

Modulation of vasodilatation to levcromakalim by adenosine analogues in the rabbit ear: an explanation for hypoxic augmentation

¹Michael D. Randall, ²Hiroshi Ujiie & Tudor M. Griffith

Department of Diagnostic Radiology, Cardiovascular Sciences Research Group, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN

1 We have used a rabbit isolated ear, buffered-perfused preparation to investigate the effects of adenosine analogues on the vasodilatation to the potassium channel opener, levcromakalim (the active (–)-enantiomer of cromakalim). We have examined the effects of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective adenosine A₁ antagonist, on vasodilatation to levcromakalim under hypoxic conditions and also following inhibition of nitric oxide synthesis.

2 Levcromakalim relaxed precontracted preparations with an EC₅₀ = 369 ± 48 nM and maximum relaxation of tone (R_{max}) = 81.0 ± 3.2%. In the presence of 1 μM N⁶-cyclohexyladenosine (CHA) a selective adenosine A₁ agonist, there was a significant (*P* < 0.01) leftward shift in the concentration-response curve with an EC₅₀ = 194 ± 54 nM and R_{max} = 93.2 ± 2.0%. Conversely, the presence of CHA did not influence vasodilatation to either pinacidil or sodium nitroprusside.

3 Hypoxia also significantly (*P* < 0.001) increased the vasodilator potency of levcromakalim (EC₅₀ = 134 ± 22 nM), and this enhancement was completely reversed (EC₅₀ = 380 ± 107 nM, *P* < 0.01) by pretreatment of the preparations with 5 μM DPCPX, a selective A₁ adenosine antagonist. However, under normoxic conditions DPCPX did not influence vasodilatation to levcromakalim.

4 Inhibition of nitric oxide synthesis with 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) caused a significant (*P* < 0.001) leftward shift in the concentration-response curve to levcromakalim (EC₅₀ = 73.0 ± 7.6 nM). Pretreatment of preparations with DPCPX partially reversed the increase in potency found in the absence of nitric oxide synthesis (EC₅₀ = 153 ± 18 nM, *P* < 0.001).

5 We have shown that an adenosine A₁ agonist may increase the potency of levcromakalim indicating that adenosine receptor activation may augment the vasodilator activity of levcromakalim. That responses to levcromakalim but not those to pinacidil were affected by CHA points to further differences in the pharmacology of these potassium channel openers. The reversal by the adenosine A₁ antagonist of the hypoxic-potential of vasodilatation to levcromakalim, and also augmentation following inhibition of nitric oxide synthesis, suggests that under these conditions there is an endogenous release of adenosine which may enhance responses to levcromakalim. The findings of this study suggest that levcromakalim may selectively dilate vessels where there is elevated adenosine release.

Keywords: Levcromakalim; pinacidil; N^G-nitro-L-arginine methyl ester; hypoxia; nitric oxide; potassium channel opener-sensitive potassium channels (KCO-channels); adenosine; 8-cyclopentyl-1,3-dipropylxanthine; N⁶-cyclohexyladenosine

Introduction

We have previously shown that vasodilatation to the potassium channel opener (KCO) levcromakalim (the active (–)-enantiomer of cromakalim, formerly designated BRL 38227) is augmented under hypoxic conditions. A similar, but even larger, effect was also observed following inhibition of nitric oxide synthesis with N^G-nitro-L-arginine methyl ester (L-NAME) (Randall & Griffith, 1993). The activity of KCO-sensitive potassium channels is regulated by purine-derivatives associated with cellular metabolism as intracellular ATP closes these channels and high ADP favours opening (for review, see Nichols & Lederer, 1991). Pharmacologically, these channels may be regulated by KCOs which reduce channel sensitivity towards ATP thereby promoting channel opening (see Edwards & Weston, 1990; Nichols & Lederer, 1991), while the hypoglycaemic sulphonylureas block the channels (Sturgess *et al.*, 1985).

