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EFFECT OF PRE-TREATING RAT ATRIA WITH POTASSIUM CHANNEL BLOCKING DRUGS ON THE ELECTRICAL AND MECHANICAL RESPONSES TO PHENYLEPHRINE

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Abstract—Action potential duration (APD) and systolic developed tension (SDT) were recorded from electrically paced rat atria before, during and after treatment with phenylephrine *in vitro*. Phenylephrine caused a prolongation of APD and an increase in SDT, the magnitude of which depended upon the frequency of pacing of the atria. Effects very similar to those elicited by phenylephrine were produced by exposing the atria to 4-aminopyridine, 3,4-diaminopyridine, CsCl, amantadine or sparteine. Atria that had been pre-treated with any of the latter five compounds showed a diminished responsiveness when subsequently treated with maximally effective concentrations of phenylephrine, and vice versa. These findings are consistent with blockade of potassium ion channels being responsible for the observed responses to all of the above agents.

Key words: action potential duration; systolic developed tension; 4-aminopyridine; 3,4-diaminopyridine; sparteine; amantadine

PISCs† of several different types occur in the plasma membrane of mammalian cardiomyocytes [1–3]. These channels are coupled via guanosine triphosphate-binding proteins to specific receptor sites, which in turn can bind certain naturally occurring chemical agents. These chemicals, therefore, may indirectly modulate plasmalemmal potassium ion conductance, and thereby alter APD and other aspects of muscle function. In general, blockade of PISC increases myocardial APD, resulting in positive inotropism by prolonging the time during which calcium ions flow into the cell during systole. Opening PISC, on the other hand, tends to cause an opposite sequence of effects.

Activation of cardiac α1-adrenoceptors has been shown to close several types of PISC. This applies to the K_{to} in rabbit atria [4–6] and rat ventricles [7–9]. Similarly, in response to α1-adrenoceptor agonists, there is a closure of the inwardly rectifying K_i in rabbit ventricles [10, 11] and the K_{Ach} in rabbit atria [12]. Closure of the K_{dr} in rat ventricles also occurred after treatment with an α1-adrenoceptor agonist [8]. Activation of the α1-adrenoceptors in rat atrial myocytes has been reported to block two distinct outward potassium currents [13, 14]. The first flowed via the previously mentioned K_{to}. The second differed from the first in remaining active when depolarization persisted, and differed from the current which flowed via K_{dr} in terms of the rapidity with which it activated at the start of depolarization. The so-called K_p, first identified in guinea-pig ventricles [15], may have carried this second outward current in rat atria. Clearly, therefore, α1-adrenoceptor agonists may delay cardiac repolarization, and thereby prolong APD, in a number of different ways. What is uncertain is the extent to which closure of any of these different types of PISC actually causes APD prolongation, and the accompanying positive inotropism, in a particular tissue. For example, positive inotropism may depend not upon the blockade of PISC of any type, but rather upon a sensitisation of the contractile proteins to normal systolic rises in cytoplasmic calcium concentration on account of a rise in intracellular pH, as reviewed recently [16, 17].

In light of the foregoing information it was of interest to compare the myocardial responses to phenylephrine, an α1-adrenoceptor agonist, with those elicited by various chemicals which are considered to block the different types of PISC more directly. If a particular directly-acting PISC blocker is selective for a certain type of PISC, and if it can be shown that responses to phenylephrine mimic those produced by the directly-acting PISC blocker, then a blockade of the relevant type of PISC becomes the most likely explanation for the observed effects of phenylephrine. Several chemicals are known which seemingly directly, but with varying degrees of selectivity, block the different types of PISC [1, 13, 18]. The present study has examined the APD-prolonging and positively inotropic effects produced by a chemically diverse range of such

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† Abbreviations: PISC, potassium ion selective channels; APD, action potential duration; K_{to}, transient outward channels; K_i, background potassium channels; K_{Ach}, acetylcholine-sensitive potassium channels; K_{dr}, delayed rectifier channels; K_p, protracted outward channels; UK-66914, *N*-(4-(1-hydroxy-2-(4-pyridyl)-1-piperizinyl)ethyl)-phenyl methane sulphonamide; K_{ATP}, adenosine triphosphate-sensitive potassium channels; SDT, systolic developed tension.

blocking agents in rat atria. Moreover, the extent to which pre-treating the atria with such agents was able to modify subsequent responses to phenylephrine, and vice versa, was examined. In this way it was hoped to clarify the possible involvement of various types of PISC in the atrial responses to phenylephrine.

