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Role of K^+ channel opening and stimulation of cyclic GMP in the vasorelaxant effects of nicorandil in isolated piglet pulmonary and mesenteric arteries: relative efficacy and interactions between both pathways

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1 The effects of the K^+ channel opener levcromakalim, the guanylate cyclase stimulant nitroprusside and the dual drug nicorandil (K^+ channel opener and guanylate cyclase stimulant) were analysed in piglet isolated endothelium-denuded pulmonary (PA) and mesenteric (MA) arteries stimulated by noradrenaline (NA) or by the thromboxane A_2 mimetic U46619.

2 Nicorandil, levcromakalim and verapamil were less potent in PA than in MA, the efficacy of levcromakalim was also reduced in PA. The effects of nicorandil and levcromakalim were similar in arteries pre-contracted by NA and U46619, whereas verapamil was more potent in arteries pre-contracted by NA. Nitroprusside was equipotent in MA pre-contracted by either NA or U46619 and in PA pre-contracted by NA whereas in PA pre-contracted by U46619, nitroprusside showed lower potency and efficacy.

3 The relaxant effects of levcromakalim and nitroprusside were inhibited by 10^{-5} M glibenclamide and 10^{-6} M ODQ, respectively. Nicorandil-induced relaxation was inhibited by ODQ in all experimental conditions, whereas glibenclamide had inhibitory effects in PA and MA pre-contracted by U46619, had no effect in PA pre-contracted by NA and in MA pre-contracted by NA it was only inhibitory in the presence of ODQ.

4 No apparent interactions were found between nitroprusside and levcromakalim as indicated by the lack of effects of pretreatment with one of them (producing 20–35% relaxation) on the potency of the relaxant response to the other. However, in PA pre-contracted by U46619, where nitroprusside or levcromakalim induced only partial relaxation, the combination of both mechanisms (either by combining nitroprusside plus levcromakalim or by nicorandil) was able to induce full vasodilatation.

5 In conclusion, K^+ channel opening and guanylate cyclase stimulation are independent pathways that induce additive vasorelaxation in piglet PA and MA. The mechanism of action of nicorandil is dependent on the artery and on the nature of the agonist employed to precontract the artery. The relative efficacy of K^+ channel opening vs guanylate cyclase stimulation may partially explain the preferential contribution of each mechanism to the relaxant effects of nicorandil.

Keywords: Nicorandil; levcromakalim; nitroprusside; verapamil; U46619; piglet pulmonary arteries; mesenteric arteries

Introduction

K^+ channels play a major role in the regulation of the resting membrane potential and modulate vascular smooth muscle tone (Nelson & Quayle, 1995). Adenosine 5′-triphosphate (ATP)-sensitive K^+ channels (K_{ATP}) are regulated by intracellular ATP levels. At physiological ATP levels K_{ATP} remain mostly in their closed state while at low ATP levels the channels open (Standen *et al*., 1989; Clapp & Gurney, 1992; Edwards & Weston, 1993). K^+ channel openers like levcromakalim activate K_{ATP} channels producing hyperpolarization of the smooth muscle membrane which results in a reduction of Ca^{2+} influx through voltage-activated L-type Ca^{2+} channels, thus producing vasodilatation (Edwards & Weston, 1993). Their actions are inhibited by the sulphonylurea, glibenclamide, which is known to selectively inhibit K_{ATP} channels (Edwards & Weston, 1993; Quast, 1993).

In recent years, nitric oxide (NO) has been demonstrated to play a prominent role in regulating vascular smooth muscle tone (Moncada *et al*., 1991). Under physiological conditions, NO is released from the endothelial cells and diffuses to the adjacent vascular smooth muscle cells where it activates soluble guanylate cyclase, increases cyclic GMP and induces vasorelaxation. Nitrovasodilators (organic nitrates and nitroprusside) exert their vasodilating effects through the spontaneous or metabolic release of NO, or a closely related compound, from their molecule (Ignarro *et al*., 1981; Ignarro & Kadowitz, 1985; Murad, 1986; Feelisch, 1991).

Nicorandil is a hybrid molecule with K^+ channel opening and nitrate-like properties (Furukawa *et al*., 1981; Hester, 1985; Hamilton & Weston, 1989) which has been successfully employed in the treatment of angina pectoris (Frampton, 1992). The relative contribution of each of these vasodilator mechanisms of action of nicorandil depends on the tissue and experimental conditions. In general, in large conductance arteries, the activation of guanylate cyclase (i.e the nitrate-like action) plays a major role, whereas in resistance arteries nicorandil produces its vasorelaxant effects mainly via its K^+ channel opening properties (Borg *et al*., 1991; Kreye *et al*., 1992; Akai *et al*., 1995). However, the reason(s) for why the mechanism varies under different circumstances is unclear.