Recent evidence, from patch clamp studies using membrane patches of rat cultured ventricular myocytes (Kirsch *et*

al., 1990) and whole-cell current recordings from porcine isolated coronary vascular smooth muscle cells (Dart & Standen, 1993), has indicated that adenosine A₁ receptors may be positively coupled, via a G-protein, to KCO-sensitive channels. The possibility that adenosine is coupled to KCO-sensitive potassium channels receives further functional support from evidence that adenosine A₁, but not A₂, receptor agonists cause sulphonylurea-sensitive vasorelaxation of porcine coronary vessels (Merkel *et al.*, 1992). Adenosine is an important mediator of blood-flow regulation which corrects an imbalance between energy production and demand (Berne *et al.*, 1983), as the supply-to-demand ratio for oxygen determines the formation of adenosine (Bardenheuer & Schrader, 1986). This has led to the so-called 'adenosine hypothesis' of local blood flow regulation, in which locally produced adenosine leads to vasodilatation and improved blood flow (Berne, 1980). In this context Marshall *et al.* (1993) have recently reported that hypoxia, via adenosine release, leads to skeletal muscle vasodilatation through the activation of sulphonylurea-sensitive potassium channels.

We have previously observed that levcromakalim selectively vasodilates collateral vessels (Randall & Griffith, 1992) and that both hypoxia and metabolic inhibitors selectively increase the vasodilator potency of this agent (Randall &

¹ Author for correspondence at present address: Department of Physiology and Pharmacology, University of Nottingham, Queen's Medical Centre, Nottingham.

² Present address: Department of Neurosurgery, Tokyo Women's Medical College, Tokyo, Japan.

Griffith, 1993). Hypoxia is associated with compromised metabolic activity leading to adenosine release (Mian & Marshall, 1991; Marshall *et al.*, 1993). This has led us now to investigate the actions of adenosine analogues on responses to levromakalim and other vasodilators in order to examine whether locally released adenosine may influence vasodilator responses. In addition to hypoxic augmentation, vasodilatation to levromakalim is also enhanced following inhibition of nitric oxide synthesis by L-NAME, and this effect is even greater than that seen in hypoxia (Randall & Griffith, 1993). Inhibition of nitric oxide activity has important consequences for vascular resistance and blood flow regulation (Griffith *et al.*, 1987). However, following inhibition of nitric oxide production in the guinea-pig heart, there is a significant compensatory increase in adenosine release, presumably as a consequence of mismatches in flow and demand (Kostic & Schrader, 1992). This compensatory release of adenosine is sufficient to exert a significant protective effect in a rabbit model of coronary occlusion, and limits the level of ischaemic damage (Patel *et al.*, 1993). We have accordingly examined whether endogenous release of adenosine, following inhibition of nitric oxide synthesis, may contribute towards the increase in potency for levromakalim in the presence of L-NAME.

A preliminary account of part of this work was communicated to the January 1994 meeting of the British Pharmacological Society (Randall *et al.*, 1994).

Methods

Preparation of the rabbit ear vascular bed

Male New Zealand White rabbits (2–2.5 kg) were killed by cervical dislocation. An ear was removed and the central artery cannulated and perfused with Holman's solution (composition, mM: NaCl 120, KCl 5, CaCl₂ 2.5, NaH₂PO₄ 1.3, NaHCO₃ 25, sucrose 10 and D-glucose 11) at a flow rate of 3.5 ml min⁻¹. The physiological buffer also contained 10 µM indomethacin to eliminate prostanoid activity. The buffer was gassed with either 95% O₂/5% CO₂ (normoxia, PO₂ = 500–600 mmHg) or 95% N₂/CO₂ (hypoxia, PO₂ = 20–30 mmHg) and maintained at 35°C.

Experimental protocols

The perfusion pressure of the intact preparation was continuously monitored by means of a pressure transducer placed close to the inflow cannula. The pressure drop across the cannula was determined at the end of each experiment and subtracted from the recorded pressure in order to determine the pressure drop across the vascular bed.

