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Increase in the vasorelaxant potency of K_{ATP} channel opening drugs by adenosine A_1 and A_2 receptors in the pig coronary artery

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Abstract

Myograph recording from ring segments of pig small coronary arteries was used to investigate the effects of adenosine receptor activation on the vasorelaxant potency of ATP-sensitive K^+ channel opening drugs. Receptor activation with 2-chloroadenosine (2-CA, 300 nM) increased the potency of both nicorandil and levcromakalim, shifting the pEC_{50}s from 4.68 ± 0.03 to 5.05 ± 0.04 and from 6.34 ± 0.06 to 6.72 ± 0.06, respectively ($P < 0.05$ in each case). Experiments with selective adenosine receptor agonists (2-chloro-N^6-cyclopentyladenosine (CCPA), 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680)) and antagonists (8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*] [1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385)) suggest that both A_1 and A_{2a} receptors can increase the potency of nicorandil, while that of levcromakalim is increased only by A_2 receptors. Adenosine receptor activation did not affect the potency of pinacidil. Thus, adenosine receptor activation can increase the potency of some K^+ channel opening drugs to relax coronary arteries, but the details of the interaction with adenosine receptors depend on the particular drug.

Keywords: Coronary artery; Adenosine; K^+ channel; Nicorandil

1. Introduction

Compounds such as nicorandil, levcromakalim, and pinacidil that open ATP-sensitive K^+ channels (K_{ATP} channels) in arterial smooth muscle cells are potent vasodilators, acting by causing hyperpolarization of the cell membrane and a consequent decrease in Ca^{2+} entry and so intracellular calcium. In addition to being targets for these K^+ channel opening drugs, K_{ATP} channels are regulated by a number of physiological vasoactive agents, and their regulation is such that they respond to the metabolic state of the tissue in which they lie, being activated under conditions of metabolic compromise, as in hypoxia or ischaemia (Quayle et al., 1997). Because of this metabolic sensitivity, certain K_{ATP} channel opening drugs are more potent as vasodilators under conditions of hypoxia or ischaemia. Metabolic inhibition or hypoxia increases the potency of levcromakalim in the rabbit ear artery (Randall and Griffith, 1993; Randall et al., 1994), and we have shown recently that metabolic inhibition also enhances the potency of nicorandil, which is used clinically for the treatment of angina, in both pig coronary and rat mesenteric small arteries (Davie and Standen, 1998; Davie et al., 1998). The increased potency of nicorandil in metabolically compromised tissue may have consequences for its effectiveness in the treatment of angina, since it might result in nicorandil exerting its maximum vasodilator effect on ischaemic regions within the heart.

In the coronary circulation, adenosine, released from cardiac myocytes in proportion to their metabolic activity, is thought to act as a vasodilator signal that matches blood delivery to the metabolic needs of the cardiac tissue (Berne, 1980; Bardenheuer and Schrader, 1986). Adenosine receptor stimulation can activate K_{ATP} channels of coronary arterial smooth muscle (Merkel et al., 1992; Dart and Standen, 1993), and activation of such channels by adenosine contributes to both resting and hypoxic vasodilation in the coronary circulation (Nakhostine and Lamontagne, 1993; Randall, 1995). Activation of adenosine receptors is also effective at increasing the vasorelaxant potency of nicorandil in pig coronary arteries (Davie and Standen, 1998).

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Adenosine receptors can be subdivided into several subtypes that differ in their molecular structure and pharmacology (Fredholm et al., 1994) and both A_1 and A_2 adenosine receptors have been identified in smooth muscle (Mills and Gewirtz, 1990). Both A_1 and A_2 adenosine receptors have been proposed to be involved in the activation of K_{ATP} channels in arterial smooth muscle of various vessels (Merkel et al., 1992; Dart and Standen, 1993; Akatsuka et al., 1994; Kleppisch and Nelson, 1995; Mutafova-Yambolieva and Keef, 1997). However, investigation of the type of adenosine receptor associated with enhanced potency of K^+ channel openers has been restricted to a study on the rabbit ear circulation by Randall et al. (1994), who found evidence that A_1 receptors were responsible for the enhanced potency of levcromakalim.

In the present study we have used subtype-selective adenosine receptor agonists and antagonists to characterize the adenosine receptors that affect the potency of K^+ channel openers in relaxing rings from pig coronary arteries. Our results show that both A_1 and A_{2a} receptor activation increased the potency of nicorandil, while that of levcromakalim was increased only by activation of A_2 receptors. The potency of pinacidil was unaffected by adenosine receptor activation.

