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^{86}RUBIDIUM EFFLUX AND NEGATIVE INOTROPY INDUCED BY P1- AND MUSCARINIC-RECEPTOR AGONISTS IN GUINEA-PIG LEFT ATRIA

EFFECTS OF POTASSIUM CHANNEL BLOCKERS

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Abstract—Guinea-pig isolated left atria, paced at 2 Hz were incubated with ^{86}rubidium (^{86}Rb) for 120 min. They were then washed every 2 min for 2 hr, each sample being retained for scintillation counting. Left atrial isometric tension was recorded simultaneously. A concentration–response curve for the muscarinic agonist carbachol or the P1-receptor agonists adenosine and L-N^6-phenylisopropyladenosine (L-PIA) was obtained. Antagonists were present from 20 min before agonist exposure. The rate constant (k) for ^{86}Rb efflux was calculated for each 2 min sample and the mean increase for each concentration of agonist determined. In the absence of drugs there was no significant alteration in the rate constant during the 2 hr experimental period. Adenosine, L-PIA and carbachol produced concentration-related increases in rate constant for ^{86}Rb efflux. The adenosine and L-PIA concentration–response curves were virtually superimposed upon the curves for the negative inotropic responses. The ^{86}Rb efflux induced by adenosine was antagonized in an apparently parallel manner by 8-phenyltheophylline (8-PT) indicating involvement of P1-receptors. Alone, the putative potassium channel blockers, 4-aminopyridine (4-AP) and bromobenzoylmethyladamantylamine (BMA) caused, respectively, no change and a reduction in resting ^{86}Rb efflux immediately prior to the agonist exposure. 4-AP reduced the L-PIA- and adenosine-induced increases in ^{86}Rb efflux and, to a lesser extent, the negative inotropic response to adenosine. BMA caused "flattening" of the dose–response curves for ^{86}Rb efflux induced by L-PIA, adenosine and carbachol with a significant reduction in response at the highest concentrations of adenosine and carbachol. The negative inotropic response to adenosine was also reduced. These results suggest that 4-AP and BMA block the P1-receptor-linked potassium channels and that BMA interacts with common K^+ channels linked to P1- and muscarinic receptors. The negative inotropic responses of the guinea-pig left atrium to P1- and muscarinic agonists can be attributed, at least in part, to the opening of outward K^+ channels.

The negative inotropic effects of P1-receptor agonists in atrial muscle are mediated, at least in part, by stimulation of potassium efflux [1, 2]. This causes a reduction in the action potential duration, thus reducing the time available for calcium influx and causing a reduction in the force of contraction. Similarly, muscarinic-receptor agonists stimulate potassium efflux in atrial muscle [1, 3, 4] and thus produce negative intropy. Indeed, it has been suggested from electrophysiological data that P1- and muscarinic-receptor agonists stimulate potassium efflux through a common population of potassium channels [1, 3, 5].

Both the electrophysiological and inotropic effects of acetylcholine and adenosine in guinea-pig left atria are antagonized by the potassium channel blocker, bromobenzoylmethyladamantylamine (BMA) [6, 7] suggesting that BMA blocks the potassium channels activated by both P1- and muscarinic-receptor activation. However, it has been claimed recently that the potassium channel blockers, tetraethylammonium (TEA) and 4-aminopyridine (4-AP) are capable of antagonizing the negative inotropic and chronotropic effects of the muscarinic-receptor agonist, carbachol, in guinea-pig atria, at concentrations which do not significantly affect responses to the P1-receptor agonist L-PIA (L-N^6-phenylisopropyladenosine) [8].

The present study was undertaken to examine the effects of P1- and muscarinic-receptor agonists upon potassium efflux from guinea-pig paced left atria. As an alternative to measuring the efflux of radioactive potassium (^{42}K), which has a very short half-life (12.4 hr), we have used radioactive rubidium (^{86}Rb). This has a longer, more manageable half-life (18.6 days) and provides a close quantitative estimate of potassium movements [9, 10]. Although adenosine and L-PIA [11–13] and carbachol [4, 12] have been examined previously upon ^{86}Rb efflux from guinea-pig left atria, the effects of K^+ channel antagonists have not. In the present study, the susceptibilities of adenosine, L-PIA and carbachol

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to the K^+ channel blockers, 4-aminopyridine and BMA, and to the P1-receptor antagonist 8-phenyltheophylline, are examined. Some of these results have been reported to the British Pharmacological Society [14].