MATERIALS AND METHODS

Chemicals. Unless otherwise stated, chemicals were purchased from Sigma Chemical Company (Poole, U.K.), and were dissolved initially in water. L-Phenylephrine, amantadine and DL-propranolol were obtained as their hydrochlorides and isoprenaline as its sulphate. 4-Aminopyridine, 3,4-diaminopyridine, Cs_2CO_3 and sparteine were first dissolved in sufficient HCl to give a neutral solution. Glibenclamide, UK-66914 and terikalant were kindly supplied by Pfizer Ltd (Sandwich, U.K.) and were first dissolved in dimethyl sulphoxide. These solutions were diluted just before use with sufficient superfusate to give the final desired concentration.

Isolation of atria. Atria were isolated from rat hearts as described previously [19]. Briefly, the atria were bathed, unless otherwise specified, with a superfusate at 34° of the following composition (mM), NaCl 138, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 10, glucose 10, and gassed with 95% O_2 plus 5% CO_2. Unless otherwise stated, atria were stimulated electrically throughout an experiment at 3 Hz with 2 msec square wave pulses each of 10 V.

Tension recordings. A thread sutured to the left atrial appendage was connected to a force displacement transducer (type SB-IT, Nihon Kohden) at a diastolic tension of 100 mg. Tension was recorded on a paper trace via a DC amplifier (type 5242) coupled to a heated stylus recorder (Multitrace 2, made by Lectromed).

Electrical recordings. Transmembrane potentials were recorded from subendocardial muscle fibres by means of standard glass microelectrodes filled with a 3 M solution of KCl and having a resistance of approximately $10^7\ \Omega$. Voltages were recorded via a Ag/AgCl wire in the lumen of the microelectrode. Voltages were led via a single-ended high input impedance coupler with facilities for capacitance neutralization (type 8124, made by C. F. Palmer) to both an oscilloscope and a transient store microprocessor (type 140, made by Bioscience). The latter permitted action potentials to be printed without distortion on the same paper trace that was used for atrial tension recordings. APD was routinely measured in the present experiments at 60% repolarization.

Calculation of the magnitude of responses. Changes in APD and systolic developed tension that were produced in response to each experimental intervention were calculated with respect to the corresponding values displayed immediately prior to the relevant intervention. Mean values from different groups of atria were considered to be significantly different from each other if the null hypothesis was rejected because $P < 0.05$ in a Student's *t*-test.

RESULTS

Effects of directly-acting PISC blockers

Atrial systolic developed tension averaged 296 ± 31 mg during pacing at 3 Hz in the standard superfusate. Positively inotropic effects were produced by adding various directly-acting PISC blockers to this superfusate. A dose–response relationship was determined for each agent, using 5-fold increments in concentration. Table 1 shows the concentration of each substance that caused the largest inotropic response when measured after 10 min of superfusion, together with a 5-fold lower concentration in each case. At the concentrations shown in Table 1 no significant difference was found

Table 1. Atrial responses to PISC blockers, with and without pre-treatment with phenylephrine

Treatment		Responses to listed treatment			
		Increase in APD (msec)		Increase in SDT (mg)	
		Pre-treatment		Pre-treatment	
Name	Concentration (μM)	Nil	Phenylephrine	Nil	Phenylephrine
4-Aminopyridine	1000	19.2 ± 3.1	4.5 ± 2.0*	302 ± 27	45 ± 9*
	200	4.2 ± 1.0	3.1 ± 1.4	68 ± 19	18 ± 5*
3,4-Diaminopyridine	100	17.1 ± 2.8	5.0 ± 2.7*	290 ± 21	47 ± 3*
	20	4.8 ± 2.3	2.6 ± 1.1	75 ± 16	26 ± 4*
CsCl	10 000	18.9 ± 2.4	6.2 ± 2.9*	316 ± 30	32 ± 10*
	2000	7.0 ± 1.2	5.0 ± 0.8	97 ± 18	19 ± 7*
Amantadine	40	16.6 ± 2.3	4.9 ± 1.3*	272 ± 25	51 ± 12*
	8	2.4 ± 1.9	1.6 ± 0.6	63 ± 14	16 ± 8*
Sparteine	500	20.5 ± 3.0	3.7 ± 1.8*	327 ± 29	42 ± 11*
	100	9.3 ± 1.5	3.2 ± 2.0*	109 ± 17	26 ± 5*

Atria were pre-treated with phenylephrine (50 μM) where indicated. Tabulated values are the means of eight observations ± SEM. APD and SDT responses were measured after exposing the atria for 10 min to each listed treatment. A significant difference existed ($P < 0.05$) between a value marked * and the corresponding response to the same treatment obtained in the absence of pre-treatment with phenylephrine.