2. Methods

2.1. Myography

Pig hearts were obtained from a local abattoir, after animals had been slaughtered in accordance with EEC regulations, and second and third order branches (mean outer diameter 303.3 ± 30.7 μm, $n = 82$) were dissected from the left anterior descending coronary artery and cut into 2-mm ring segments. Dissection was done in ice cold saline containing (in mM): 125 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.4 with NaOH. Two rings were mounted in a small vessel myograph (Mulvany and Halpern, 1976; Mulvany et al., 1980). The rings were placed in a 10-ml bath and maintained at 37°C. All chemicals were added directly to the bath. All solutions contained 20 μM L-*N*ω-nitro-L-arginine methyl ester (L-NAME) to eliminate endogenous nitric oxide activity. In agreement with the results of White and Angus (1987) using dog small coronary arteries, we found that pig small coronary arteries did not give steady contractions to the thromboxane A_2 mimetic U46619. To contract vessels, we therefore used a solution containing 200 nM of the Ca^{2+} channel agonist BAYK 8644 and in which $[K^+]$ was increased to 20 mM. As described previously (Davie and Standen, 1998), this solution gave a very stable contraction while keeping the K^+ equilibrium potential sufficiently negative for K^+ channel activation to cause hyperpolarization and relaxation. The solution contained 200 nM BAYK 8644 in either 20 mM K^+ saline (in mM: 125 NaCl, 20 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.4 with NaOH) or 20 mM K^+ sucrose saline (in mM: 95 NaCl, 20 KCl, 60 sucrose, 10 HEPES, 10 glucose, 1.8 $CaCl_2$, 1 $MgCl_2$, pH 7.4 with NaOH). Similar results were obtained with either solution, and so data with both solutions were pooled. The maximal tonic force achieved was 22.4 ± 2.4 mN and all measurements were calculated as the reduction in this tone. Once a stable level of contraction had been reached, cumulative concentration–response curves to nicorandil (1–300 μM), levcromakalim (10 nM–10 μM) and pinacidil (10 nM–30 μM) were measured. 2-Chloroadenosine (2-CA, 300 nM), 2-chloro-N^6-cyclopentyladenosine (CCPA, 100 nM) and 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680, 100 nM) were applied prior to the measurement of the cumulative concentration–response curve. Similarly, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*] [1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385) were added to the bath with 300 nM 2-CA to give final concentrations of 100 nM.

2.2. Data analysis

The decrease in tone (relaxation) was measured from the maximal contraction generated with 20K/BAYK. The difference between the maximum tone and the baseline just prior to the contraction was calculated and used to convert the values to a percentage relaxation. Individual concentration–response curves were fitted with the expression

$$y = M\left[1 + (k/x)^H\right]^{-1} \quad (1)$$

where y is the percent relaxation, x is the K^+ channel opener concentration, M is the maximum response, k is the EC_{50} for the channel opener, and H is the Hill coefficient, using the least squares algorithm in Sigmaplot (Jandel Scientific). EC_{50} values were obtained as the concentration at which half maximal reduction in tone occurred, and pEC_{50}s calculated as $-\log EC_{50}\,(M)$. pEC_{50} values are given throughout as mean ± S.E.M. Statistical significance was assessed using Student's *t*-test for simple comparisons, and analysis of variance (ANOVA) with Duncan's post hoc test for multiple comparisons.

2.3. Drugs

Nicorandil was a gift from Rhône-Poulenc Rorer and levcromakalim a gift from SmithKline Beecham. Sources of other drugs were as follows: pinacidil (Sigma); 2-CA, BAYK 8644, CGS 21680 HCl and CCPA (RBI); DPCPX and ZM 241385 (Tocris).

3. Results

3.1. Adenosine receptor activation

Fig. 1 illustrates the effect of 2-CA, a stable analogue of adenosine that acts at both A_1 and A_2 adenosine receptors (Fredholm et al., 1994), on the vasorelaxant actions of nicorandil, levcromaklim and pinacidil in pig coronary arterial rings. Under control conditions all three K^+ channel openers produced concentration dependent relaxations with pEC_{50}s of 4.68 ± 0.03, 6.34 ± 0.06 and 6.30 ± 0.09 for nicorandil, levcromakalim and pinacidil, respectively. The calculated Hill coefficients for nicorandil, levcromakalim and pinacidil were 3.8 ± 0.3, 1.3 ± 0.3 and 1.8 ± 0.2, respectively.

Addition of 2-CA (300 nM) caused a small reduction of 19.4 ± 3.0% of the maximal contractile force achieved by 20K/BAYK. Fig. 1A shows that nicorandil was more potent at causing relaxation in the presence of 2-CA, its pEC_{50} being increased to 5.05 ± 0.04 (Table 1). Similarly, 2-CA enhanced the potency of levcromakalin (Fig. 1B), raising the pEC_{50} for levcromakalim to 6.72 ± 0.06 (Table 2). However, Fig. 1C and Table 3 show that 2-CA did not affect the potency of pinacidil.

3.2. A_1 and A_2 receptor agonists

3.2.1. CCPA (A_1)

The A_1 adenosine receptor agonist CCPA shows approximately 10,000-fold selectivity for A_1 receptors compared to A_2 receptors in striatal membranes from rat brain (Lohse et al., 1988). CCPA has been reported to activate glibenclamide-sensitive K_{ATP} currents in cells isolated from the pig coronary artery, but to be without effect in rabbit mesenteric arterial cells (Dart and Standen, 1993; Kleppisch and Nelson, 1995).