In the present study we have analysed the vasorelaxant actions mediated through K^+ channel opening and guanylate

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cyclase stimulation, the possible interactions between them and the contribution of each mechanism to the relaxant effects of nicorandil in piglet isolated endothelium-denuded pulmonary (PA) and mesenteric (MA) arteries stimulated by noradrenaline (NA) or by the thromboxane A_2 mimetic U46619. Part of these results has been presented at the British Pharmacological Society (Pérez-Vicaíno *et al*., 1997b).

Methods

Tissue preparation

Two week old male piglets (10–17 days, 3–5 kg) were used in this study. Piglets were killed in the local abattoir by exsanguination and the lungs and mesenteric vascular beds were rapidly immersed in cold (4°C) Krebs solution (composition in mM: NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 2.0, KH_2PO_4 1.2 and glucose 11) and transported to the laboratory. The PA (third branch) and MA from piglets (all with an internal diameter 1–2 mm) were carefully dissected free of surrounding tissue and cut into rings of 2–3 mm of length (Villamor *et al*., 1995; Pérez-Vizcaíno *et al*., 1996; 1997a). The endothelium was removed by gently rubbing the intimal surface of the rings with a metal rod. The endothelium removal procedure was verified by the inability of 10^{-6} M acetylcholine to relax arteries pre-contracted with 10^{-6} M NA. Two L-shaped stainless-steel wires were inserted into the arterial lumen and the rings were introduced into Allhin organ chambers filled with Krebs solution (gassed with 95% O_2 and 5% CO_2 at 37°C). One wire was attached to the chamber and the other to an isometric force-displacement transducer coupled to a signal amplifier (Model PRE 206-4, Cibertec, Madrid) and connected to a Hewlett Packard computer via an A/D interface. Contractile tension was recorded by a REGXPC computer programme (Cibertec, Madrid). The rings were stretched to their optimal resting tension (0.5 g for pulmonary rings and 2 g for mesenteric rings) and allowed to equilibrate for 60–90 min. During this period, tissues were restretched and washed every 30 min with warm Krebs solution.

Experimental protocols

After equilibration, rings were pre-contracted by equi-effective concentrations of NA or U46619. Unless otherwise stated, the concentrations were 10^{-5} M NA (this concentration induced about a 95% of the maximal response to NA, E_{max}) and 10^{-7} M U46619 (80% of E_{max}) for PA and 10^{-6} M NA (75% of E_{max}) and 10^{-6} M U46619 (95%, of E_{max}) for MA. When the contractile response to each agonist reached a stable tension, cumulative concentration-response curves to nicorandil, nitroprusside, levcromakalim or verapamil were carried out. In some experiments, after the contractile response had been induced, pulmonary rings were treated with the inhibitor of K_{ATP} glibenclamide (10^{-5} M), the guanylate cyclase inhibitor ODQ (10^{-6} M) or both for 20 min before the concentration-response curve to nicorandil, nitroprusside or levcromakalim was performed. Since glibenclamide is a competitive inhibitor of thromboxane A_2 receptors (Cocks *et al*., 1990), it induced a relaxant effect (30–50%) in PA pre-contracted by 10^{-7} M U46619. Therefore, for the experiments with 10^{-5}M glibenclamide the concentration of U46619 was increased to 3×10^{-7} M. Under these conditions, glibenclamide relaxed PA by 26 ± 4%, so that the final tone was not significantly different from that induced by 10^{-7} M U46619 alone. In MA pre-contracted by 10^{-6} M U46619, 10^{-5} M glibenclamide produced only minimal relaxant effects (<15%) and therefore, the concentration of U46619 was not increased.

The effects of the combination of levcromakalim plus nitroprusside were tested in MA pre-contracted by NA (3×10^{-6} M) and in PA pre-contracted by U46619 (10^{-6} M). When the contractile response reached a steady-state, rings were treated with a single concentration of levcromakalim (3×10^{-7} M in PA and 3, 5 or 7×10^{-8} M in MA) to induce a 20–35% relaxation (34 ± 5% in PA pre-contracted by U46619 and 23 ± 2% in MA pre-contracted by NA). Following this, a concentration-response curve to nitroprusside was carried out. In another set of rings, the opposite experiments were performed by pretreating with a single concentration of nitroprusside (3×10^{-7} M in PA and 3×10^{-8} M in MA) to induce a 20–35% relaxation (34 ± 4% in PA pre-contracted by U46619 and 20 ± 4% in MA pre-contracted by NA) before the concentration-response to levcromakalim. The results of these combination studies were compared with the concentration-response curves in the absence of pretreatment in (a) arteries pre-contracted by the same concentration of NA or U46619 (i.e. concentration control) or (b) arteries pre-contracted with a concentration of agonist (10^{-6} M NA in MA and 10^{-7} M U46619 in PA) which induced a similar tone to that induced by the combination of agonist plus pretreatment (i.e. tone control).