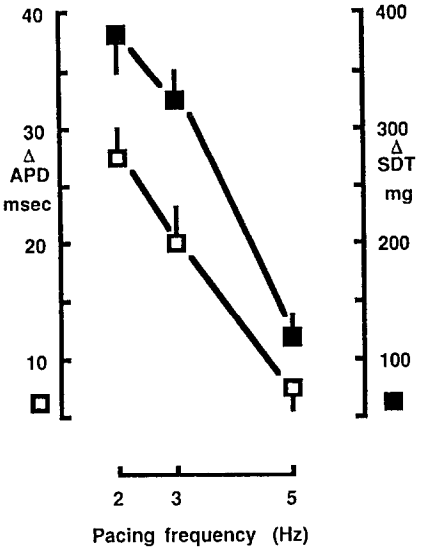

Fig. 1. Changes in APD and SDT produced by exposing atria to sparteine (500 μM) for 10 min while being paced at different frequencies. There were eight observations at each data point. Vertical bars represent SEM.

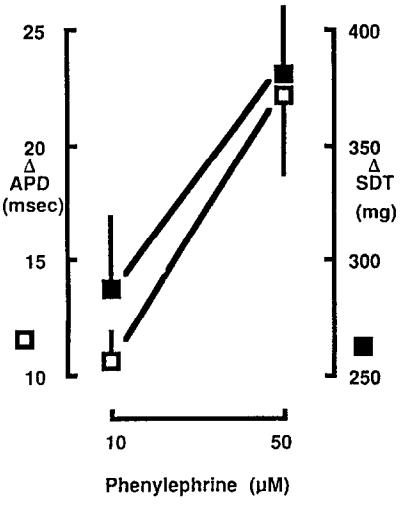

Fig. 2. Changes in APD and SDT produced by exposing atria to different concentrations of phenylephrine for 10 min. There were eight observations at each data point. Vertical bars represent SEM.

between the inotropic responses to a particular agent when measured after 10 min and again after 15 min of superfusion. At higher concentrations than those shown in Table 1, however, the inotropic responses to some of these agents faded significantly between 10 and 15 min from the start of superfusion. This was most marked with sparteine.

During pacing at 3 Hz the diastolic membrane potential of atrial muscle was found to be Minus 84 ± 5 mV. Action potentials showed a 15 ± 7 mV overshoot and had an APD at 60% repolarization of 19.2 ± 2.0 msec. Table 1 shows that each of the listed compounds significantly prolonged APD. No statistically significant change in diastolic membrane potential was found with these compounds during 10 min of superfusion at the concentrations shown in Table 1. Maximal prolongation of APD was attained within the first 10 min of exposure in all cases, and was well maintained for at least 15 min. The higher of the two listed concentrations of each substance prolonged APD significantly more than the corresponding lower concentration. The prolongation of APD and accompanying inotropic response with each agent showed similar time-course relationships. Attainment of the peak response occurred fastest with sparteine (4–6 min) and slowest with CsCl (7–9 min). Repeated 15 min periods of exposure of the atria to each agent in Table 1 at 45 min intervals for up to 8 hr gave a series of electrical and mechanical responses that did not change significantly with time. Returning the atria to a drug-free superfusate caused APD and systolic developed tension to return to baseline values over the course of the next 15–30 min. The experiments described so far were all performed during atrial pacing at 3 Hz, but they were repeated during pacing at 2 and 5 Hz. The magnitude of APD prolongation and positive inotropy were dependent upon the pacing rate, being smallest at 5 Hz and largest at 2 Hz (Fig. 1), although the degree of suppression at 5 Hz was different for each compound. Thus, 4-aminopyridine (1 mM) lost all of its effects at 5 Hz, whereas sparteine (500 μM) still had some potency at 5 Hz (Fig. 1).

Exposure of the atria to glibenclamide, UK-66914 or terikalant (each at 10 μM for 10 min) failed to prolong APD significantly, and produced no significant change in inotropism. Higher concentrations were not tested, since these three agents are known to substantially block K_{ATP}, K_{dr} and K_i at 10 μM (see Discussion).

Effects of phenylephrine and isoprenaline

Superfusion of the atria with phenylephrine (10–50 μM) produced a rapidly developing and concentration-dependent positively inotropic response (Fig. 2) that was maximal within 6 min and was well maintained thereafter for up to 12 min of exposure, but which disappeared over the course of 20–30 min after returning the atria to drug-free superfusate. Exposure to phenylephrine (50 μM) for periods longer than 12 min caused small and variable rises in diastolic tension. These were more common in atria that had been isolated for several hours than in those more recently obtained, but the cause was not investigated further.