CCPA caused a small reduction, of less than 10%, in the maximal force produced by 20K/BAYK. Fig. 2A and Table 1 show that 100 nM CCPA caused a significant increase in the potency of nicorandil, raising its pEC_{50} to 5.01 ± 0.08. In contrast, 100 nM CCPA did not effect the potency of levcromakalim (Fig. 2B, Table 2). The pEC_{50}s for levcromakalim in the presence of CCPA was 6.30 ±

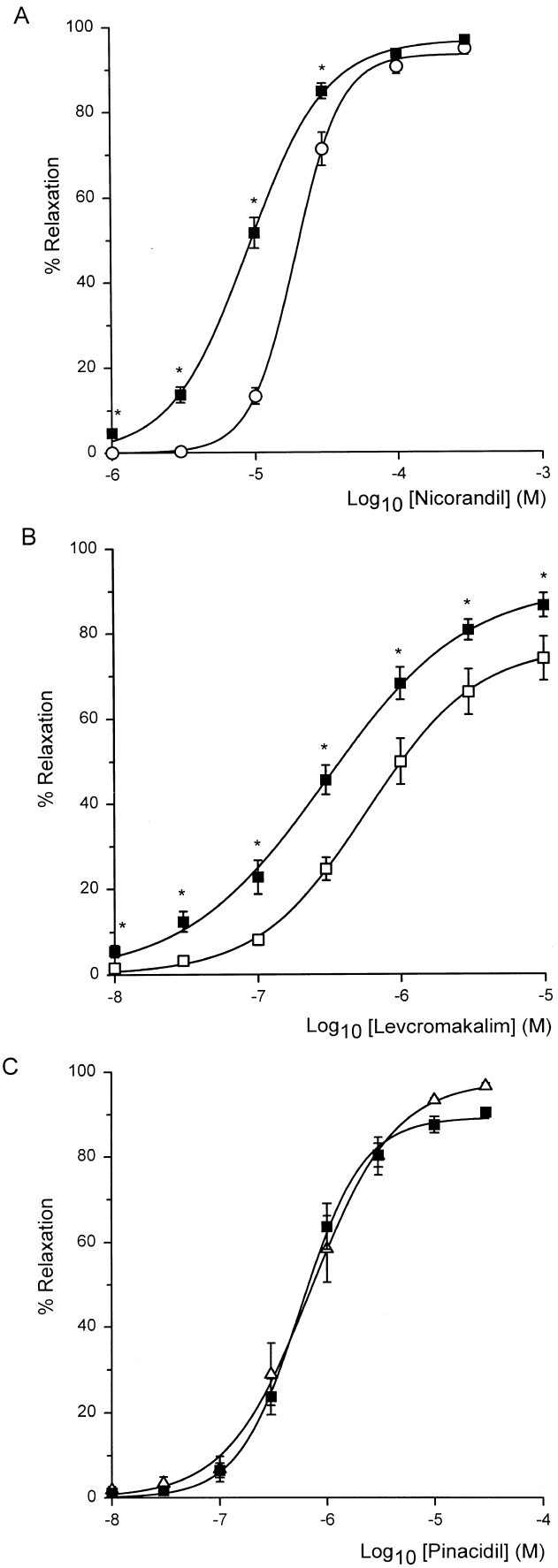

Fig. 1. Effect of 2-CA on the vasorelaxant action of nicorandil, levcromakalim and pinacidil. (A) Concentration–response curves for nicorandil under control conditions (○; $n = 20$) and in the presence of 300 nM 2-CA (■; $n = 18$). Pig coronary arterial rings were contracted with 20K/BAYK as described in the text, and the points show mean ± S.E.M. * Indicates in this figure and in Fig. 1B responses which are significantly different ($P < 0.05$) from those under control conditions. The curves were drawn using Eq. (1). (B) Concentration–response curves for the relaxation of pig coronary arterial rings by levcromakalim under control conditions (□; $n = 7$), and in the presence of 300 nM 2-CA (■; $n = 7$). (C) Concentration–response curves for pinacidil under control conditions (△; $n = 6$) and in the presence of 300 nM 2-CA (■; $n = 6$).

Table 1
$p\mathrm{EC}_{50}$s and Hill coefficient (H) values for nicorandil relaxation of 20K/BAYK contractions under control conditions and in the presence of 2-CA (300 nM), CCPA (100 nM) and CGS 21680 (100 nM). $p\mathrm{EC}_{50}$ and H values are also given for nicorandil relaxation in the presence of 2-CA with DPCPX (100 nM), ZM 241385 (100 nM), or both. Values are given as mean ± S.E.M.

	$p\mathrm{EC}_{50}$ nicorandil	H	n
Control	4.68 ± 0.03	3.8 ± 0.3	39
2-CA	5.05 ± 0.04*	2.0 ± 0.1	25
CCPA	5.01 ± 0.08*	2.2 ± 0.2	8
CGS 21680	5.10 ± 0.07*	2.5 ± 0.4	6
2-CA + DPCPX	4.82 ± 0.07	2.9 ± 0.5	6
2-CA + ZM 241385	4.62 ± 0.12	2.4 ± 0.3	7
2-CA + DPCPX + ZM 241385	4.71 ± 0.04	2.5 ± 0.2	5

*$P < 0.05$ compared to control, ANOVA followed by Duncan's multiple range test.

0.05 ($P = 0.54$ vs. control). Consistent with the lack of effect of 2-CA, CCPA also had no effect on the concentration–response curves for pinacidil (Table 3).