MATERIALS AND METHODS

Isolated tissue preparation. Male Dunkin–Hartley guinea-pigs (240–450 g) were used, and for experiments with 4-aminopyridine they were pretreated with reserpine (5 mg/kg i.p.) 24 hr before. Animals were killed by a blow to the back of the neck and exsanguinated under running water. The rib cage was opened and the heart displaced to the right. The left atrium was removed with cottons attached to the tip of the left atrial appendage and the atrioventricular junction. The latter cotton was used to secure the atrium to the electrode tips of a Harvard bipolar platinum electrode which was coated with a waterproof plastic sheath and the lower end sealed with a plug of water resistant silicone elastomer (Silgard®). The elastomer was pared away to reveal the electrode tips and allow their direct contact with the tissue. This coating was found to be essential to prevent the electrodes from retaining radioactivity. The first cotton was attached to an isometric transducer (Harvard Ultra High Sensitivity, 0–5 g) connected to a Grass Model 79D polygraph for recording isometric tension. A resting diastolic tension of 0.5–0.75 g was applied. The tissue was immersed in a 4 mL organ bath containing Krebs-bicarbonate solution of composition (mM) in double distilled water: NaCl 118, KCl 4.69, $CaCl_2$ $2H_2O$ 2.52, $MgSO_4$ $7H_2O$ 1.18, KH_2PO_4 $2H_2O$ 1.18, $NaHCO_3$ 25, glucose 11.7, which was maintained at 37.5 ± 0.5° and gassed with 5% CO_2 in oxygen. The bathing medium could be rapidly collected by suction into a syringe and replaced by the same method with prewarmed (37.5°) and pregassed Krebs solution.

The atria were paced at 2 Hz with square-wave pulses of 5 msec duration at threshold voltage (+50%) delivered by a Harvard Research 50–75 stimulator.

Measurement of ^{86}rubidium efflux. An initial equilibrium period (approximately 45 min) was allowed with changes of bathing medium at 15 min intervals. The tissue was then loaded with ^{86}rubidium (^{86}Rb) by emptying the bath and refilling with 4 mL of Krebs solution containing ^{86}Rb (1 μCi/mL, 37 mBq/L) and incubating for 120 min. The tissue was then washed every 2 min with ^{86}Rb-free Krebs solution for a further 120 min. Each 2 min sample was transferred to a Zinsser scintillator vial for subsequent scintillation counting. At 120 min, the bath was emptied, the left atrium removed from the electrode and placed in a scintillation vial containing 1 mL of Packard-Soluene tissue solubilizer. Dissolution of the tissue was complete at 24 hr, after which 10 mL of Packard Pico-Fluor 15 scintillation fluid was added. The activity remaining in the tissue and that in the 60 Krebs samples was counted in a Packard Tri-Carb 460 scintillation counter.

As no scintillant was added to the Krebs samples, they were counted in the low energy Cerenkov mode at approximately 45% efficiency. To the tissue sample, however, scintillation fluid had been added and activity was measured by classical scintillation counting at greater than 90% efficiency. To avoid differences in background count arising from the use of different energy ranges, a common wide range of energy values (0–2000 mV) was used to measure both aqueous (Krebs) and non-aqueous (tissue) samples. The background count was less than 50 cpm.

To permit comparison between counts derived from the dissolved tissue (non-aqueous, scintillation mode) and the Krebs samples (aqueous, Cerenkov mode), a correction factor was obtained by performing control experiments. In these, a non-radioactive left atrium was dissolved in solubilizer and then 'spiked' with a known amount of ^{86}Rb before adding the scintillation fluid and counting in the scintillation mode. A 4 mL sample of Krebs was also 'spiked' with the known amount of ^{86}Rb and counted in the Cerenkov mode. Dividing the latter aqueous count by the non-aqueous scintillation count of the tissue sample yielded a correction factor (0.464). This was applied to all tissue counts.

Drug administration. All drugs were added to the Krebs solution in the required concentration and then warmed to 37.5° and gassed with 5% CO_2 in oxygen before addition to the organ bath. For construction of agonist concentration–response curves, four increasing concentrations were added in turn, each concentration being in contact with the atria for four of the 2 min washout periods. The first additions of each agonist concentration were at 58, 66, 74 and 82 min after the end of the loading period. Antagonists were added 20 min before the first agonist addition and remained present in each Krebs sample until after the final addition of the highest concentration of agonist.

