Interaction of potassium channel openers and blockers in canine atrial muscle

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1 The possibility that the interaction between potassium channel openers, e.g. cromakalim, pinacidil and nicorandil, and some potassium channel blockers involves a common site was investigated in canine atrial muscle.

2 Cromakalim, pinacidil and nicorandil produced a negative inotropic effect, their pD_2 ($-\log EC_{50}$) values being 6.11 ± 0.07 , 5.37 ± 0.09 and 4.55 ± 0.07 , respectively.

3 The potassium channel blockers, tetraethylammonium (TEA), tetrabutylammonium (TBA), 3,4-diaminopyridine (DAP), CsCl and BaCl₂ all produced a positive inotropic effect.

4 The concentration-effect curves for the negative inotropic actions of pinacidil were shifted in a parallel way to the right by low concentrations of TEA, TBA or BaCl₂. Maximum responses to pinacidil were depressed by higher concentrations of the blockers. An analysis of the non-competitive antagonism by TEA yielded pK_A ($-\log K_A$) values of 4.00–4.05 for pinacidil.

5 The concentration-effect curves for cromakalim and nicorandil were shifted by TEA similarly to those for pinacidil, and a similar analysis yielded pK_A values of 4.47–4.68 for cromakalim and 3.47–3.74 for nicorandil.

6 The K_A values of cromakalim, pinacidil and nicorandil were about 10–30 times greater than their EC_{50} values, indicating that there are non-linear stimulus-effect relationships between the binding of the three potassium channel openers to their binding sites at potassium channels and their negative inotropic effects.

7 The dissociation constants for TEA could also be estimated from pA_2 and pK_B values for antagonizing competitively and non-competitively the negative inotropic effects of the three potassium channel openers; they were 3.47–3.89, and did not differ between the potassium channel openers.

8 The concentration-effect curves for the three potassium channel openers were not affected by DAP or CsCl.

9 These results suggest the following: (i) quaternary ammonium compounds like TEA and TBA antagonize the negative inotropic effect of cromakalim, pinacidil and nicorandil by binding to potassium channels, thus preventing binding of the channel openers to the same sites or closely related sites in canine right atrial muscles. (ii) The potassium channels responsible for the negative inotropic effects of the three potassium channel openers seem to be akin to apamin-insensitive and calcium-insensitive potassium channels in smooth muscle and are opened at the resting membrane potential.

Introduction

Recently increasing attention has been paid to vasodilator drugs which activate or open potassium channels in vascular smooth muscle and eventually lower blood pressure. These drugs called 'potassium channel activators or openers' include nicorandil (Taira *et al.*, 1979), pinacidil (Arrigoni-Martelli *et al.*, 1980; Bray *et al.*, 1987) and cromakalim (BRL 34915; Hamilton *et al.*, 1986; Weir & Weston, 1986a,b), although nicorandil seems also to have an

additional mechanism of action similar to classical nitrates (Taira, 1987). Potassium channel opening as one of the mechanisms underlying the cardiac action of vasodilator drugs was first suggested for nicorandil by us (Yanagisawa *et al.*, 1979) and also demonstrated by us (Yanagisawa & Taira, 1980). Nonetheless, studies of the cardiac effects of potassium channel openers are scant compared with those of their vasodilator effects. In a previous study (Yanagisawa *et al.*, 1988), we investigated the effects of cromakalim, pinacidil and nicorandil on the force