3.2.2. CGS 21680 (A_{2a})

CGS 21680 is a selective A_{2a} adenosine receptor agonist, showing about 170-fold selectivity for A_{2a} over A_1 receptors and over 5000-fold selectivity for A_{2a} over A_{2b} receptors (Collis and Hourani, 1993; Fredholm et al., 1994). CGS 21680 has been reported to activate K_{ATP} currents in rabbit mesenteric arterial smooth muscle (Kleppisch and Nelson, 1995). In our experiments, the initial addition of CGS 21680 caused a decrease of 29.3 ± 4.7% of the maximal contraction elicited with 20K/BAYK. Fig. 3 shows the effect of 100 nM CGS 21680 on concentration–response curves to nicorandil and levcromakalim. CGS 21680 increased the potency of nicorandil, raising its $p\mathrm{EC}_{50}$ to 5.10 ± 0.07 (Fig. 3A). Fig. 3B and Table 2 show that CGS 21680 also caused a significant increase in the potency of levcromakalim. Finally, CGS 21680 did not affect the potency of pinacidil (Table 3).

Table 2
Values of $p\mathrm{EC}_{50}$ and Hill coefficient (H) for levcromakalim relaxation of 20K/BAYK contractions under control conditions and in the presence of 2-CA (300 nM), CCPA (100 nM) and CGS 21680 (100 nM). $p\mathrm{EC}_{50}$ and H values are also given for levcromakalim relaxation in the presence of 2-CA and either DPCPX (100 nM) or ZM 241385 (100 nM), and for relaxation in the combined presence of CGS 21680 (100 nM) and ZM 241385 (100 nM). Values are given as mean ± S.E.M.

	$p\mathrm{EC}_{50}$ levcromakalim	H	n
Control	6.34 ± 0.06	1.3 ± 0.3	21
2-CA	6.72 ± 0.06*	1.4 ± 0.1	19
CCPA	6.30 ± 0.05	1.2 ± 0.1	7
CGS 21680	6.68 ± 0.10*	1.3 ± 0.3	9
2-CA + DPCPX	6.63 ± 0.06*	1.7 ± 0.2	5
2-CA + ZM 241385	6.76 ± 0.07*	1.6 ± 0.1	5
CGS 21680 + ZM 241385	6.44 ± 0.05	1.5 ± 0.8	5

*$P < 0.05$ compared to control, ANOVA followed by Duncan's multiple range test.

Table 3
Values of $p\mathrm{EC}_{50}$ and Hill coefficient (H) for pinacidil relaxation of 20K/BAYK contractions under control conditions and in the presence of 2-CA (300 nM), CCPA (100 nM) and CGS 21680 (100 nM). Values are given as mean ± S.E.M. There is no significant difference between groups at the 0.05 level, ANOVA.

	$p\mathrm{EC}_{50}$ pinacidil	H	n
Control	6.30 ± 0.09	1.8 ± 0.2	15
2-CA	6.30 ± 0.07	1.9 ± 0.2	6
CCPA	6.15 ± 0.13	2.0 ± 0.4	5
CGS 21680	6.41 ± 0.16	1.2 ± 0.2	4

3.3. Adenosine receptor antagonists

To investigate further the receptor subtype involved in enhancing the potency of the K_{ATP} channel openers in pig small coronary arteries, we tested the effects of selective A_1 and A_2 adenosine receptor antagonists on the increase in vasorelaxant potency of nicorandil and levcromakalim induced by 2-CA.

3.3.1. DPCPX

We used the A_1 adenosine receptor antagonist DPCPX which has a 500-fold greater selectivity for the A_1 than A_2 adenosine receptor (Halleen et al., 1987). In six different preparations 100 nM DPCPX prevented the increase in potency of nicorandil produced with 2-CA (Fig. 4A), lowering the $p\mathrm{EC}_{50}$ value to 4.82 ± 0.07 (Table 1). In contrast, the antagonist had no significant effect on the 2-CA enhancement of the potency of levcromakalim (Fig. 4B). The $p\mathrm{EC}_{50}$ values were 6.72 ± 0.06 and 6.63 ± 0.06 in the presence of 2-CA alone and in 2-CA + DPCPX, respectively ($P = 0.28$, Table 2).

3.3.2. ZM 241385

We also studied the effect of the selective A_{2a} adenosine receptor antagonist ZM 241385 on the enhancement of potency of nicorandil and levcromakalim produced by 2-CA. This antagonist shows a 100-fold greater selectivity for the A_{2a} adenosine receptor subtype compared to the A_{2b} adenosine receptor, and a 1000-fold selectivity compared to the A_1 adenosine receptor (Keddie et al., 1996). As Fig. 5A illustrates, the increase in potency of nicorandil elicited by 2-CA was inhibited by 100 nM ZM 241385, decreasing the $p\mathrm{EC}_{50}$ to 4.62 ± 0.12 (Table 1). Simultaneous application of both DPCPX and ZM 241385 exerted no greater effect than did either antagonist alone (Table 1). Surprisingly, ZM 241385 had no effect on the 2-CA enhancement of the potency of levcromakalim, the $p\mathrm{EC}_{50}$ for levcromakalim remaining high at 6.76 ± 0.07, not significantly different from that in the presence of 2-CA alone (Table 2). Fig. 5B shows that ZM 241385 was, however, able to inhibit the enhancement of levcromakalim potency produced by the A_{2a} agonist CGS 21680. The $p\mathrm{EC}_{50}$s for levcromakalim in the presence of CGS 21680 alone and in