To characterize vasodilator responses, preparations were equilibrated for 1 h. Perfusion pressure was raised pharmacologically with the combination of 5-hydroxytryptamine and histamine in equimolar concentrations (1 µM) to achieve submaximal (ca. 60% of maximal tone, Randall & Griffith, 1991). Cumulative vasodilator concentration-response curves were obtained in different preparations for levromakalim, pinacidil and sodium nitroprusside by addition of each agent to the perfusion fluid in volumes less than 100 µl.

The effects of the selective adenosine A₁ agonist, N⁶-cyclohexyladenosine (CHA), were investigated by inclusion of this agent at a concentration of 1 µM in the perfusion fluid after steady state precontraction, but 15 min prior to construction of the concentration-response curves for levromakalim, pinacidil or sodium nitroprusside.

In subsequent experiments, concentration-response curves for levromakalim were constructed under hypoxic perfusion and the results were compared with control responses obtained from different preparations perfused with normoxic buffer. The influence of the selective adenosine A₁ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX),

was examined on the vasodilatation to levromakalim under both normoxic (control) and hypoxic conditions. In these experiments the preparations were equilibrated for 1 h with the buffer of the appropriate oxygen tension after which time the preparations were precontracted and then continuously perfused with 5 µM DPCPX. The concentration-response curve to levromakalim was constructed 15 min after inclusion of DPCPX.

In order to investigate the influence of inhibition of nitric oxide synthesis on vasodilatation to levromakalim, different preparations were perfused with 100 µM L-NAME which was added to the perfusion fluid 30 min prior to precontraction. We have previously shown that perfusion with 100 µM L-NAME selectively abolishes endothelium-dependent relaxations to acetylcholine and inhibits basal nitric oxide activity (Randall & Griffith, 1991). Inhibition of basal nitric oxide production leads to augmented constrictor responses and, therefore, in these experiments the equimolar concentrations of the vasoconstrictor agents were reduced to 300 nM to give an equivalent level of tone (Randall & Griffith, 1993). In further experiments, preparations were perfused with 5 µM DPCPX 30 min after inclusion of L-NAME and subsequent precontraction but 15 min prior to constructing concentration-response curves for levromakalim.

Data and statistical analysis

All data are given as the mean ± s.e.mean and were compared by either paired or unpaired Student's *t*-tests or analysis of variance, as appropriate. EC₅₀ values for vasodilator responses were obtained from individual concentration-response curves as the concentration at which half-maximal reduction of established tone occurred. These values were converted to the logarithmic values (pD₂) for statistical analysis.

Drugs

All solutions were prepared on the day of the experiment. N^G-nitro-L-arginine methyl ester, 5-hydroxytryptamine as creatinine sulphate complex, histamine dihydrochloride and sodium nitroprusside (all from Sigma Chemical Company, Poole, Dorset), were dissolved in saline. Levromakalim (a generous gift from Smith Kline Beecham, Surrey, U.K.), pinacidil (a generous gift from Leo, Bucks), N⁶-cyclohexyladenosine and indomethacin (both from Sigma) and 8-cyclopentyl-1,3-dipropylxanthine (from Research Biochemicals Incorporated, Natick, MA, U.S.A.) were dissolved in 70% (v/v) ethanol. All drugs were then diluted to the required concentrations in the Holman's solution.

Results

Effect of N⁶-cyclohexyladenosine on vasodilator responses to levromakalim and pinacidil

In the 15 control preparations basal perfusion pressure was 32.4 ± 5.5 mmHg and was increased by 113 ± 10 mmHg following addition of the vasoconstrictor agents. The concentration-response curve for the vasodilator effects of levromakalim (10 nM–10 µM) under control conditions is shown in Figure 1a and is described by an EC₅₀ of 369 ± 48 nM and the maximum relaxation of tone (R_{max}) was 81.0 ± 3.2% (*n* = 15) (Table 1).