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of contraction of canine atrial muscles in relation to their effects on cyclic nucleotides levels in canine atrial muscle, because nicorandil had been shown to increase guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels and a possible involvement of an increase in cyclic GMP levels in the negative inotropic effect of nicorandil had been suggested (Endoh & Iijima, 1983). We concluded that these three potassium channel openers reduce the force of contraction in canine atrial muscles exclusively by opening potassium channels. The increases in cyclic GMP were not involved in the negative inotropic effect, because their negative inotropic effects were blocked by the potassium channel blocker, tetraethylammonium (TEA; Coraboeuf & Vassort, 1968; Harder & Sperelakis, 1978), whereas the increase in cyclic GMP levels produced by nicorandil remained unchanged. It has also been shown that in trachealis muscle the relaxant and hyperpolarizing effects of cromakalim are suppressed by potassium channel blockers such as TEA, 4-aminopyridine and procaine but not by apamin (Allen *et al.*, 1986). Thus, it was of interest to know whether the negative inotropic effects of the three potassium channel openers were specifically blocked by potassium channel blockers. If the effects are specifically blocked by a certain class of potassium channel blockers, it is possible that the three potassium channel openers and their specific blockers would interact at the same site or closely related sites at potassium channels. The present experiments were designed to explore this possibility. For this purpose, we investigated how the negative inotropic effects of the three potassium channel openers on the force of contraction in canine atrial muscles could be modified by various potassium channel blockers, TEA, tetrabutylammonium (TBA; French & Shoukimas, 1981), CsCl (Hagiwara *et al.*, 1976; Isenberg, 1976), BaCl₂ (Harder & Sperelakis, 1978; Ehara & Inazawa, 1980), and 3,4-diaminopyridine (DAP). We further analysed the antagonism of TEA and TBA against potassium channel openers by applying Schild analysis (Arunlakshana & Schild, 1959) or procedures devised to analyse non-competitive antagonism (Kenakin, 1987).

Methods

Hearts were excised from mongrel dogs of either sex, (weight 5 to 13 kg) anaesthetized with pentobarbitone sodium (30 mg kg⁻¹ i.v.). Trabecular muscles of the right atrial wall were isolated from the heart in oxygenated cold (ca. 7°C) Krebs-Henseleit solution and mounted in 20 ml organ baths. The composition

(mmol l⁻¹) of the solution was as follows: NaCl 118, NaHCO₃ 24.9, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 2.5, MgSO₄ 1.2, glucose 11.1, ascorbic acid 0.057 and Na₂EDTA, 0.027. The solution was equilibrated with 95% O₂ and 5% CO₂ at a temperature of 37°C (pH 7.4). Muscles were stretched to a resting tension of about 0.5 g and were stimulated by square wave pulses of twice threshold voltage and 5 ms duration at a frequency of 0.5 Hz. During an equilibration period of about 1 h, the length of the muscle was adjusted to that producing maximum contractile force. The force of isometric contraction was recorded on a thermal pen-writing oscillograph (NEC San-ei, Recti-Horiz-8K) by means of strain-gauge transducers (Shinkoh, UL-10230). The concentrations of nicorandil or pinacidil were increased at 5 min intervals and those of cromakalim and nifedipine at 10 or 30 min intervals, respectively. Usually 4–8 muscles were isolated from each heart and run in parallel, one of them being used as control. All of the potassium channel blockers were administered 20 min before the potassium channel openers were applied. All muscles were treated with both 10⁻⁶ M nadolol and 10⁻⁶ M atropine to eliminate possible effects of noradrenaline and acetylcholine released from autonomic nerve terminals by electrical stimuli or indirectly by potassium channel blockers (Harvey & Marshall, 1977; Yanagisawa *et al.*, 1978).

Drugs and chemicals were obtained from the following sources: cromakalim (Beecham Research Laboratories), pinacidil (Shionogi), nicorandil hydrochloride (Chugai), nifedipine (Bayer), tetraethylammonium chloride (TEA, Wako), tetrabutylammonium chloride (TBA, Wako), 3,4-diaminopyridine (DAP, Wako), (±)-nadolol base (Squibb) and atropine sulphate (Wako). Cromakalim was dissolved in 70% ethanol to give a concentration of 10 mmol l⁻¹. Pinacidil was dissolved in 0.1 N HCl to give a concentration of 200 mmol l⁻¹. Nifedipine concentration 289 µmol l⁻¹. Nadolol base was dissolved in 0.5 N HCl to give a concentration of 32 mmol l⁻¹. Other drugs were dissolved in distilled water in the desired concentrations. From these stock solutions the desired concentrations were obtained by diluting with distilled water.

The concentration-negative inotropic effect curves for potassium channel openers were expressed as % reduction in the basal force and computer fitted to a logistic equation:

$$E = M \times A^p / (A^p + K^p)$$

where E is normalized response, M is the maximum response of each drug, A is drug concentration, K is EC₅₀ value of each drug and p is the slope parameter (Parker & Waud, 1971). EC₅₀ values were presented as pD₂ (pD₂ = -log EC₅₀).