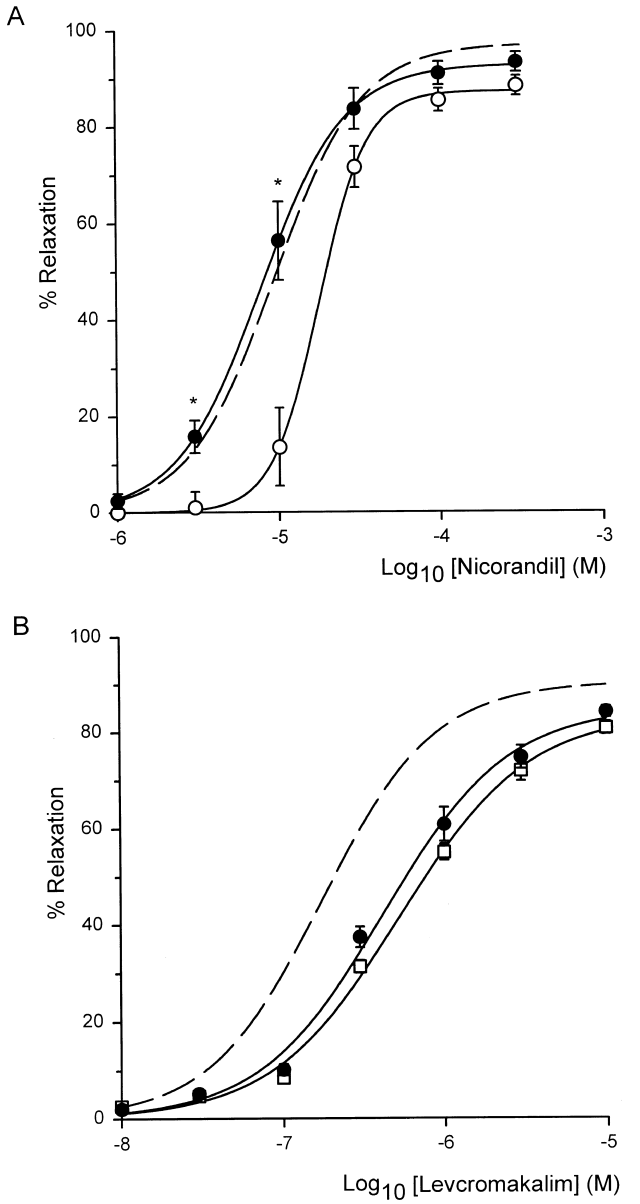

Fig. 2. Effect of CCPA on the potency of nicorandil and levcromakalim. (A) Concentration–response curves for the relaxation of pig coronary arterial rings by nicorandil under control conditions (○; $n = 8$) and in the presence of 100 nM CCPA (●; $n = 7$). The broken line shows the mean nicorandil + 2-CA concentration–response curve for comparison. Rings were contracted using 20K/BAYK and the points show mean ± S.E.M. * Indicates responses significantly different ($P < 0.05$) from those under control conditions. The curves were drawn using Eq. (1). (B) Concentration–response curves for levcromakalim relaxation in the absence (□; $n = 5$) and presence of (●; $n = 5$) of 100 nM CCPA. The broken line represents the levcromakalim + 2-CA response curve for comparison.

CGS 21680 plus ZM 241385 were 6.76 ± 0.10 and 6.44 ± 0.05 (Fig. 5B, Table 2).

4. Discussion

Our results show that the potencies of both nicorandil and levcromakalim to relax the pig small coronary artery were enhanced by activation of adenosine receptors with 2-CA. However, adenosine receptor activation did not affect the potency of another K^+ channel opener, pinacidil. The finding for nicorandil is in agreement with our previ-