Calculation of rate constant for ^{86}Rb efflux. The rate constant for fractional release of ^{86}Rb was used to assess ^{86}Rb efflux. The rate constant (k_i) at time $t = t_i$; is $k = C_i/\Delta t \times C_i$), where C_i is the radioactivity released from the tissue in the time interval (min) from t_i to $t_i + \Delta t$. C_i, the radioactivity remaining in the tissue at time t_i, may be calculated backwards as the sum of the radioactivity remaining in the tissue at the end, C_f, plus all the radioactivity released from time t_i to the end [15]. For the sake of simplicity, the counts obtained and the corrected tissue count were used in place of the actual radioactivity present. Thus, from each experiment, a plot of how k varied every 2 min during the 120 min efflux period was obtained. Each experiment was repeated at least four times and the mean values (± SEM) determined (see Fig. 1).

Construction of concentration–response curves. In individual experiments, the changes in rate constant for ^{86}Rb efflux at each concentration of agonist were calculated as the mean of the four values for each 2 min sample obtained in the presence of each agonist concentration. The mean of the corresponding values from each experiment was calculated ($N \geq 4$) to obtain the increase in k value produced by each agonist concentration (±SEM), which was plotted against molar concentration on the log scale. The increase in rate constant in individual experiments, averaged for the four washout periods at each concentration, was also expressed as a percentage

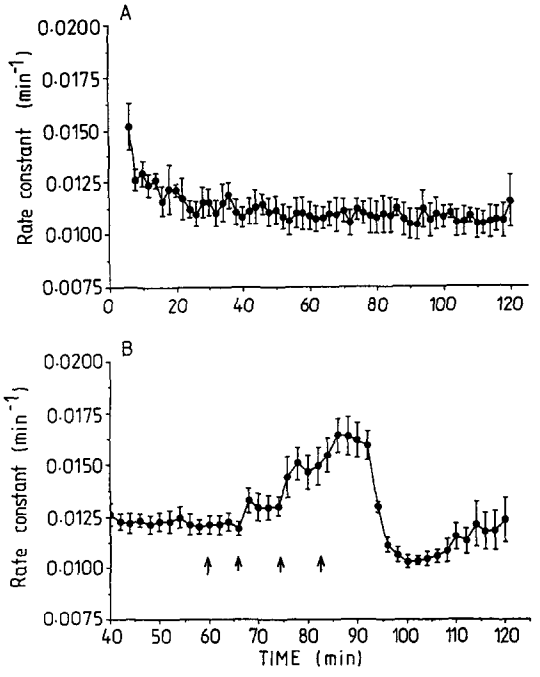

Fig. 1. Mean (N = 4) rate constants (k, min^{-1}) for ^{86}Rb efflux from guinea-pig left atria measured at 2 min intervals (A) in the absence of any drug addition and (B) during the course of an experiment to determine the response to four increasing concentrations of adenosine. The first exposure to each concentration of adenosine was at 60 min (2.25 μM), 68 min (22.5 μM), 76 min (225 μM) and 84 min (2.25 nM), indicated by the arrows. Each concentration was present for 4 × 2 min. Drug-free Krebs solution was present up to 60 min and after 92 min. Time (min) represents the time after the end of the loading period. Vertical bars represent the SEM.

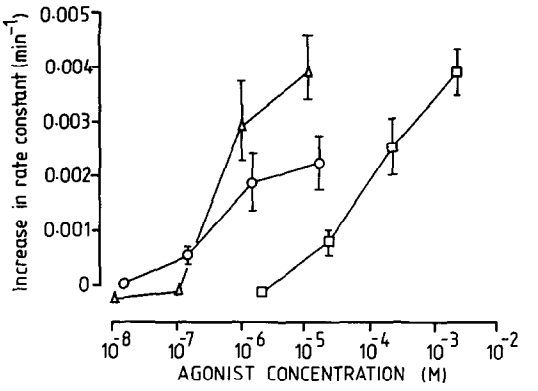

Fig. 2. Mean (N ≥ 4) concentration–response curves for the increase in rate constant (k, min^{-1}) for ^{86}Rb efflux from guinea-pig left atria produced by carbachol (△), L-PIA (○) and adenosine (□). Increases in k are expressed as absolute increases and vertical bars represent the SEM.

of the value immediately preceding the start of the concentration–response curve. The mean (N ≥ 4) percentage increases (± SEM) were then plotted against molar concentration on a log scale.