The antagonism by TEA and TBA of the negative inotropic effects of the three potassium channel openers was analysed in the following way: Schild analysis (Arunlakshana & Schild, 1959) was performed at the concentration range of TEA (10^{-4} to 10^{-3} M) which produced parallel rightward shifts of the concentration-negative inotropic effect curves and at that of TBA (3×10^{-5} to 3×10^{-4} M) which produced parallel rightward shifts, although the maxima were suppressed only slightly at 3×10^{-4} M TBA. With a concentration of TEA (3×10^{-3} M) which produced a suppression of the maxima of the concentration-negative inotropic effect curves, the procedures were employed which analyse non-competitive antagonism (Kenakin, 1987).

Experimental values are given as mean or mean $\pm$ s.e.mean. Statistical significance of differences between mean values was estimated by Student's *t* test. A *t* test for the paired comparison was used when it was applicable. A *P* value less than 0.05 was considered to be significant.

Results

Antagonism by TEA of the negative inotropic effects of potassium channel openers and nifedipine

As in a previous study (Yanagisawa *et al.*, 1988), cromakalim (10^{-8} to 10^{-4} M), pinacidil (10^{-7} to 3×10^{-4} M) and nicorandil (10^{-6} to 10^{-3} M) caused a concentration-dependent decrease in the force of contraction (up to 90%) in canine right atrial muscles. Nifedipine (10^{-8} to 3×10^{-6} M) produced a negative inotropic effect leading to complete abolition of contractions. Figure 1 shows the modification by TEA of concentration-negative inotropic effect curves for cromakalim, pinacidil, nicorandil and nifedipine. TEA (10^{-4} to 10^{-2} M) *per se* produced a concentration-dependent positive inotropic effect which amounted to 140% at its maximum effect in canine atrial muscles (Table 1), in which the pD_2 value of TEA was 3.33. These concentrations of TEA antagonized the negative inotropic effects of the potassium channel openers and nifedipine (Figure 1, Table 2). However, the mode and magnitude of antagonism by TEA were different between the potassium channel openers and nifedipine. TEA at 10^{-4} to 10^{-3} M shifted the concentration-negative inotropic effect curves for the three potassium channel openers in a parallel fashion to the right; the maxima and slope factors remained unchanged (Figure 1, Table 2). With 10^{-3} to 10^{-2} M TEA, however, the curves were shifted to the right and downward. In contrast TEA only shifted the

concentration-negative inotropic effect curves for nifedipine to the right. The pD_2 values of nifedipine in producing a negative inotropic effect in the absence and presence of 10^{-3} and 10^{-2} M TEA were 7.14 ± 0.08 , 6.78 ± 0.08 and 6.60 ± 0.08 , respectively. Thus, although the shift was indeed produced by TEA, its magnitude was very small and the maxima were not changed.

Quantitative analysis of the antagonism by TEA of the negative inotropic effects of potassium channel openers

Since the concentration-negative inotropic effect curves for the three potassium channel openers were shifted to the right in a parallel way by 10^{-4} to 10^{-3} M TEA, the data obtained with these concentrations of TEA were subjected to Schild analysis. Each Schild regression was linear and had a slope of unity (Figure 2). This analysis yielded pA_2 values of 3.64, 3.47 and 3.66 for TEA against cromakalim, pinacidil and nicorandil, respectively. Since the pA_2 values of TEA were not different for the three potassium channel openers, it seemed that TEA competed for the same binding sites with the three potassium channel openers to antagonize their negative inotropic effect.

TEA at 3×10^{-3} M suppressed the maximum effects of the potassium channel openers (Figure 1, Table 2). To analyse the non-competitive antagonism by TEA, equieffective concentrations of pinacidil were obtained from the concentration-effect curves in the absence and presence of 3×10^{-3} M TEA and the dissociation constant (K_A) of pinacidil and the binding constants (K_B) of TEA were estimated (Figure 3, Table 3). The pK_A ($= -\log K_A$) values of pinacidil estimated in three ways were 4.05, 4.00 and 4.03. These K_A values were about 20 times greater than EC_{50} values of pinacidil (Table 2). The pK_B ($= -\log K_B$) values of TEA estimated in three ways were 3.84, 3.89 and 3.86. Similar analyses were performed for the concentration-effect curves of cromakalim and nicorandil in the absence and presence of 3×10^{-3} M TEA (Table 3). The K_A values determined for cromakalim and nicorandil were also 10 to 30 times greater than their EC_{50} values (Table 3). The pK_B values of TEA against cromakalim and nicorandil were 3.86 and 3.51, respectively. These pK_B values of TEA were very close to the pA_2 values of TEA.