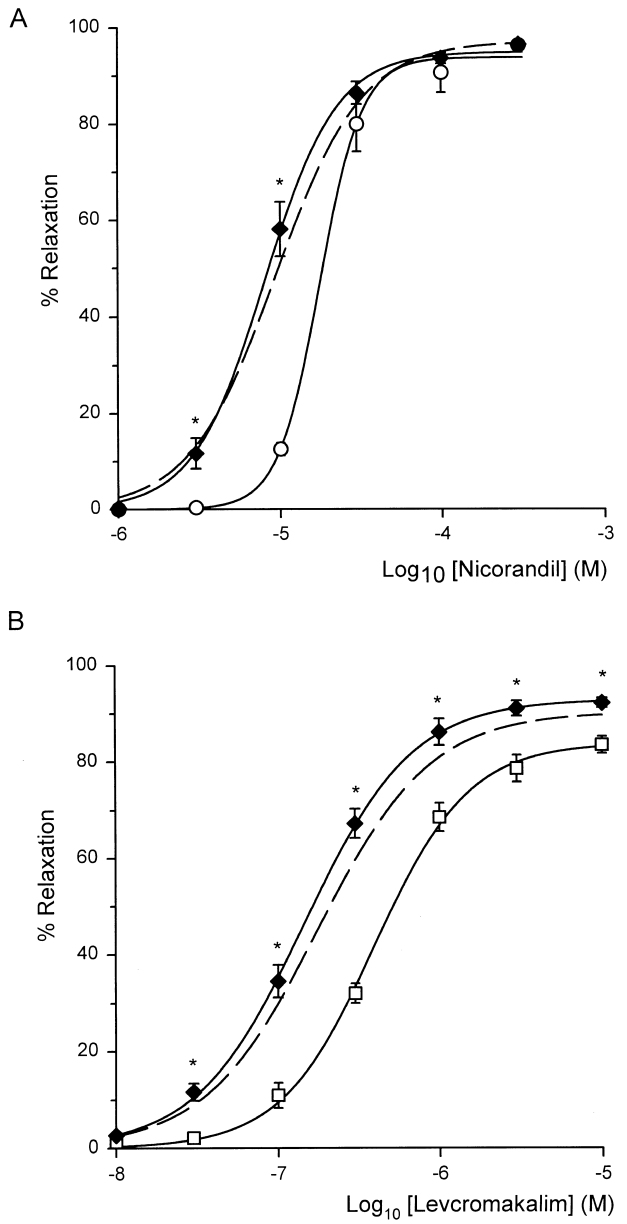

Fig. 3. Increase in the potency of nicorandil and levcromakalim by CGS 21680. (A) Concentration–response curves for nicorandil relaxations of 20K/BAYK induced contractions in the absence (○; $n = 6$) and presence (♦; $n = 6$) of 100 nM CGS 21680. The points show mean ± S.E.M., and curves were drawn using Eq. (1). The broken line shows the nicorandil + 2-CA response for comparison. (B) Concentration–response curves for levcromakalim under control conditions (□; $n = 9$) and in the presence of 100 nM CGS 21680 (♦; $n = 9$). Rings were contracted with 20K/BAYK. The broken line represents the mean levcromakalim + 2-CA curve for comparison.

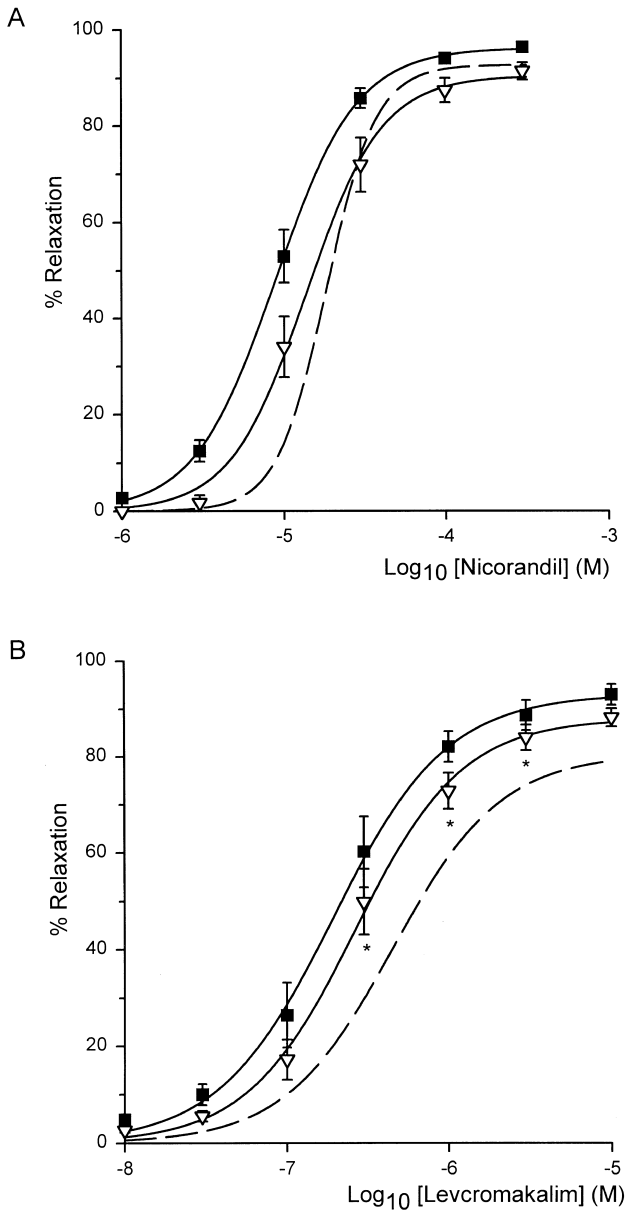

Fig. 4. Nicorandil and levcromakalim relaxations in the presence of DPCPX. (A) Concentration–response curves for nicorandil in the presence of 2-CA alone (■; $n = 6$) and 2-CA + 100 nM DPCPX (▽; $n = 6$). The broken line shows the mean control concentration–response curve for comparison. Arterial rings were contracted using 20K/BAYK, and the points represent mean ± S.E.M. The curves were drawn using Eq. (1). (B) Concentration–response curves for the relaxation of pig coronary arteries by levcromakalim in the presence of 2-CA alone (■; $n = 7$) and 2-CA + 100 nM DPCPX (▽; $n = 7$). The broken line is the mean control concentration–response curve. * Indicates responses significantly different ($P < 0.05$) from those under control conditions.