Measurement of isometric contractions of the left atria did not always yield usable data because of the high degree of "bubble noise" and the disturbance caused by the frequent washing of the tissue. When it was usable, however, the negative inotropic response to each concentration of agonist, in an individual experiment, was measured as the mean of the developed tension obtained at the four washout periods. This was subtracted from the resting developed tension immediately preceding the concentration–response curve and expressed as a percentage of this resting level. Mean percentage reductions at each agonist concentration were then plotted against molar concentration on the log scale.

Statistical analysis. Arithmetic mean values (± SEM) for the increases in ^{86}Rb efflux rate constant and percentage reduction of isometric tension at each concentration of agonist were calculated. Differences between untreated controls and antagonist-treated tissues were compared by a two-tailed Student's unpaired t-test, where $P < 0.05$ was considered significant.

Drugs used. Adenosine (Sigma), 4-aminopyridine (Sigma), bromobenzoylmethyladamantylamine (BMA; EGYT, Budapest), carbamylcholine chloride (carbachol; Sigma) and L-N^6-phenylisopropyladenosine (L-PIA; Sigma) were dissolved in distilled water. 8-Phenyltheophylline (Sigma) was dissolved in sodium hydroxide (0.05 M) and further diluted in distilled water. Reserpine (Sigma) was dissolved by adding benzyl alcohol (0.08 mL) to reserpine (10 mg) and citric acid (10 mg). To this was added Tween 80 (0.4 mL) and the concentration of reserpine adjusted to 2.5 mg/mL by addition of distilled water.

^{86}Rubidium was obtained as an aqueous solution of rubidium chloride (Amersham International) containing a maximum activity of 8 mCi/mg of ^{86}rubidium and 1 mCi/mL.

RESULTS

Variation in rate constant (k) *for* ^{86}Rb *efflux without drug addition*

During the initial 20 min after the loading period, the rate constant (k) for ^{86}Rb efflux rapidly decreased from very high values to plateau at approximately 0.011 min^{-1}. Subsequently, in the absence of drug addition, no significant change occurred in the value of k between 20 and 120 min after the loading period (Fig. 1). Thus, no time-dependent alterations in ^{86}Rb efflux rate constant occurred during the course of the experiment and any changes can be attributed to the presence of added drug.

Effects of adenosine, L-PIA and carbachol upon ^{86}Rb *efflux*

Adenosine, L-PIA and carbachol produced significant concentration-related increases in the rate constant for ^{86}Rb efflux (Fig. 2). The mean experimental data for adenosine is shown in Fig.

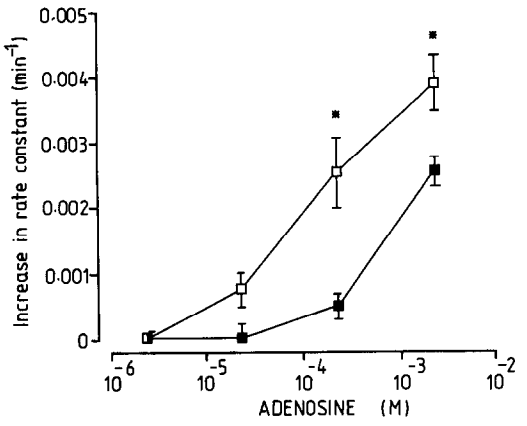

Fig. 3. Effect of 8-phenyltheophylline (8-PT) on the mean ($N \geq 4$) concentration–response curves for the increase in ^{86}Rb efflux from guinea-pig left by adenosine. Curves were obtained in the absence (□) or presence (■) of 8-PT (10 μM). Increases in rate constant for ^{86}Rb efflux (k, min^{-1}) above baseline are plotted and vertical bars represent the SEM. *Indicates a significant difference between values in the absence and presence of 8-PT.