Modification by other potassium channel blockers of the negative inotropic effect of pinacidil

The potassium channel blockers, TBA (3×10^{-5} to 3×10^{-4} M), $BaCl_2$ (3×10^{-4} and 10^{-3} M), DAP (10^{-5} to 3×10^{-3} M) and CsCl (10^{-2} M) all produced a positive inotropic effect in canine atrial muscles,

Figure 1 Modification by tetraethylammonium (TEA) of the concentration-negative inotropic effect curves for (a) cromakalim, (b) pinacidil, (c) nicorandil and (d) nifedipine in canine atrial muscles. (○) Control, (●) 10^{-4} M, (□) 3×10^{-4} M, (■) 10^{-3} M, (▲) 10^{-2} M TEA.

although their potencies and maximum effects differed (Table 1). Figures 4 and 5 show differential modification by these potassium channel blockers of the negative inotropic effect of pinacidil. TBA (3×10^{-5} to 3×10^{-4} M) and BaCl_2 (3×10^{-4} and

10^{-3} M), like TEA, shifted the concentration-effect curves of pinacidil to the right and slightly downward in a concentration-dependent manner (Figure 4, Table 2). Since the suppression of the maximum effect of pinacidil by 3×10^{-4} M TBA was marginal,

Table 1 Positive inotropic effects of various potassium channel blockers in canine atrial muscles

	10^{-5}	3×10^{-5}	10^{-4}	<i>Concentrations (M)</i>		3×10^{-4}	10^{-3}	3×10^{-3}	10^{-2}
TEA			(16) 7.4 ± 2.3	(16)			(28) 137.2 ± 32.7	(16) 171.7 ± 38.9	(19) 142.7 ± 32.2
TBA		(5) 31.3 ± 12.4	(6) 41.1 ± 10.2	(6)					
DAP	(7) 49.2 ± 2.3		(8) 69.7 ± 9.4				(6) 55.1 ± 32.7	(7) 120.6 ± 38.9	
BaCl_2				(5) 35.0 ± 16.3			(5) 75.1 ± 17.6		
CsCl									(17) 99.5 ± 26.9

Positive inotropic effects expressed as % increase above basal force are given as means $\pm$ s.e.mean. Numbers of muscles are given in parentheses.

TEA, tetraethylammonium; TBA, tetrabutylammonium; DAP, 3,4-diaminopyridine.

Table 2 Influences of tetraethylammonium (TEA), tetrabutylammonium (TBA) or BaCl₂ on the negative inotropic effects of potassium channel openers in canine atrial muscles

		Concentrations of potassium channel blockers (M)				
		Control	3 × 10 ⁻⁵	10 ⁻⁴	3 × 10 ⁻⁴	10 ⁻³
TEA						
Cromakalim	(6)			(5)	(5)	(12)
Max	93.7 ± 2.4			94.5 ± 1.5	94.5 ± 0.9	93.5 ± 2.0
pD ₂	6.11 ± 0.07			5.97 ± 0.05	5.74 ± 0.08*	5.27 ± 0.13*
Pinacidil	(17)			(6)	(5)	(5)
Max	87.9 ± 2.1			82.9 ± 4.0	86.9 ± 2.0	75.3 ± 4.6*
pD ₂	5.37 ± 0.09			5.31 ± 0.07	5.09 ± 0.07*	4.57 ± 0.14*
Nicorandil	(12)			(5)	(5)	(5)
Max	91.6 ± 1.8			85.4 ± 3.7	79.3 ± 5.0	64.0 ± 7.2*
pD ₂	4.55 ± 0.07			4.19 ± 0.05*	3.99 ± 0.07*	3.76 ± 0.16*
TBA						
Pinacidil	(4)		(5)	(6)	(6)	
Max	87.7 ± 5.2		80.8 ± 3.6	76.8 ± 3.6	72.8 ± 5.0*	
pD ₂	5.10 ± 0.15		4.75 ± 0.07*	4.26 ± 0.13*	3.67 ± 0.09*	
BaCl ₂						
Pinacidil	(6)				(5)	(5)
Max	88.1 ± 4.7				90.6 ± 2.8	79.7 ± 4.1*
pD ₂	5.31 ± 0.08				4.80 ± 0.11*	4.61 ± 0.19*

Maximum effects (Max) and pD₂ values were obtained by computer fitting to the logistic equation of the concentration-effect curves for cromakalim, pinacidil and nicorandil (see Methods), and are given as means ± s.e.mean.