Accompanying the inotropic response to phenylephrine there was a marked prolongation of APD (Fig. 3), with both responses showing similar time-course relationships. Phenylephrine at 250 μM gave electrical and mechanical responses after 10 min that were not significantly different from those elicited at 50 μM, indicating that the latter were maximal. Accompanying the prolongation of APD was a small and variable diastolic depolarization which averaged 5 ± 2 mV. Repeated 10 min periods of exposure of

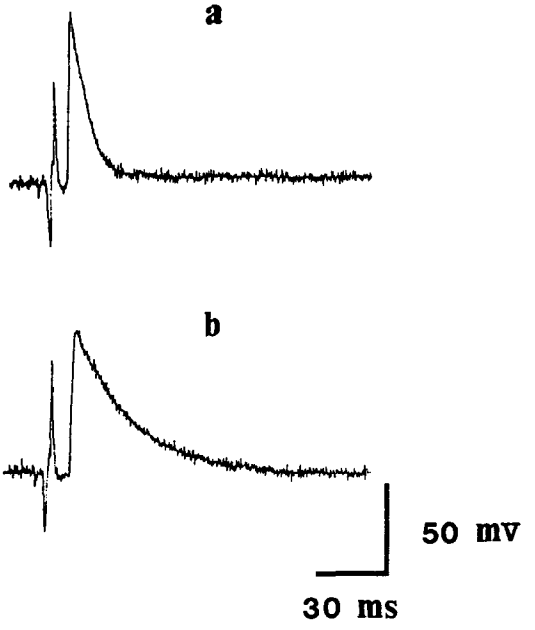

Fig. 3. Representative atrial action potentials recorded from a single cell (a) during exposure to the standard superfusate and (b) after 10 min of exposure to phenylephrine (50 μM).

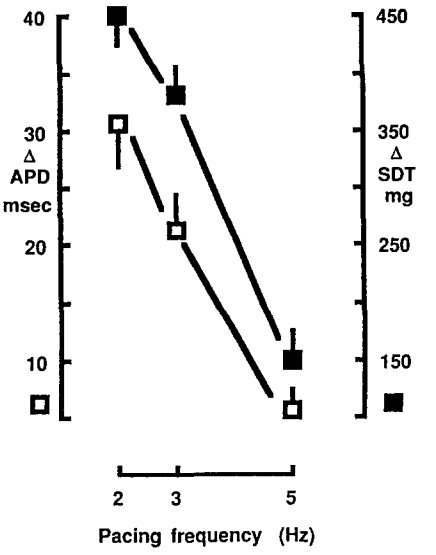

Fig. 4. Changes in APD and SDT produced by exposing atria to phenylephrine (50 μM) for 10 min while being paced at different frequencies. There were eight observations at each data point. Vertical bars represent SEM.

the atria to phenylephrine (50 μM) at 50 min intervals for up to 10 hr gave a series of electrical and mechanical responses that did not change significantly with time and which were not significantly altered by pre-treating the atria for 10 min with propranolol (5 μM). For this reason, except where specified below, propranolol (5 μM) was routinely added to the superfusate in order to block any β-adrenoceptor-mediated effects that might have been caused by noradrenaline released into the atria by the electrical pacing stimuli.

The experiments with phenylephrine described above were all conducted at a pacing frequency of 3 Hz, but were repeated at 2 and 5 Hz. Both the prolongation of APD and the positive inotropy that were produced by phenylephrine (50 μM) were significantly greater at 2 than at 3 Hz, and significantly less at 5 than at 3 Hz (Fig. 4). In these respects, therefore, responses to phenylephrine resembled those to the PISC blockers (see Fig. 1).

Atria which has been pre-treated for 10 min with the directly-acting PISC blockers that are listed in Table 2 gave responses to subsequent applications of phenyephrine (50 μM) that were significantly smaller in each case than the responses obtained without the pre-treatment. Nevertheless, responses obtained in the presence of the PISC blockers were maximal, because phenylephrine at 250 μM gave responses that were not significantly greater than those elicited at 50 μM. This indicated that the apparent antagonism shown towards phenylephrine by the PISC blockers was unsurmountable in nature. One would expect, therefore, that antagonism would also operate in the reverse direction. Table 1 showed that atria which had been pre-treated for 10 min with phenylephrine (50 μM), and then exposed to each of the directly-acting PISC blockers in turn, gave responses to these latter agents that were significantly smaller than those elicited in the absence of pre-treatment with phenylephrine. Once again, however, the responses were maximal, because with each of the directly-acting PISC blockers a 5-fold higher concentration than the higher of the two shown in Table 1 gave electrical and mechanical responses after pre-treatment with phenylephrine (50 μM) for 10 min that were no larger than those tabulated. In some cases, and particularly with sparteine, the inotropic responses to these high concentrations were actually smaller than those tabulated, although the same was not true for the accompanying APD changes. In summary, therefore, atrial responses to phenylephrine were very similar to those elicited by the PISC blockers listed in Table 1, and the similarity extended to mutual interference with each others maximal responses, both electrically and mechanically.