ous results on the pig coronary artery (Davie and Standen, 1998), while the results with levcromakalim and pinacidil are consistent with those of Randall et al. (1994) in the rabbit ear. The effects of adenosine receptor activation appear to occur through a mechanism independent of endogenous NO, since all experiments were done in the presence of L-NAME to inhibit NO synthesis. Adenosine receptor activation caused a decrease in contractile tone, but this does not appear to account for the observed increase in potency of K_{ATP} channel opening drugs, since such increases have been reported when initial tone is kept

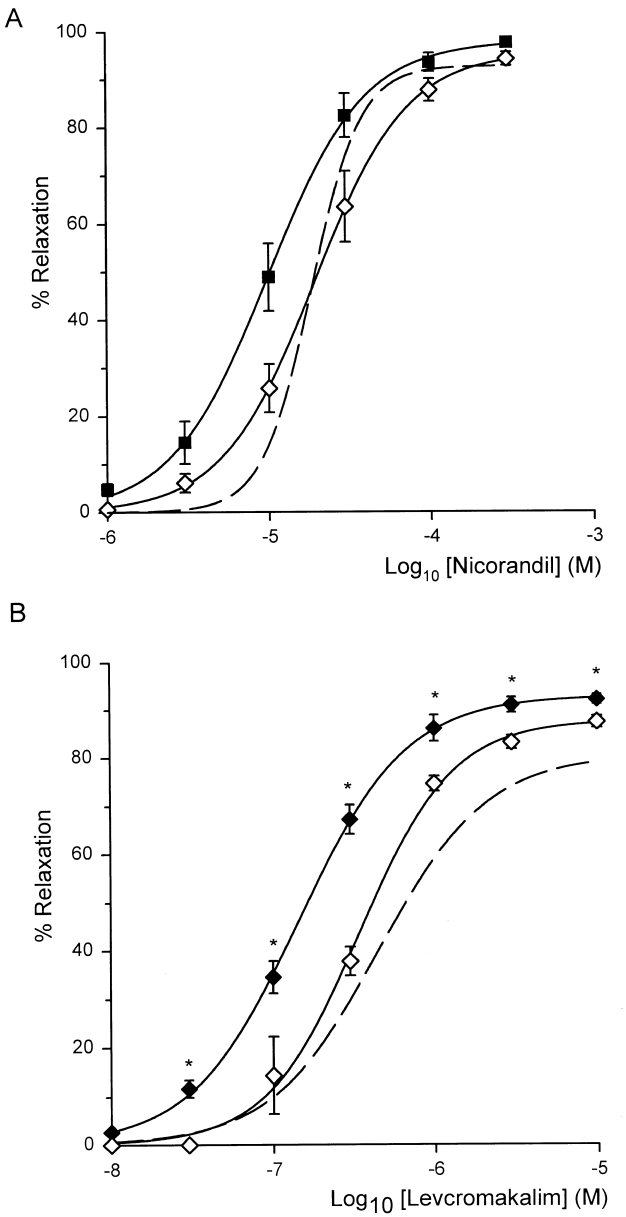

Fig. 5. Effect of ZM 241385 on nicorandil and levcromakalim response curves. (A) Nicorandil concentration–response curves in the presence of 2-CA alone (■; $n = 7$) or 2-CA and 100 nM ZM 241385 (◇; $n = 7$). The broken line represents the mean control concentration–response curve. (B) Levcromakalim concentration–response curves in the presence of CGS 21680 alone (◆; $n = 10$) or CGS 21680 + 100 nM ZM 241385 (◇; $n = 5$). Rings were contracted with 20K/BAYK, and the points represent mean ± S.E.M. The curves were drawn using Eq. (1). The broken line shows the mean control concentration–response curve for comparison. * Indicates values significantly different ($P < 0.05$) from those under control conditions.

constant by alteration of the concentration of contractile agonist (Randall et al., 1994), while we have shown that, in spite of the decreased initial tone, adenosine receptor activation did not affect the potency of the nitrovasodilator glyceryl trinitrate (Davie and Standen, 1998).

Both levcromakalim and pinacidil are thought to relax arteries solely by opening K_{ATP} channels of arterial smooth muscle, so leading to membrane hyperpolarization and reducing intracellular $[Ca^{2+}]$ and thus contractile force both through a decreased open probability of voltage operated calcium channels and a reduction in inositol trisphosphate dependent calcium release (reviewed by Quayle et al.,1997). Nicorandil, however, is known to cause vasodilation by two mechanisms, acting both as a K_{ATP} channels opener and as a nitrovasodilator (Holzmann et al., 1992). The increase in potency of nicorandil by adenosine receptor activation appears to involve its K_{ATP} channel opening action rather than its nitrovasodilator action since we have shown previously that the effect of 2-CA was abolished either by the K_{ATP} channel inhibitor glibenclamide, or by raising extracellular $[K^+]$ to 80 mM (Davie and Standen, 1998). It is likely that the dual action of nicorandil underlies the fall in Hill coefficient that occurs when the potency of nicorandil is increased by adenosine receptor activation (Fig. 1A, Table 1). K_{ATP} channel opening is thought to contribute more to the vasorelaxant action of nicorandil at low concentrations of the drug (Holzmann et al., 1992), and it is this action which is enhanced by adenosine receptor stimulation, resulting in a flattening of the concentration–response curve (Davie and Standen, 1998).