In subsequent experiments addition of 1 µM CHA to the perfusion fluid did not influence the level of induced tone (Table 1). However, in the presence of CHA levromakalim was significantly (*P* < 0.01) more potent as a vasodilator (Figure 1a) with an EC₅₀ value of 194 ± 54 nM and there was a significant (*P* < 0.05) increase in the maximum relaxation of tone (93.2 ± 2.0%) (Table 1).

In 11 control preparations, pinacidil (10 nM–30 µM)

Table 1 Vasodilator properties of levcromakalim and pinacidil in the absence and presence of 1 μM N⁶-cyclohexyladenosine (CHA)

	<i>Levcromakalim</i>	<i>Levcromakalim</i> + CHA	<i>Pinacidil</i>	<i>Pinacidil</i> + CHA
<i>n</i>	15	8	11	7
Basal perfusion pressure (mmHg)	32.4 \pm 5.5	17.5 \pm 2.2	26.8 \pm 5.3	21.4 \pm 3.4
Increase in perfusion pressure (mmHg)	113 \pm 10	129 \pm 10	106 \pm 9	112 \pm 8
Total increase in perfusion pressure in presence of CHA (mmHg)	—	132 \pm 12	—	112 \pm 5
EC ₅₀ (nM)	369 \pm 48	194 \pm 54**	1,783 \pm 336	973 \pm 150
Maximum relaxation (%)	81.0 \pm 3.2	93.2 \pm 2.0*	93.5 \pm 2.7	95.8 \pm 4.4

For each vasodilator the statistical differences between the presence and absence of N⁶-cyclohexyladenosine are indicated by *($P < 0.05$) and **($P < 0.01$).

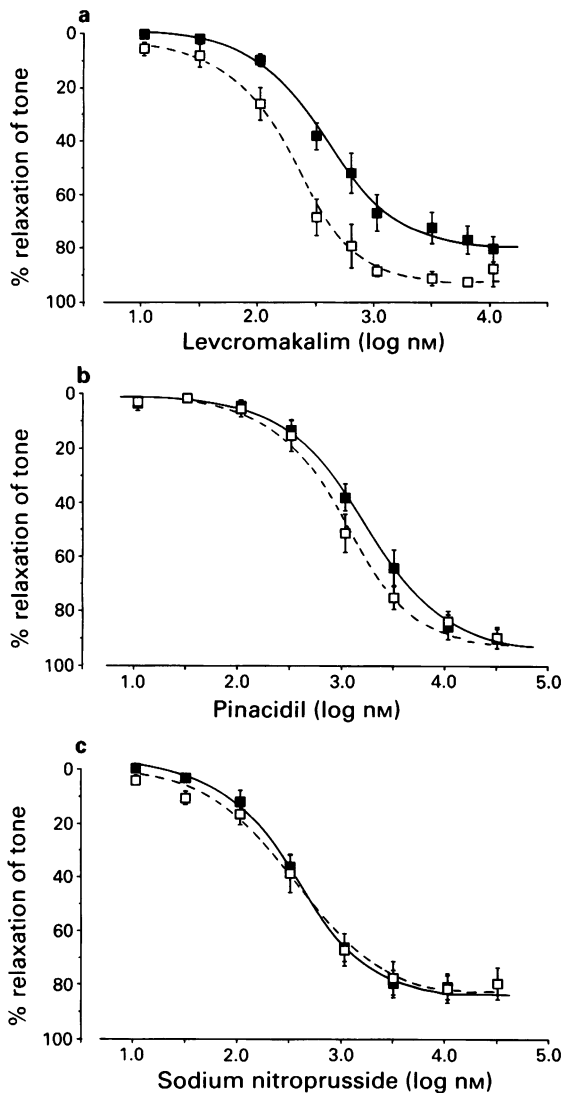


Figure 1 Concentration-response curves for the relaxation of established tone in rabbit ear isolated perfused preparations by (a) levcromakalim in the absence (■, $n = 15$) and presence (□, $n = 8$) of 1 μM N⁶-cyclohexyladenosine; (b) pinacidil in the absence (■, $n = 8$) and presence (□, $n = 7$) of 1 μM N⁶-cyclohexyladenosine; (c) sodium nitroprusside in the absence (■, $n = 6$) and presence (□, $n = 7$) of N⁶-cyclohexyladenosine. The vertical bars indicate \pm s.e.mean.

similarly gave concentration-related relaxations of tone, but was some 5 fold less potent than levcromakalim (Figures 1a,b and Table 1). In 7 different preparations CHA did not influence either the level of established tone (Table 1) or vasodilatation to pinacidil (Figure 1b and Table 1).