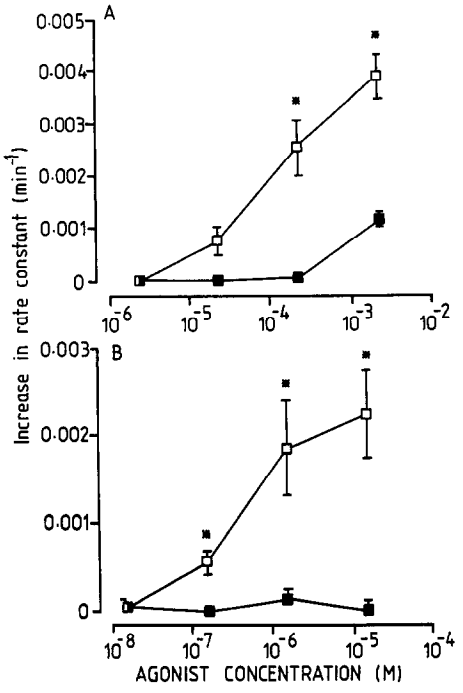

Fig. 4. Effects of 4-aminopyridine (4-AP) on the mean ($N \geq 4$) concentration–response curves for the increase in ^{86}Rb efflux from guinea-pig left atria by (A) adenosine and (B) L-PIA. Curves were obtained in the absence (□) or presence (■) of 4-AP (10 mM). Increases in rate constant for ^{86}Rb efflux (k, min^{-1}) above baseline are plotted and vertical bars represent the SEM. *Indicates a significant difference between values in the absence and presence of 4-AP.

1B. The increase in rate constant in response to the maximum concentrations of carbachol and adenosine were not significantly different, however, both produced significantly greater stimulation than the maximum concentration of L-PIA. The concentration–response curve for L-PIA was, however, to the left of that for adenosine indicating its greater potency.

Effects of 8-phenyltheophylline (8-PT), 4-aminopyridine (4-AP) and BMA upon ^{86}Rb efflux

BMA (100 μM), alone, significantly reduced the rate constant for ^{86}Rb efflux from 0.0104 to 0.0087 min^{-1} during the 20 min exposure. 4-AP (10 mM) caused a transient increase in the rate constant which had returned to the resting level at the end of the antagonist equilibration period. 8-PT (10 μM), alone, had no effect on ^{86}Rb efflux.

Effect of 8-PT upon the stimulation of ^{86}Rb efflux by adenosine

8-PT (10 μM) produced an apparently parallel rightward shift of the adenosine concentration–response curve (Fig. 3), with a significant reduction in the response to the maximum adenosine concentration used (Table 1).

Effects of 4-AP upon the stimulation of ^{86}Rb efflux by adenosine and L-PIA

In the presence of 4-AP (10 mM), the ability of both adenosine and L-PIA to increase the rate constant for ^{86}Rb efflux was greatly reduced (Fig. 4), the responses to the maximum concentrations of adenosine and L-PIA used being significantly reduced (Table 1). Adenosine was only able to significantly increase the value of k at the highest concentration used (2.25 mM) and the response to L-PIA was virtually abolished.

Effects of BMA upon the stimulation of ^{86}Rb efflux by adenosine, L-PIA and carbachol

The presence of BMA (100 μM) significantly reduced the increases in rate constant for ^{86}Rb efflux by the maximum concentrations of adenosine and carbachol and reduced, although not significantly, the response to L-PIA (Table 1). In addition, the shape of the agonist concentration–response curves was altered in the presence of BMA (Fig. 5). The curves were not shifted in parallel fashion, rather they seemed to be "flattened".

Comparison of stimulation by ^{86}Rb efflux and inhibition of atrial tension produced by adenosine and L-PIA

Over the same concentration range, adenosine produced significant concentration-related increases in rate constant for ^{86}Rb efflux and inhibitions of left atrial tension. The maximum percentage reduction was 90.6 ± 2.8% of the initial resting tension (Fig. 6A). The increases in rate constant and reduction in atrial tension by L-PIA were also over a common concentration range, the maximum reduction of resting tension being 82.9 ± 9.9% (Fig. 6B).

Effects of 8-PT, 4-AP and BMA upon the inhibition of left atrial tension by adenosine and L-PIA

In the presence of 8-PT (10 μM), 4-AP (10 mM)

Table 1. The effects of 8-phenyltheophylline (8-PT, 10 μM), BMA (100 μM) and 4-aminopyridine (4-AP, 10 mM) upon the mean increases in ^{86}Rb efflux rate constant (k) observed at the highest concentrations of adenosine (2.25 mM), L-PIA (15.6 μM) and carbachol (11 μM) used in guinea-pig left atria

	Increase in rate constant (min^{-1})		
	Adenosine	L-PIA	Carbachol
Control	0.00389 ± 0.0004	0.00223 ± 0.0005	0.00400 ± 0.0006
8-PT	0.00255 ± 0.0002*	—	—
BMA	0.00185 ± 0.0001*	0.00101 ± 0.0004	0.00179 ± 0.0002*
4-AP	0.00116 ± 0.0001*	0.00015 ± 0.0001*	—

Arithmetic mean values (N ≥ 4) ± SEM are shown.
* Indicates a significant different ($P < 0.05$) from the relevant control value.