Atria which has been pre-treated for 10 min with the K_{to} blocker 3,4-diaminopyridine (100 μM) gave responses to subsequent applications of amantadine (40 μM) or sparteine (500 μM) that were significantly smaller in each case than corresponding responses (shown in Table 1) that were obtained in the absence of any pre-treatment. Indeed, in the presence of this concentration of 3,4-diaminopyridine, both the prolongation of APD and the positive inotropism normally produced by exposure to amantadine or sparteine were effectively abolished.

Atria which had been pre-treated for 10 min with glibenclamide, UK-66914 or terikalant (each at 10 μM) gave responses to subsequent applications of phenylephrine (50 μM) that were insignificantly

Table 2. Effects of various pre-treatments on responses to phenylephrine

Pre-treatment		Responses to phenylephrine (50 μM)	
Name	Concentration (μM)	Increase in APD (msec)	Increase in SDT (mg)
Control	—	22.0 ± 4.2	383 ± 39
4-Aminopyridine	1000	6.6 ± 0.9*	52 ± 16*
	200	17.5 ± 2.3	277 ± 25*
3,4-Diaminopyridine	100	7.4 ± 1.2*	63 ± 20*
	20	16.8 ± 3.4	312 ± 52
CsCl	10 000	5.9 ± 1.1*	74 ± 18*
	2000	19.7 ± 3.8	290 ± 32*
Amantadine	40	9.5 ± 2.0*	85 ± 15*
	8	20.3 ± 2.9	305 ± 34
Sparteine	500	7.6 ± 1.4*	17 ± 5*
	100	15.6 ± 3.5	286 ± 27*
Adenosine	1000	24.7 ± 5.4	395 ± 47

Tabulated values are the means of eight observations ± SEM. APD and SDT responses were measured after 10 min of exposure to phenylephrine, either without pre-treatment (control) or after 10 min of pre-treatment with the listed agent. A significant difference existed ($P < 0.05$) between control responses to phenylephrine and corresponding responses to phenylephrine marked * that were obtained from atria pre-treated with the listed agents.

different from those elicited in the absence of such pre-treatment.

Superfusing the atria with isoprenaline (0.1–0.5 μM), in the absence of propranolol, elicited a concentration-dependent positive inotropism, the maximal value of which was somewhat greater than that produced by a maximally inotropic concentration of phenylephrine, in confirmation of previous findings in this tissue [20]. The changes in APD elicited by isoprenaline (0.5 μM) were small and rather variable, and were not investigated further. Atria that were paced at 5 Hz gave inotropic responses to isoprenaline (0.5 μM) that were not significantly different from those elicited at the usual pacing frequency of 3 Hz. In this respect, therefore, inotropic responses to isoprenaline and phenylephrine differed markedly (see Fig. 3). Atria that had been pre-treated for 10 min with the PISC blockers listed in Table 1 gave positively inotropic responses to subsequent applications of isoprenaline (0.5 μM) that were insignificantly different from those elicited in the absence of such pre-treatment. Once again, therefore, the inotropic responses to isoprenaline and phenylephrine differed markedly (see Table 2).

Effects of adenosine

Exposing the atria to adenosine (1 mM) rapidly elicited a negatively inotropic response, accompanied by a decrease in APD from 19.2 ± 2.0 msec in the controls to 12.1 ± 1.1 msec. Both of these effects of adenosine disappeared over the course of 15–20 min on return to a drug-free superfusate. Concentrations of adenosine greater than 1 mM failed to produce any greater responses. Repeated 10 min periods of exposure of the atria to adenosine (1 mM) at 50 min intervals for up to 10 hr gave consistent responses. There was insignificant change in diastolic membrane potential in response to the application of adenosine (1 mM). Pre-treatment of the atria with adenosine (1 mM) for 10 min had only a small and statistically insignificant effect on both the positively inotropic and the APD-prolonging responses that could be elicited by a subsequent application of phenylephrine (Table 2).