Effects of N⁶-cyclohexyladenosine on vasodilatation to sodium nitroprusside

In 6 control preparations, basal perfusion pressure was 19.8 \pm 2.3 mmHg and was increased by 102 \pm 16 mmHg following addition of the vasoconstrictors. Sodium nitroprusside (10 nM–10 μM) gave rise to concentration-related relaxations of established tone with an EC₅₀ = 367 \pm 32 nM and R_{max} = 84.9 \pm 4.7% (Figure 1c). In another 6 preparations basal perfusion pressure was 18.6 \pm 3.7 mmHg, while after addition of the vasoconstrictors perfusion pressure was increased by 124 \pm 12 mmHg. Addition of 1 μM CHA had no effect on vascular tone (124 \pm 14 mmHg). In the presence of CHA the vasorelaxant potency of sodium nitroprusside was 335 \pm 70 nM and the R_{max} was 82.9 \pm 5.1% (Figure 1c), these parameters were not significantly different from those in the absence of CHA (EC₅₀ = 367 \pm 32 nM, R_{max} = 84.9 \pm 4.7%).

Effects of hypoxia on vasodilatation to levcromakalim in the absence and presence of 5 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX)

In 12 hypoxic preparations basal perfusion pressure was similar to that under normoxic conditions (Table 2). Similarly the oxygen tension did not influence the increase in perfusion pressure induced by the combination of vasoconstrictors.

Figure 2a and Table 2 indicate that levcromakalim was approximately 3 times ($P < 0.001$) more potent as a vasodilator under hypoxic compared to normoxic conditions. However, Figure 2a and Table 2 show that in preparations which are pretreated with 5 μM DPCPX the hypoxic augmentation of vasodilator responses to levcromakalim is abolished completely. DPCPX had no effects on vascular tone in the precontracted preparations in any of the groups to which it was added (Table 2). In normoxic preparations pretreatment with DPCPX did not influence vasodilatation to levcromakalim (Figure 2b and Table 2).

Effects of 5 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on vasodilatation to levcromakalim in the presence of L-NAME

Pretreatment with L-NAME did not influence perfusion pressure (28.0 \pm 3.6 mmHg v. 32.4 \pm 4.5 mmHg, $n = 9$). In the presence of L-NAME there was a significant ($P < 0.001$) five fold leftward shift in the concentration-response curve for the relaxation of tone by levcromakalim (Figure 3 and Table 3). In 7 different preparations this shift was partially ($P < 0.001$) attenuated by pretreatment of the preparations with 5 μM DPCPX such that the EC₅₀ was intermediate between control and that obtained in the presence of DPCPX alone. In these preparations addition of DPCPX did not significantly alter the level of established tone (146 \pm 20 v. 130 \pm 21 mmHg).

Table 2 Vasodilator properties of levcromakalim under normoxic and hypoxic conditions in the absence and presence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX)

	<i>Normoxia</i>	<i>Normoxia</i> + <i>DPCPX</i>	<i>Hypoxia</i>	<i>Hypoxia</i> + <i>DPCPX</i>
<i>n</i>	15	7	12	6
Basal perfusion pressure (mmHg)	32.4 ± 5.5	23.6 ± 4.6	20.3 ± 3.0	21.7 ± 1.7
Increase in perfusion pressure (mmHg)	113 ± 10	112 ± 14	111 ± 6	119 ± 12
Total increase in perfusion pressure in presence of DPCPX (mmHg)	–	111 ± 17	–	120 ± 8
EC ₅₀ (nM)	369 ± 48	310 ± 74	134 ± 22***	380 ± 107††
Maximum relaxation (%)	81.0 ± 3.2	76.7 ± 6.6	90.1 ± 2.5	93.2 ± 2.9

The statistical differences between the vasodilator responses under normoxic conditions are shown by ***($P < 0.001$) and the difference between the absence and presence of DPCPX under hypoxic conditions are shown by ††($P < 0.01$). The control data for vasodilatation to levcromakalim under normoxic conditions is taken from Table 1 and is included for comparison.

