risk of cardiac arrhythmia [Attwell and Lee, 1988], but despite this we found no evidence of arrhythmia in this study. When salbutamol was infused with BRL38227, BRL38227 prevented salbutamol-induced QTc shortening for reasons that are unclear at present. The effect of salbutamol alone, and in combination with aminophylline, on T-wave amplitude was more evident. Salbutamol infusion elicited a marked flattening of T-wave which was significant after 45 min of infusion. Reduction in T-wave height by other β_2-adrenoceptor agonists has also been reported in normal volunteers [Flatt et al., 1990]. These changes in T-wave height are probably related to hypokalaemia; this has been demonstrated elsewhere [Scheinen et al., 1987; Burgess et al., 1989]. Although aminophylline infusion reduced T-wave amplitude, but to a slightly lesser degree than salbutamol, it served to potentiate the effect of salbutamol such that an 80% reduction in T-wave height was observed after 15 min of infusion. This correlates with the observed increase in the degree of hypokalaemia and cardiac stimulation and provides further evidence for increased risk of ECG changes as a result of this combination. On the other hand, BRL38227 infusion had only slight

effects on T-wave amplitude and did not modify the salbutamol-induced effect. The mechanism for the aminophylline-induced reduction in T-wave is clearly not a consequence of reduced plasma potassium, and some other, as yet undefined, mechanism must therefore be responsible. An intriguing finding was the apparent reversal of aminophylline-induced reduction in T-wave height by BRL38227, the mechanism of which is also unclear at present.

In conclusion, we have shown that salbutamol induced hypokalaemia, cardiac stimulation, ECG changes, in particular T-wave, and their exacerbation by aminophylline, can be demonstrated in the anaesthetised cat. There is no evidence from the present study to suggest that combination of BRL38227 with salbutamol would worsen these effects of salbutamol alone should co-administration of these drugs be necessary. Also, no evidence has been obtained to suggest that combination of BRL38227 with aminophylline would result in adverse effects on plasma potassium, cardiac, and ECG parameters.

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