* *P* < 0.05 compared with values in the absence of potassium channel blockers (control). Numbers of muscles are given in parentheses.

the data obtained with TBA (3 × 10⁻⁵ to 3 × 10⁻⁴ M) were subject to Schild analysis. The analysis yielded a pA₂ value of TBA of 4.70 (Figure 4b). DAP (10⁻⁵ to 3 × 10⁻³ M) and CsCl (10⁻² M) failed to affect the concentration-effect curves of pinacidil (Figures 4 and 5).

No modification by CsCl of the negative inotropic effects of nicorandil and cromakalim

As with pinacidil, the concentration-negative inotropic effect curves for nicorandil and cromakalim in atrial muscles were not modified by 10⁻² M CsCl (Figure 5).

Discussion

As previously observed (Yanagisawa *et al.*, 1988), cromakalim, pinacidil and nicorandil, whose mechanism of action is thought to be the activation or opening of potassium channels in vascular (Weston & Abbot, 1987) and cardiac muscle (Yanagisawa & Taira, 1980; Cain & Metzler, 1985; Iijima & Taira, 1987; Scholtysik, 1987), produced a negative inotropic effect in canine atrial muscles. Their maximum negative inotropic effects were about 90% as against

100% with the calcium channel blocker, nifedipine (Yanagisawa *et al.*, 1988). The mechanism for such negative inotropic effects is thought to be due secondarily to the shortening of the action potential of atrial muscle produced by increased outward potassium currents (Yanagisawa & Taira, 1980). As discussed in our previous article (Yanagisawa *et al.*, 1988), the decreased force of contraction and duration of action potential obtained with nicorandil were consistent with what has previously been demonstrated in voltage-clamp experiments (Morad & Trautwein, 1968).

It is known that potassium channel blockers produce a positive inotropic effect by prolongation of the action potential duration due to blockade of potassium channels in cardiac muscle (Coraboeuf & Vassort, 1968 for TEA; Yanagisawa & Taira, 1979 for 4-aminopyridine; Ehara & Inazawa, 1980 for BaCl₂). Indeed, in the present study all the potassium channel blockers used, TEA, TBA, BaCl₂, DAP and CsCl, produced a positive inotropic effect which, however, differed in magnitude. The negative inotropic effects of three potassium channel openers were antagonized differently by these potassium channel blockers; the negative inotropic effect of pinacidil, which was subject to all of the potassium channel blockers used, was antagonized by TEA, TBA and BaCl₂ but not by DAP and CsCl. Thus,

Figure 2 Schild plots of the antagonism of the negative inotropic effects of (a) cromakalim, (b) pinacidil and (c) nicorandil by tetraethylammonium (TEA) in canine atrial muscles. (a) Cromakalim: pA_2 value, 3.64; the slope of regression line, -1.07 ; the correlation coefficient, -0.78 ($n = 21$). (b) Pinacidil: pA_2 value, 3.47; the slope of regression line, -1.00 ; the correlation coefficient, -0.85 ($n = 16$). (c) Nicorandil: pA_2 value, 3.66; the slope of regression line, -0.88 ; the correlation coefficient, -0.87 ($n = 15$).

Table 3 Dissociation constants (K_A), pD_2 values and ratios of K_A to EC_{50} of the three potassium channel openers and the binding constants (K_B) and pA_2 values of tetraethylammonium (TEA)

	Potassium channel openers			TEA	
	pK_A	pD_2	K_A/EC_{50}	pK_B	pA_2
Cromakalim	4.68			3.78	
	4.47	6.11	35	3.86	3.64
	4.57			3.86	
Pinacidil	4.05			3.84	
	4.00	5.37	22	3.89	3.47
	4.03			3.86	
Nicorandil	3.74			3.47	
	3.47	4.55	9	3.65	3.66
	3.58			3.51	

K_A of the three potassium channel openers and K_B of TEA were estimated by the analysis of non-competitive antagonism in three ways. The pA_2 values of TEA were obtained by Schild analysis (Figure 2). Ratios of K_A (mean of the values) to the EC_{50} (pD_2 ; Table 2) of the potassium channel openers indicate that there are nonlinear stimulus-effect relationships between the binding of the three potassium channel openers to their binding sites and their negative inotropic effects.