DISCUSSION

Previous experiments showed that phenylephrine caused positively inotropic responses in rat atria which were mediated by the α1A subgroup of adrenoceptors [20], which were associated with both a blockade of outward potassium ion current and a prolongation of APD [13], and which were accompanied by an augmented systolic rise in cytoplasmic calcium ion concentration [20, 21]. The present study sets out to determine the type of PISC involved.

Glibenclamide is known to block cardiac K_{ATP} [22]. Terikalant is known to block cardiac K_i [23], and UK-66914 selectively blocks K_{dr}[24]. With each of these three agents a block of the relevant type of PISC has been reported to occur at 10 μM or less. In the present experiments, however, none of these three agents at 10 μM prolonged APD, and none exerted a positively inotropic effect, suggesting that K_{ATP}, K_i and K_{dr} provided little or no repolarizing current during normal action potentials in rat atria. It seemed unlikely, therefore, that the effects of phenylephrine on APD in the present experiments

Table 3. Properties of PISC blockers that were described in previously published articles

Agent	APD prolongation	Positive inotropism	Type of PISC blocked			
			K_i	K_{dr}	K_{Ach}	K_{to}
4-Aminopyridine	Yes [31]	Yes [31]	Yes [32]		Yes [33]	Yes [34]
3,4-Diaminopyridine	Yes [35]					Yes [35]
CsCl	Yes [1]		Yes [1]			Yes [1]
Amantadine	Yes [36]	Yes [36]			Yes [37]	
Sparteine	Yes [38]	Yes [39]		Yes [38]		

The reported ability (yes) to exert each effect is based on the reference indicated. Blanks indicate the absence of published data.

were due to a closure, or a failure to open normally, of one of those three types of PISC. The inability of a period of pre-treatment with these three agents to modify subsequent responses to phenylephrine in the present experiments points in the same direction.

Table 3 summarizes previous reports showing that chemical agents of diverse structure and potency block the various types of PISC, prolong cardiac APD and exert positive inotropism. Although Table 3 indicates that some of these agents can block K_{ATP}, K_i and K_{dr}, it is unlikely, for the reasons given above, that those types of PISC actually conducted significant amounts of repolarizing current during normal action potentials in rat atria. Hence, they were also unlikely to have been able to mediate the APD-prolonging and positively inotropic effects shown in Table 1. Moreover, it is unlikely that K_{Ach} was involved [25]. That is because, if phenylephrine or those agents listed in Table 3 had prolonged APD and caused positive inotropy by blocking K_{Ach}, then phenylephrine would be expected to have exerted a greater maximal APD-prolonging effect in atria that had been pre-treated with adenosine than in those not so pre-treated, and that did not occur. By exclusion, therefore, the type of PISC listed in Table 3 that seemed most likely to have mediated the effects of phenylephrine was K_{to}.

The ability of 4-aminopyridine to reduce the positively inotropic response to phenylephrine in the present experiments confirmed previous reports in rat ventricular muscle [9, 26] and in rat atria [20]. Interactions involving the other agents listed in Tables 1 and 2, however, have not been reported previously. Pre-treating the myocardium with a blocker of one particular type of PISC might have been expected to antagonise subsequently elicited maximal responses to any other agent that normally exerted its own effects via closure of that same type of PISC, because the first agent would be expected to have left open fewer of the relevant ion channels for the second agent to close. Under such circumstances the first agent would appear to inhibit maximal responses to the second agent, and do so unsurmountably. Tables 1 and 2 indicated that unsurmountable antagonism did exist between phenylephrine and some of the directly-acting PISC blockers, suggesting a common mode of action. However, 4-aminopyridine also has been reported to block α-adrenoceptors in the brain competitively at 1 mM [27], although a similar effect has not been reported with any other agent listed in Table 3. In such a chemically diverse group of compounds it seemed unlikely that the ability to block adrenoceptors would occur at precisely the concentration necessary in each case to prolong APD, since the latter effect was due to an unrelated property, namely the blockade of PISC. Furthermore, competitive antagonism at adrenoceptors would not explain the unsurmountable antagonism that was found in the present experiments.