Table 3 Vasodilator properties of levcromakalim in the presence and absence of 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) with or without 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) pretreatment

	<i>Levcromakalim</i>	<i>Levcromakalim</i> + <i>L-NAME</i>	<i>Levcromakalim</i> + <i>L-NAME</i> + <i>DPCPX</i>
<i>n</i>	15	9	7
Basal perfusion pressure (mmHg)	32.4 ± 5.5	28.0 ± 3.6	26.7 ± 2.6
Increase in perfusion pressure (mmHg)	113 ± 10	124 ± 7	146 ± 20
Total increase in perfusion pressure in presence of DPCPX (mmHg)	–	–	130 ± 21
EC ₅₀ (nM)	369 ± 48	73.0 ± 7.6***	153 ± 18**(†††)
Maximum relaxation (%)	81.0 ± 3.2	88.6 ± 3.3	87.8 ± 3.8

Statistical differences for the vasodilator potency between the absence and presence of L-NAME are indicated by **($P < 0.01$) and ***($P < 0.001$) while †††($P < 0.001$) indicates statistical differences between the absence and presence of DPCPX in the L-NAME pretreated preparations.

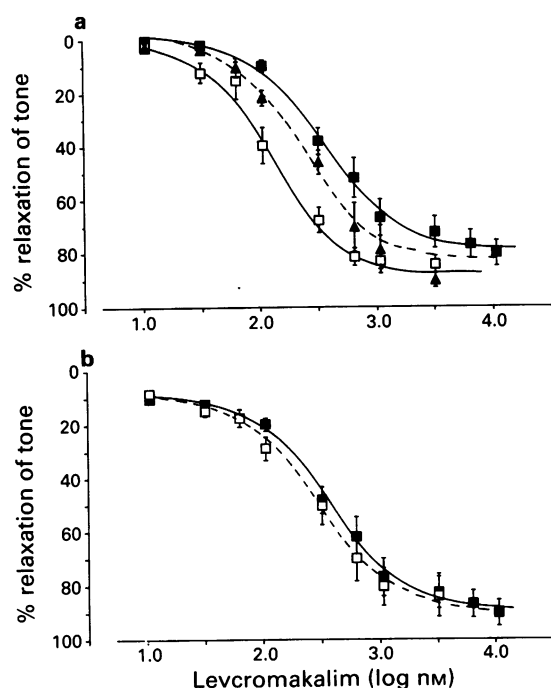


Figure 2 Concentration-response curves for the relaxation of established tone by levcromakalim in rabbit ear isolated perfused preparations; (a) (■, $n = 15$) indicates control data obtained under normoxic conditions and is taken from Figure 1a for the purposes of comparison, (□, $n = 12$) show the data obtained under hypoxic perfusion and (▲, $n = 6$) show the data obtained under hypoxic perfusion in the presence of 5 μM 8-cyclopentyl-1,3-dipropylxanthine; (b) (■, $n = 15$) indicates data obtained under normoxic conditions that is taken from Figure 1a for the purposes of comparison; (□, $n = 7$) show the data obtained under normoxic perfusion in the presence of 5 μM 8-cyclopentyl-1,3-dipropylxanthine. Their vertical bars indicate ± s.e.mean.