the antagonism by TEA, TBA or $BaCl_2$ of the negative inotropic effect of pinacidil, and that by TEA of the negative inotropic effects of cromakalim and nicorandil, should not simply be ascribed to functional antagonism between the positive inotropic effects produced by potassium channel blockade on the one hand and the negative inotropic effect caused by potassium channel opening on the other. Indeed, the specific antagonism between TEA and the three potassium channel openers and that between TBA and pinacidil suggests that TEA and TBA interact with the same or closely related sites of actions as the potassium channel openers. In the present study Schild analysis applied to the concentration range of TEA (10^{-4} to 10^{-3} M) which produced rightward parallel shifts of the concentration-negative inotropic effect curves for the three potassium channel openers yielded pA_2 values of 3.47–3.66 for TEA which were not different for the three potassium channel openers (Figure 2 and Table 3). Analysis of the non-competitive antagonism by the high concentration of TEA (3×10^{-3} M) of the concentration-effect curves of the three potassium channel openers yielded pK_B values of 3.47–3.89 for TEA which were also not different for the three potassium channel openers (Table 3). Thus, the dissociation constants for TEA were determined to be 1.3 – 3.4×10^{-4} M. The values were close to EC_{50} values (4.7×10^{-4} M; $pD_2 = 3.33$) which caused a positive inotropic effect but far lower than the concentrations of TEA usually used

Figure 3 The non-competitive antagonism of the negative inotropic effects of pinacidil by tetraethylammonium (TEA, 3×10^{-3} M) in canine atrial muscles. (a) Concentration-negative inotropic effect curves for pinacidil in the absence (○) and presence (●) of 3×10^{-3} M TEA. Equieffective concentrations of pinacidil expressed by horizontal lines were obtained from the logistic equation of the concentration-effect curves for pinacidil in the absence and presence of 3×10^{-3} M TEA. (b) Double-reciprocal plot of equieffective concentrations of pinacidil in the absence (ordinate scale, $1/[A]$) and presence (abscissa scale, $1/[A']$) of 3×10^{-3} M TEA. Slope, 21.8; intercept, 2.70×10^5 ; dissociation constant (K_A) of pinacidil, 8.9×10^{-5} M; dissociation constant (K_B) of TEA, 1.45×10^{-4} M. (c) Regression of $[A']/[A]$ vs. $[A']$. Slope, 2.43×10^5 ; intercept, 24.2; dissociation constant (K_A) of pinacidil, 9.9×10^{-5} M; dissociation constant (K_B) of TEA, 1.29×10^{-4} M. (d) Regression $[A]/[A']$ vs. $[A]$. Slope, -89.7 ; intercept, 3.94×10^{-6} ; dissociation constant (K_A) of pinacidil, 9.4×10^{-5} M; dissociation constant (K_B) of TEA, 1.38×10^{-4} M.

(more than 10^{-2} M) to block potassium conductance by external application. The dissociation constants of TEA determined in the present study were also close to those (K_D) for the reaction between TEA and its receptors to inhibit potassium conductance ($K_D = 4 \times 10^{-4}$ M, $-\log K_D = 3.40$) in the frog node or molluscan neurone (Stanfield, 1983). TBA was about 10 times more potent than TEA in shifting the concentration-effect curves of pinacidil to the right. A similar difference in potency between TEA and TBA has been obtained for blockade of potassium channels in neurones of various species (French & Shoukimas, 1981; Stanfield, 1983). Thus, the binding of quaternary ammonium compounds to their binding sites at potassium channels could preclude

potassium channel openers from binding to the same sites or closely related sites.