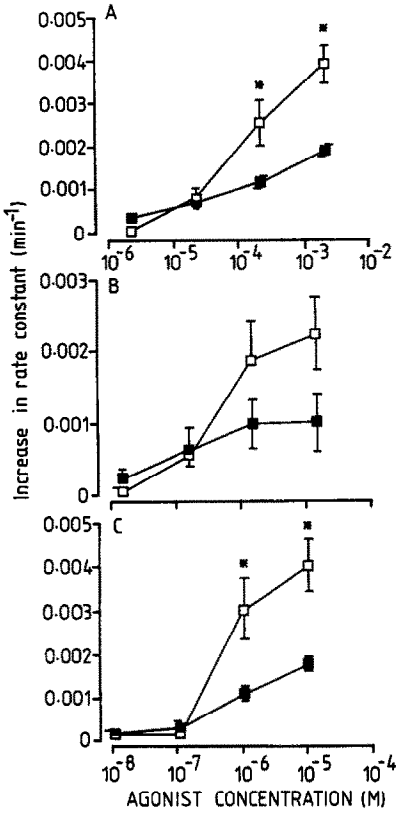

Fig. 5. Effects of BMA on the mean (N ≥ 4) concentration–response curves for the increase in ^{86}Rb efflux from guinea-pig left atria by (A) adenosine, (B) L-PIA and (C) carbachol. Curves were obtained in the absence (□) or presence (■) of BMA (100 μM). Increases in rate constant for ^{86}Rb efflux (k, min^{-1}) above baseline are plotted and vertical bars represent the SEM. *Indicates a significant difference between values in the absence and presence of BMA.

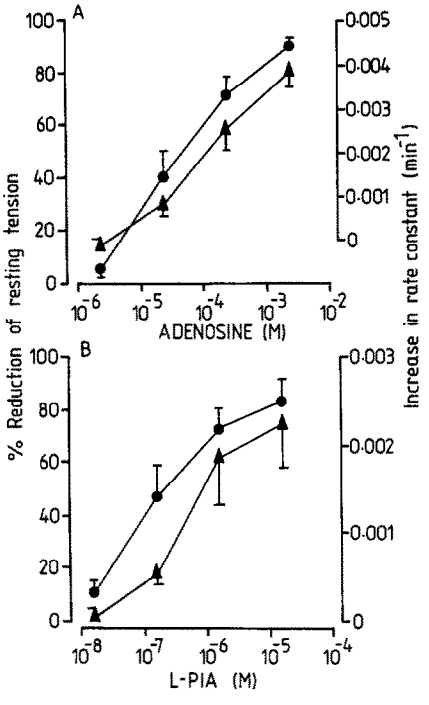

Fig. 6. Mean (N ≥ 4) concentration–response curves for the increase in ^{86}Rb efflux from guinea-pig left atria (▲) and the inhibition of tension (●) by (A) adenosine and (B) L-PIA. Increases in rate constant for ^{86}Rb efflux (k, min^{-1}) above baseline and inhibition of tension as a percentage of the pre-agonist resting tension are plotted. Vertical bars represent the SEM.

or BMA (100 μM), the left atrial negative inotropic responses to adenosine were reduced (Fig. 7), the responses to the maximum adenosine concentration used being significantly reduced by all three antagonists. The residual inhibitions of left atrial tension by the highest concentration of adenosine employed were 69.6 ± 4.4, 56.6 ± 2.6 and 50.1 ± 5.8%, respectively, in the presence of 8-PT, 4-AP and BMA. In the case of 4-AP, this represents a reduction of the adenosine response to 62.5% of the control value in the absence of antagonist. This compares with a much greater inhibition of the corresponding ^{86}Rb efflux to 29.5% of the control value.

Only two or three tissues provided measurable negative inotropic responses to L-PIA in the presence of 4-AP and BMA. Although these showed reduced

responses in the presence of the antagonists, the results are not presented.

DISCUSSION

The P1-receptor agonists, adenosine and L-PIA, and the muscarinic-receptor agonist carbachol all stimulated ^{86}Rb efflux from guinea-pig left atria in a dose-dependent manner. This agrees with previous reports for P1- [11–13] and muscarinic-receptor agonists [4, 12, 16, 17]. The greater potency of L-PIA than adenosine was matched by its greater potency in producing negative inotropy shown here and previously [18, 19] and is consistent with both responses being mediated via the A_1-subtype of P1-receptor. The maximum response to adenosine was, however, significantly greater than for L-PIA, which was surprising since both were full agonists for the negative inotropic responses of the left atria [18, 19].