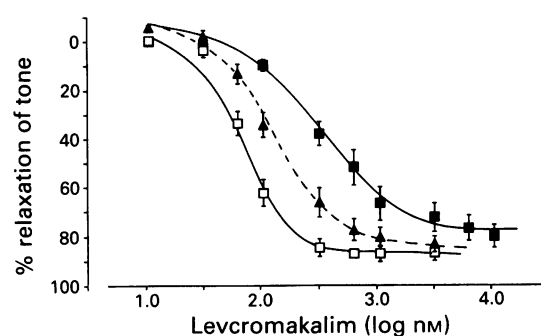


Figure 3 Concentration-response curves for the relaxation of established tone by levcromakalim in rabbit ear isolated perfused preparations; (■, $n = 15$) indicate control data obtained in the absence of N^G-nitro-L-arginine methyl ester (L-NAME) and is taken from Figure 1a for the purposes of comparison, (□, $n = 9$) show the data obtained in the presence of 100 μM L-NAME and (▲, $n = 7$) show the data obtained in the presence of both 100 μM L-NAME and 5 μM 8-cyclopentyl-1,3-dipropylxanthine. The vertical bars indicate ± s.e.mean.

Discussion

The results of the present study clearly point to an interaction between the KCO, levcromakalim, and adenosine. This interaction provides an explanation as to the augmentation of vasodilatation to levcromakalim in both hypoxia and following nitric oxide synthase inhibition. The findings corroborate our previous findings concerning the different pharmacology of levcromakalim and pinacidil (Randall & Griffith, 1993).

That the potency of levcromakalim is augmented by the adenosine agonist CHA accords with reports indicating that the adenosine A₁ receptor may be coupled, via a G-protein,

to KCO-sensitive potassium channels (Kirsch *et al.*, 1990; Dart & Standen, 1993). Not only are our data compatible with adenosine A₁ receptors interacting with KCO-sensitive potassium channels, but more importantly, they demonstrate that adenosine may modulate the activity of KCOs. Since CHA did not have any direct vascular actions at the reasonably high concentrations used, then an independent, direct action on the rabbit ear vasculature may be excluded. Furthermore, the lack of interaction between CHA and sodium nitroprusside and pinacidil confirms that the effects observed with levcromakalim are not due to a non-specific interaction between the different vasodilators. That CHA did not augment vasodilatation to pinacidil, a structurally different KCO, may point to differences in the pharmacology of these agents. This would appear to accord with other evidence in the literature pointing to differences in their pharmacology (see Cook & Quast, 1990). For example there are haemodynamic differences between cromakalim and pinacidil (Longman *et al.*, 1988), while McPherson & Angus (1990) also identified differences in the pharmacology of cromakalim and pinacidil, in that glibenclamide, phen-tolamine and alinidine non-competitively inhibited the actions of cromakalim on the canine coronary artery while having competitive actions against pinacidil. More recently, Lawson *et al.* (1992) have demonstrated that endothelin-1 discriminates between the actions of levcromakalim and pinacidil, and proposed that these agents interact with different sites on the potassium channel. In our previous study we also proposed that there were differences in their comparative pharmacology, since we observed that the potency of levcromakalim, but not that of pinacidil, was augmented by both hypoxia and L-NAME (Randall & Griffith, 1993). In view of the lack of effect of L-NAME and hypoxia on responses to pinacidil (Randall & Griffith, 1993) we examined only the influence of the adenosine antagonist on vasodilatation to levcromakalim under these conditions.

Our previous finding that vasodilatation to levcromakalim was augmented by hypoxia was of interest in view of the specific potentiation of responses by CHA, a mimetic of adenosine. We therefore repeated these experiments with the inclusion of the adenosine A₁ antagonist, DPCPX in the perfusate. Under normoxic control conditions DPCPX did not influence vascular tone but more importantly did not alter vasodilatation to levcromakalim. The lack of effect on vascular tone indicates that this antagonist is acting specifically and that any basally released adenosine does not contribute significantly towards vascular regulation under any of the conditions used. However, under hypoxic perfusion DPCPX caused a rightward shift of the concentration-response curve for relaxation by levcromakalim, thereby abolishing the hypoxic augmentation. DPCPX did not influence vasodilatation to levcromakalim under normoxic conditions and this excludes a direct antagonist action against levcromakalim. Since we have demonstrated functionally that the adenosine-mimetic may interact with KCO-sensitive potassium channels, then the most likely explanation for these results is that adenosine activity is increased sufficiently to enhance KCO activity. Although the most probable change in adenosine activity in hypoxia is increased release, other potential mechanisms may operate and they include receptor upregulation and reduced reuptake. The present experiments do not discriminate between these alternatives.