The analysis of non-competitive antagonism by TEA yielded dissociation constants (K_A) of 8.9 – 9.9×10^{-5} M ($-\log K_A = 4.0$ – 4.1) for pinacidil (Figure 3, Table 3) which were about 20 times greater than the EC_{50} of the negative inotropic effect of pinacidil ($4.3 \pm 0.1 \times 10^{-6}$ M, $pD_2 = 5.37 \pm 0.09$; Table 2). There were also similar differences between K_A values and EC_{50} values for cromakalim and nicorandil (Table 3). These results suggest that there is nonlinear stimulus-effect relationships between the binding of the three potassium channel openers to their binding sites at potassium channels and their negative inotropic effects. Thus, the possibility that

Figure 4 Differential modification by (a) tetrabutylammonium (TBA), (c) BaCl_2 and (d) 3,4-diaminopyridine (DAP) of the concentration-negative inotropic effect curves for pinacidil in canine atrial muscle. Each point represents mean value of 4–8 muscles. (a) (○) Control, (●) $3 \times 10^{-5} \text{ M}$, (□) 10^{-4} M , (■) $3 \times 10^{-4} \text{ M}$ TBA. (b) Schild plot of the antagonism of the negative inotropic effects of pinacidil by TBA in canine atrial muscles. pA_2 value, 4.70; the slope of regression line, -1.17 ; the correlation coefficient, -0.93 ($n = 17$). (c) (○) Control, (●) $3 \times 10^{-4} \text{ M}$, (□) 10^{-3} M BaCl_2 . (d) (○) Control, (●) 10^{-5} M , (□) 10^{-4} M , (■) 10^{-3} M , (Δ) $3 \times 10^{-3} \text{ M}$ DAP.

the three potassium channel openers bind to only a fraction of these channels to produce their negative inotropic effects seems to be expressed by the preclu-

sion of potassium channel openers from binding to the same channels previously bound by TEA.

Specific antagonism of the negative inotropic effects of the three potassium channel openers by quaternary ammonium compounds, demonstrated in the present study, is consistent with the finding that in guinea-pig trachealis muscle the relaxant and hyperpolarizing effects of cromakalim are suppressed by TEA (Allen *et al.*, 1986). The failure of DAP, a congener of 4-aminopyridine (4-AP, Harvey & Marshall, 1977), to block the negative inotropic effect of pinacidil in the present study, however, is inconsistent with the finding that in trachealis muscle the effects of cromakalim were suppressed by 4-AP. In cardiac muscles, 4-AP has been shown to block the transient outward current which is activated partly by an increase in intracellular Ca (Kenyon & Gibbons, 1979; Simurda *et al.*, 1988). The failure of DAP to antagonize the negative inotropic effects of pinacidil suggests that the potassium channels opened by pinacidil are different from channels for 4-AP- and DAP-sensitive transient outward current.

Figure 5 Negative inotropic effects of cromakalim (○, ●), pinacidil (□, ■) and nicorandil (Δ, ▲) in canine atrial muscle in the absence (○, □, Δ) and presence (●, ■, ▲) of 10^{-2} M CsCl. Each point represents mean value of 5–6 muscles.

Although quaternary ammonium compounds are known to block potassium currents in cardiac muscle (Coraboeuf & Vassort, 1968), the kind of potassium channels they block has not been elucidated; because they are usually effective when applied internally (Stanfield, 1983). In the guinea-pig superior mesenteric artery both BaCl₂ and TEA are known to reduce resting potassium conductance (Harder & Sperelakis, 1978). As is the case with quaternary ammonium compounds, the kind of potassium channels opened by the potassium channel openers in cardiac sarcolemma has not been elucidated; opening of the inward rectifying potassium channels (I_{K1} channels) (Taira, 1987) and opening of a new class of potassium channels (Kakei *et al.*, 1986) are currently suggested. In intestinal smooth muscle of the guinea-pig, nicorandil has been shown to activate apamin-insensitive and Ca-insensitive potassium channels (Yamanaka *et al.*, 1985). In the guinea-pig taenia caeci, too, cromakalim and nicorandil are capable of opening apamin-insensitive potassium channels (Weir & Weston, 1986a). Thus, the potassium channels in canine atrial muscles opened by cromakalim, pinacidil and nicorandil seem to be akin to apamin-insensitive and Ca-

insensitive channels in smooth muscles and to be opened at the resting membrane potential by these drugs (Kakei *et al.*, 1986; Iijima & Taira, 1987). The binding of these potassium channel openers to their binding sites at these potassium channels seems to be modified either directly or indirectly by quaternary ammonium compounds, like TEA and TBA.

In the present study CsCl was entirely ineffective in antagonizing the negative inotropic effects of the three potassium channel openers. This is understandable since potassium channel openers are effective when potassium ions are present, even if caesium ions as an impermeable potassium ion substitute (Hagiwara *et al.*, 1976) are included in the medium.

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