8-PT caused an apparently parallel rightwards shift of the adenosine concentration–response curve for the ^{86}Rb efflux response. This agrees with previous findings [11] and suggests that adenosine stimulates ^{86}Rb efflux by P1-receptor activation. The greater maximum ability of adenosine to stimulate ^{86}Rb efflux compared to L-PIA cannot therefore be attributed to stimulation of ^{86}Rb efflux by a mechanism other than P1-receptor agonism. The fact that L-PIA and adenosine inhibited left atrial tension and stimulated ^{86}Rb efflux over the same concentration ranges supports the proposal that the negative inotropic effects of P1-receptor agonists are mediated by a stimulation of potassium efflux.

The receptor mediating the carbachol-induced increase in ^{86}Rb efflux was not investigated in the present study. However, since the effects of acetylcholine upon ^{42}K efflux are antagonized by atropine, it can be assumed that the effect of carbachol is also muscarinic-receptor-mediated [20].

The direct effects of the P1-receptor and potassium channel antagonists upon ^{86}Rb efflux were examined during the 20 min exposure prior to commencing the agonist concentration–response curves. 8-PT had no effect upon ^{86}Rb efflux, which confirmed that if any endogenous adenosine was present, it had a negligible effect upon ^{86}Rb efflux. 4-AP caused an unexpected transient *increase* in efflux which returned to baseline levels after 20 min. This increase was not due to the release of catecholamines reported in a variety of tissues [21] since the atria were from reserpine-pretreated guinea-pigs. It could have been linked to the increase in pH that is associated with addition of 4-AP to the organ bath [22]. However, since the rate constant for ^{86}Rb efflux returned to basal levels before exposure to the agonists, it is unlikely that any pH changes were responsible for the effects of 4-AP upon the agonist-induced changes in ^{86}Rb efflux. We have also shown that blockade of the negative inotropic response to adenosine by 4-AP is identical whether or not the pH effect is neutralized. The experiments were not therefore repeated after pH neutralization. The failure to reduce basal ^{86}Rb was surprising since it increases cardiac action potential duration and exerts positive inotropy [23, 24], consistent with blockade of potassium channels.

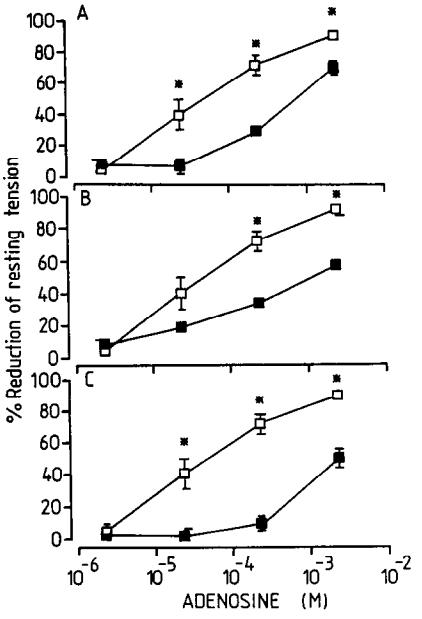

Fig. 7. Mean ($N \geq 4$) concentration–response curves for the inhibition of guinea-pig left atrial tension by adenosine in the absence (□) or presence (■) of (A) 8-phenyltheophylline (10 μM), (B) 4-aminopyridine (10 mM) and (C) BMA (100 μM). Inhibition of tension is expressed as a percentage of the pre-adenosine resting tension. Vertical bars represent the SEM. *Indicates a significant difference between values in the absence and presence of antagonist.

In spite of the lack of effect on resting efflux, 4-AP virtually abolished the increase in ^{86}Rb efflux induced by L-PIA and substantially attenuated that induced by adenosine. The reason for this apparently greater antagonism of L-PIA is not evident. It is possible that the antagonism of adenosine is distorted by its uptake which was not inhibited. To resolve this question, the experiments would require repetition in the presence of uptake blockade. 4-AP concomitantly antagonized the negative inotropic response to adenosine, a result at variance with De Biasi *et al.* [8] who obtained no antagonism of L-PIA.