Our experiments with agonists and antagonists selective for different adenosine receptor subtypes also point to differences in the details of interaction between adenosine receptors and the K^+ channel openers nicorandil and levcromakalim in causing activation of K_{ATP} channels. The A_1 receptor agonist CCPA increased the potency of nicorandil, but did not affect that of levcromakalim (Tables 1 and 2). However, the A_{2a} receptor agonist CGS 21680 increased the potency of both nicorandil and levcromakalim. This suggests that activation of either A_1 or A_{2a} receptors can enhance the potency of nicorandil, while A_1 receptors are ineffective in enhancing that of levcromakalim. The effects of the A_1 receptor antagonist DPCPX are consistent with this, since DPCPX abolished the enhancement of nicorandil potency by 2-CA, but did not block the increase in potency of levcromakalim. As expected, the relatively selective A_{2a} receptor antagonist ZM 241385 also reduced the effect of 2-CA on the potency of nicorandil, but surprisingly, it had no effect on the enhancement by 2-CA of the potency of levcromakalim. It did, however, abolish the effect of the selective A_{2a} agonist CGS 21680. Thus it appears clear that A_{2a} receptor activation is able to enhance the potency of levcromakalim, but that when the non-selective agonist 2-CA is applied, activation of receptors other than A_{2a} (possibly A_{2b}, see below,) is able to cause such enhancement when A_{2a} receptors are blocked.

Although Randall et al. (1994), found evidence that the enhanced potency of levcromakalim in rabbit ear was associated with A_1 receptor activation, A_1, A_{2a}, and A_{2b} receptors have all been associated with K_{ATP} channel activation in different vascular preparations. Channel activation has been measured either as activation of K_{ATP} current or inferred from glibenclamide-sensitive vasorelaxation. In the pig coronary artery Merkel et al. (1992) suggested that glibenclamide-sensitive relaxation to adenosine occurred through A_1 receptor stimulation, and support for this came from patch clamp studies in isolated cells by Dart and Standen (1993) who reported that K_{ATP} current was activated by CCPA, but not by the A_{2a} agonist CGS 21680, while consistent results were obtained with selective antagonists. In the rabbit coronary circulation, adenosine induced vasodilation during hypoxia also appears to involve A_1 receptors (Nakhostine and Lamontagne, 1993). In contrast, A_2 receptor activation has been reported to lead to vasodilation through K_{ATP} channels in the dog coronary circulation (Akatsuka et al., 1994), and A_{2a} receptor activation with CGS 21680 activated K_{ATP} currents in rabbit mesenteric artery (Kleppisch and Nelson, 1995). However, in the guinea-pig coronary circulation CGS 21680 was ineffective in producing glibenclamide-sensitive hyperpolarization, and the receptor involved appears to be A_{2b} (Mutafova-Yambolieva and Keef, 1997). These findings may help explain the disparate nature of the adenosine receptors that can interact with the effects of K_{ATP} channel opening drugs.

It is also clear that the openers themselves show differences in the mechanisms by which they cause channel activation, though these mechanisms are only partially understood. K_{ATP} channel openers are thought to act in general by modulating the nucleotide sensitivity of the channel (Terzic et al., 1995; Shyng et al., 1997). K_{ATP} channels are heteromers of Kir6 subunits, forming the K^+ conductive pore, and sulphonylurea receptor (SUR) subunits, thought to possess the binding sites for openers and blockers (Aguilar-Bryan et al., 1998). The definitive structure of K_{ATP} channels of vascular smooth muscle is unknown, but is likely to involve the SUR2B form of the SUR since, when expressed in heterologous cells, this confers similar sensitivities to openers and blockers to those of native smooth muscle (Isomoto et al., 1996). Channels involving SUR2B are much more sensitive to activation by nicorandil than are those with SUR2A, which is thought to form K_{ATP} channels of cardiac muscle. Since SUR2B and SUR2A differ only by 42 amino acids at their C-terminal ends, Shindo et al. (1998) have suggested that this region is critical for activation by nicorandil. It has also been suggested recently that the Kir6 subunit of the channel may play a role in channel activation by pinacidil, since pinacidil attenuated ATP-inhibition of channels formed by co-expression of Kir6.2 and SUR2B (Kir6.2/

SUR2B), but enhanced nucleoside diphosphate activation of Kir6.1/SUR2B channels (Satoh et al.,1998).

In summary, the sensitivity of the action of K_{ATP} channel openers in arterial smooth muscle to enhancement by activation of adenosine receptors may depend both on the tissue and on the nature of the opener. Adenosine receptor activation can enhance the potency of both nicorandil and levcromakalim, though the receptors involved may differ. In contrast, channel activation by pinacidil appears not to interact with the effect of adenosine receptors. Enhancement of vasodilator potency by locally released adenosine may have significance for the therapeutic use of current and future openers, since it may result in openers whose potency is enhanced in this way being effectively targeted to metabolically compromised regions of tissue, where adenosine release is greatest.

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