The major repolarizing current that flows during an action potential in most mammalian atria is known to be carried by K_{to} [28–30], and each agent in Table 3 tested so far for an ability to block K_{to} was active. Moreover, the ability of 3,4-diaminopyridine in the present experiments to inhibit subsequent responses to both amantidine and sparteine suggested that the latter two compounds could also block K_{to}. Clearly, therefore, blockade of K_{to} would adequately explain the observed effects of both phenylephrine and the agents listed in Table 3. In addition to K_{to}, however, a second type of channel exists in rat atria which may carry repolarizing current during an action potential, and which is blocked by phenylephrine (see Introduction). The latter type of PISC, which has been likened to K_p in other issues, has not been tested so far for ability to be blocked by the agents listed in Table 3. If voltage clamp experiments reveal that such compounds can block K_p then this would become an alternative explanation for the effects observed in the present experiments. Nevertheless, there is now indirect evidence that blockade of some type of PISC is an adequate explanation for the positively inotropic effect of phenylephrine in rat atria.

REFERENCES

1. Rudy B, Diversity and ubiquity of K channels. *Neuroscience* **25**: 729–749, 1988.
2. Trube G, Potassium currents in isolated adult cardiac myocytes. In: *Isolated Adult Cardiomyocytes* (Eds. Piper HM and Isenberg G), Vol. 2, pp. 75–95. CRC Press, Boca Raton, FL, U.S.A., 1989.
3. Carmeliet E, K^+ channels and control of ventricular repolarization in the heart. *Fund Clin Pharmacol* **7**: 19–28, 1993.
4. Fedida D, Shimoni Y and Giles WR, A novel effect