The antagonism of the negative inotropic response to carbachol in the guinea-pig left atria by 4-AP has also been proposed to be mediated by blockade of potassium channels [8, 25]. However, 4-AP has also been shown to block the response of the trachea to carbachol, a response which is not dependent upon potassium channel activation (unpublished observation) and to displace the muscarinic receptor ligand, [^{3}H]dexetimide from rat striatal binding sites [26]. These observations suggest that 4-AP may be a muscarinic receptor antagonist as well as a potassium channel antagonist. Therefore, an assessment of the ability of 4-AP to block carbachol-induced increases in ^{86}Rb efflux was not attempted, since the results would be difficult to interpret.

The potassium channel antagonist, BMA, is capable of reversing both the inotropic and

electrophysiological effects of adenosine [7] and acetcylcholine [6] in guinea-pig isolated left atria. In the present study, BMA reduced the increases in ^{86}Rb efflux induced by the maximum concentrations of adenosine, L-PIA and carbachol. BMA appeared to "flatten" the concentration–response curves, an effect that was probably due to the reduction of basal ^{86}Rb efflux by BMA as well as the inhibition of agonist-induced ^{86}Rb efflux. The fact that BMA affected the P1-receptor and muscarinic receptor agonists similarly, is consistent with the theory that in left atria they stimulate the opening of a common population of potassium channels [1, 5, 3].

If the direct negative inotropic effects of the P1-receptor agonists are totally dependent upon the stimulation of potassium efflux, then a near total blockade of ^{86}Rb efflux by 4-AP or BMA would be expected to be accompanied by a near total blockade of the direct negative inotropic responses to the P1-receptor agonists. However, although the negative inotropic responses to the P1-receptor agonists were reduced in the presence of 4-AP and BMA, left atrial tension was still inhibited by 50% or more at the maximum effect of adenosine. The preferential inhibition of the ^{86}Rb efflux response to adenosine (to 29.8%) compared with the tension response (to 62.5%) was most marked with 4-AP. This suggests either that only very small changes in potassium efflux, too small for detection, are necessary to mediate the negative inotropic effects of P1-receptor stimulation or that the negative inotropic responses are not totally dependent upon stimulation of potassium efflux. The former possibility is unlikely as a study of the control responses shows that ^{86}Rb efflux and negative inotropic responses increase over a common concentration range. If only a small increase in efflux was required, the entire inotropic response would be expected to be seen after only a small stimulation of ^{86}Rb efflux had occurred. Thus, the results are consistent with only a portion of the direct negative inotropic response to P1-receptor agonists being dependent upon an increase in potassium efflux. The non-potassium efflux-dependent portion of the response is mediated through P1-receptors since 8-PT antagonized the negative inotropic response to P1-receptor agonists in guinea-pig left atria; here and in previous studies this was shown to be competitive [11, 27]. If two mechanisms were responsible for the negative inotropy, the concentration–response curve for the inotropic response might be expected to be slightly to the left of that for ^{86}Rb efflux, depending on the proportion that was potassium efflux-dependent. This was in fact the case, but not of the order expected for a substantial reserve capacity.

The apparently parallel rightward shift by 8-PT of the adenosine concentration–response curve for ^{86}Rb efflux is consistent with a competitive antagonism between 8-PT and adenosine. However, the effects of both the potassium channel antagonists upon the agonist concentration–response curves for ^{86}Rb efflux suggest that the antagonism is not competitive. 4-AP almost completely abolished the increases in ^{86}Rb efflux produced by the P1-receptor agonists, although the possibility that greater P1-receptor agonist concentrations would have produced greater increases in ^{86}Rb efflux cannot be discounted. BMA "flattened" the concentration–response curves to adenosine, L-PIA and carbachol, an effect clearly not the result of competitive antagonism. P1- and muscarinic-receptors in atrial muscle are linked directly to the potassium channels they activate by a GTP-binding protein, without the involvement of a second messenger [3, 28, 29]. This arrangement appears to have little potential for spare capacity which would enable the agonists to overcome the effect of functional antagonists, such as potassium channel antagonists. Lack of a substantial spare capacity is also indicated by the closeness of the ^{86}Rb efflux and negative inotropy concentration–response curves for L-PIA and adenosine. Thus, potassium channel antagonists capable of blocking the P1- and muscarinic-receptor-linked potassium channels would be expected to produce non-parallel shifts of the agonist concentration–response curves for ^{86}Rb efflux typical of functional antagonism.

This study has therefore demonstrated that 4-AP and BMA block P1- and muscarinic-receptor-linked potassium channels in guinea-pig paced left atria. These antagonists may be used to further evaluate the role of these channels in the negative inotropic responses to agonists at these receptors.

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