Vasorelaxant responses to levcromakalim are also augmented by inhibition of nitric oxide synthases by L-NAME, an effect that is completely reversed by exogenous L-arginine (Randall & Griffith, 1993). Interestingly the enhancement following inhibition of nitric oxide synthase is more pronounced than that in hypoxia. Recent studies have shown that inhibition of nitric oxide production leads to mismatches of perfusion and demand which promotes a compensatory release of adenosine (Griffith *et al.*, 1987; Kostic & Schrader, 1992). That DPCPX partially reversed the augmen-

tation of vasodilatation to levcromakalim in the presence of L-NAME indicates that following inhibition of nitric oxide synthesis in the rabbit ear there is an increase in adenosine release. The shift of the concentration-response curve produced by DPCPX was comparable to that produced by the adenosine antagonist under hypoxic conditions and would indicate that the release of adenosine following blockade of nitric oxide synthesis occurs at a comparable level to that found in hypoxia. The high concentration of DPCPX used only partially reversed the augmentation due to inhibition of nitric oxide synthesis and this suggests that mechanisms other than the interaction between adenosine and the KCO may also be in operation.

Additional mechanisms for the potentiation of vasodilatation to levcromakalim in the absence of nitric oxide production are not apparent from the present data. Nitric oxide, in some (Tare *et al.*, 1990; Garland & McPherson, 1992), but not all (Komori *et al.*, 1988), vascular preparations causes hyperpolarization. Associated with this is the cyclic GMP-dependent activation of calcium-sensitive potassium channels leading to hyperpolarization and relaxation (Fujino *et al.*, 1991). If nitric oxide exerts a hyperpolarizing effect in the rabbit ear, then inhibitors of this input may lead to vascular smooth muscle depolarization, which might enable a potassium channel opener to have a greater hyperpolarizing effect leading to augmented vasodilatation. This action of nitric oxide may account for its synergism with cromakalim reported by others (Rae & Corrêa, 1992). In the present context, loss of nitric oxide activity following treatment with L-NAME may therefore potentially enable levcromakalim to have greater impact. However, since vasodilatation to pinacidil is unaffected by L-NAME (Randall & Griffith, 1993) then the above mechanisms would appear doubtful.

Potentiation of responses to levcromakalim has been reported in other circumstances. In a recent report by Pavlovic *et al.* (1993) the relaxant action of levcromakalim on rat tracheal smooth muscle was selectively potentiated by destruction of the airway epithelium leading to loss of the epithelium-derived inhibitory factor.

Despite our observations of augmented vasodilatation to levcromakalim in the presence of L-NAME, others, in different preparations, have not observed such a change in activity (Gardiner *et al.*, 1991). Differences between preparations may be accounted for by differences in adenosine release and reuptake and also differences in adenosine receptor populations between vascular beds.

The results of the present study clearly indicate that the increase in vasodilator potency of levcromakalim in hypoxia, and to a lesser extent in the presence of L-NAME, can be explained by local increases in adenosine activity leading to potentiated responses. The apparent enhancement of the vasodilator activity of levcromakalim by adenosine may perhaps explain the selectivity of cromakalim for chronically ischaemic tissues (Angersbach & Nicholson, 1988), and the ability of levcromakalim to improve substantially collateral flow after acute arterial occlusion (Randall & Griffith, 1992). In conclusion, we have reported an important and significant interaction between adenosine, an endogenous mediator associated with hypoperfusion and hypoxia, and levcromakalim. This interaction may enable levcromakalim to exert selective vasodilator effects on ischaemic tissues.

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