of norepinephrine on cardiac cells is mediated by alpha1-adrenoceptors. *Am J Physiol* **256**: H1500–H1504, 1989.
5. Fedida D, Shimoni Y and Giles WR, Alpha-adrenergic modulation of the transient outward current in rabbit atrial myocytes. *J Physiol* **423**: 257–277, 1990.
6. Braun AP, Fedida D, Clark RB and Giles WR, Intracellular mechanism for alpha1-adrenergic regulation of the transient outward current in rabbit atrial myocytes. *J Physiol* **431**: 689–712, 1990.
7. Apkon M and Nerbonne JM, Alpha1-adrenergic agonists selectively suppress voltage-dependent K^+ currents in rat ventricular myocytes. *Proc Natl Acad Sci USA* **85**: 8756–8760, 1988.
8. Ravens U, Wang XL and Wettwer E, Alpha adrenoceptor stimulation reduces outward currents in rat ventricular myocytes. *J Pharmacol Exp Ther* **250**: 364–370, 1989.
9. Tohse N, Nakaya H, Hattori Y, Endoh M and Kanno M, Inhibitory effect mediated via alpha1-adrenoceptors on transient outward current in isolated rat ventricular cells. *Eur J Physiol* **415**: 575–581, 1990.
10. Shah A, Cohen IS and Rosen MR, Stimulation of cardiac alpha receptors increases Na/K pump current and decreases G_K via a pertussis toxin-sensitive pathway. *Biophys J* **54**: 219–225, 1988.
11. Fedida D, Braun AP and Giles WR, Alpha1-adrenoceptors reduce background K^+ current in rabbit ventricular myocytes. *J Physiol* **441**: 673–684, 1991.
12. Braun AP, Fedida D, and Giles WR, Activation of alpha1-adrenoceptors modulates the inwardly rectifying potassium currents of mammalian atrial myocytes. *Eur J Physiol* **421**: 431–439, 1992.
13. Ertl R, Jahnel U, Nawrath H, Carmeliet E and Vereecke J, Differential electrophysiologic and inotropic effects of phenylephrine in atrial and ventricular heart muscle preparations from rats. *Naunyn-Schmiedebergs Arch Pharmacol* **344**: 574–581, 1991.
14. Jahnel U, Nawrath H, Carmeliet E and Vereecke J, Depolarisation-induced influx of sodium in response to phenylephrine in rat atrial heart muscle. *J Physiol* **432**: 621–637, 1991.
15. Yue DT and Marban E, A novel cardiac potassium channel that is active and conductive at depolarised potentials. *Eur J Physiol* **413**: 127–133, 1988.
16. Fedida D, Braun AP and Giles WR, Alpha1-adrenoceptors in myocardium: functional aspects and transmembrane signaling mechanisms. *Physiol Rev* **73**: 469–487, 1993.
17. Terzic A, Puceat M, Vassort G and Vogel SM, Cardiac alpha1-adrenoceptors: an overview. *Pharmacol Rev* **45**: 147–175, 1993.
18. Robertson DW and Steinberg MI, Potassium channel modulators: scientific applications and therapeutic promise. *J Med Chem* **33**: 1529–1541, 1990.
19. Northover BJ, Continuous fluorimetric assessment of the changes in cytoplasmic calcium concentration during exposure of rat isolated myocardium to conditions of simulated ischaemia. *Br J Pharmacol* **100**: 477–482, 1990.
20. Northover BJ, Phorbol 12,13-dibutyrate produces negative inotropism and selective antagonism of responses to adrenoceptor agonists in rat atria. *Biochem Pharmacol* **45**: 1183–1189, 1993.
21. Jahnel U, Nawrath H, Shieh RC, Sharma VK, Williford DJ and Sheu SS. Modulation of cytosolic free calcium concentration by alpha1-adrenoceptors in rat atrial cells. *Naunyn-Schmiedebergs Arch Pharmacol* **346**: 88–93, 1992.
22. Ashcroft FM, Adenosine 5′-triphosphate-sensitive potassium channels. *Annu Rev Neuroscience* **11**: 97–118, 1988.
23. Escande D, Mestre M, Cavero I, Brugada J and Kirchhof C, RP58866 and its active enantiomer RP 62719 (terikalant): blockers of the inward rectifier K^+ current acting as pure class III antiarrhythmic agents. *J Cardiovasc Pharmacol* **20**: Supl 2, S106–S113, 1992.
24. Gwilt M, Dalrymple HW, Burges RA, Blackburn KJ, Dickinson RP, Cross PE and Higgins AJ, Electrophysiological properties of UK-66914, a novel class III antiarrhythmic agent. *J Cardiovasc Pharmacol* **17**: 376–385, 1991.
25. Belardinelli L, Klockner U and Isenberg G, Modulation of potassium and calcium currents in atrial and nodal cells. In: *Isolated Adult Cardiomyocytes* (Eds. Piper HM and Isenberg G), Vol. 2, pp. 155–180. CRC Press, Boca Raton, FL, U.S.A., 1989.
26. Fedida D and Bouchard RA, Mechanisms for the positive inotropic effect of alpha1-adrenoceptor stimulation in rat cardiac myocytes. *Circ Res* **71**: 673–688, 1992.
27. Drukarch B, Kits KS, Leysen JE, Schepens E and Stoof JC, Restricted usefulness of tetraethylammonium and 4-aminopyridine for the characterization of receptor-operated K^+ channels. *Br J Pharmacol* **98**: 113–118, 1989.
28. Giles WR and Imaizumi Y, Comparison of potassium currents in rabbit atrial and ventricular cells. *J Physiol* **405**: 123–145, 1988.
29. Shibata EF, Drury T, Refsum H, Aldrete V and Giles W, Contributions of a transient outward current to repolarization in human atrium. *Am J Physiol* **257**: H1773–H1781, 1989.
30. Fermini B, Wang Z, Duan D and Nattel S, Differences in rate dependence of transient outward current in rabbit and human atrium. *Am J Physiol* **263**: H1747–H1754, 1992.
31. Hilgemann DW, Extracellular calcium transients and action potential configuration changes related to post-stimulatory potentiation in rabbit atrium. *J Gen Physiol* **87**: 675–706, 1986.
32. Van Bogaert PP and Snyders DJ, Effects of 4-aminopyridine on inward rectifying and pacemaker currents of cardiac Purkinje fibres. *Eur J Physiol* **394**: 230–238, 1982.
33. Urquhart RA, Ford WR and Broadley KJ, Potassium channel blockade of atrial negative inotropic responses to P1-purinoceptor and muscarinic receptor agonists and to cromakalim. *J Cardiovasc Pharmacol* **21**: 279–288, 1993.
34. Campbell DL, Qu Y, Rasmusson RL and Strauss HC, The calcium independent transient outward potassium current in isolated ferret right ventricular myocytes: II Closed state reverse use-dependent block by 4-aminopyridine. *J Gen Physiol* **101**: 603–626, 1993.
35. Thompson S, Aminopyridine block of transient potassium current. *J Gen Physiol* **80**: 1–18, 1982.
36. Freeman SE, Dawson RM, Culvenor AJ and Keeghan AM, Interactions of amantadine with the cardiac muscarinic receptor. *J Mol Cell Cardiol* **17**: 9–21, 1985.
37. Freeman SE and Lau WM, Regulation of potassium channels in guinea-pig atrium by adenosine and potassium channel blocking drugs. *Asia Pacific J Pharmacol* **3**: 149–156, 1988.
38. Ohta M and Narahashi T, Sparteine interaction with nerve membrane potassium conductance. *J Pharmacol Exp Ther* **187**: 47–55, 1973.
39. Zetler G and Strubelt O, Actions of antiarrhythmic drugs on refractory period and contractility of isolated rat and guinea-pig atria. *Naunyn-Schmiedebergs Arch Pharmacol* **271**: 335–345, 1971.