Drugs

The following drugs were used: (−)-NA bitartrate, U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ methyl acetate solution), acetylcholine chloride, sodium nitroprusside, glibenclamide (Sigma Chemical Co., London), ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, Tocris Cookson Ltd, Bristol), nicorandil (Merck Farma y Química, S.A., Barcelona) and verapamil (Knoll AG, Ludwigshafen, Germany). All drugs were dissolved initially in distilled deionized water (except levcromakalim which was dissolved in ethanol and glibenclamide and ODQ in dimethylsulphoxide (DMSO)) to prepare a 1 or 10 mM stock solution and further dilutions were made in Krebs solution.

Analysis of the results

Results are expressed as means ± s.e. means of measurements in *n* arteries. Individual cumulative concentration-response (CR) curves were fitted to a logistic equation. The maximal relaxant effect (E_{max}, expressed as a percentage of the initial contractile response), which is an index of the efficacy of the vasodilator, and the drug concentration exhibiting 50% of the E_{max} (EC_{50}, expressed as negative log molar, pD_2), which is an index of the potency of the vasodilator, were calculated from the fitted concentration-response curves for each ring. For the curves carried out in the presence of antagonists (glibenclamide or ODQ) in which a maximal relaxation was not achieved with the maximal concentration of vasodilator tested, the E_{max} was assumed to be the same as that in the absence of antagonist. The apparent pK_B value was calculated following the Schild equation: $pK_B = -\log[\text{antagonist}] + \log(\text{CR}-1)$, the CR being the ratio between the EC_{50} in the presence and absence of the antagonist. This equation is valid for competitive antagonists, which is not the case for ODQ and glibenclamide. Glibenclamide follows the Schild equation (e.g. Pérez-Vicaíno *et al*., 1993) in spite of it not being a truly competitive antagonist (Bray & Quast, 1992). ODQ also behaved as an apparent competitive antagonist against the

effects of nicorandil. However, more detailed studies have revealed that ODQ is a mixed (competitive and non-competitive) type of inhibitor (Schrammel *et al*., 1996). Therefore, apparent pK_B values were only calculated as indicators of the potency of the antagonist and do not imply any type of antagonism of the drugs. Apparent pK_B for an antagonist in the presence of a second antagonist was calculated by the CR between the EC_{50} of nicorandil in the presence of both antagonists and in the presence of the second antagonist. Statistically significant differences between groups were calculated by unpaired Student's *t* test or for multiple comparisons by an ANOVA followed by a Newman Keuls test. $P<0.05$ was considered statistically significant.

Results

Relaxant effects of levcromakalim, nitroprusside, nicorandil and verapamil in PA and MA

Following equilibration, rings were pre-contracted by equi-effective concentrations of NA or U46619 which in parallel time controls remained stable throughout the time the experiments were being carried out. Unless otherwise stated, the concentrations used were 10^{-5} M NA and 10^{-7} M U46619 for PA (904 ± 52 mg, $n=70$ and 982 ± 60 mg, $n=48$, respectively) and 10^{-6} M NA and 10^{-6} M U46619 for MA (1732 ± 142 mg, $n=35$ and 2096 ± 207 mg, $n=22$, respectively).

The relaxant effects of nicorandil, levcromakalim, nitroprusside and verapamil in piglet PA and MA pre-contracted by NA or U46619 are shown in Figure 1 and Table 1. Figure 1a shows that nicorandil (10^{-7} M -3×10^{-4} M) produced a concentration-dependent relaxation of the contractions induced by NA and U46619 in both PA and MA, but it was less potent at relaxing PA when compared with MA (3.3 and 2.7 fold less potent for NA and U46619, respectively). Furthermore, at the highest concentration tested, nicorandil produced a full relaxant response in both arteries. Levcromakalim also induced a concentration-dependent relaxant response in both PA and MA (Figure 1b). In both tissues this relaxant effect was independent of the agonist (i.e. similar results were obtained with NA and with U46619) and although levcromakalim tended to relax NA-induced contractions more potently than those induced by U46619 in PA, this difference did not reach statistical significance. However, levcromakalim induced a full relaxant response in MA whereas it only partially (E_{max} about 60%) and less potently relaxed PA (3.3 and 6.8 fold less potent for NA and U46619, respectively). Nitroprusside induced a full and similar concentration-dependent relaxant response in PA and MA, except in PA pre-contracted by U46619, where it only partially and less potently (3.3 fold less potent) relaxed these contractions (Figure 1c). The relaxant response to verapamil was dependent on both the artery and the agonist, the concentrations induced by NA in MA being more sensitive than those induced by NA in PA or by U46619 in both MA and PA (Figure 1d). The parameters of most of the

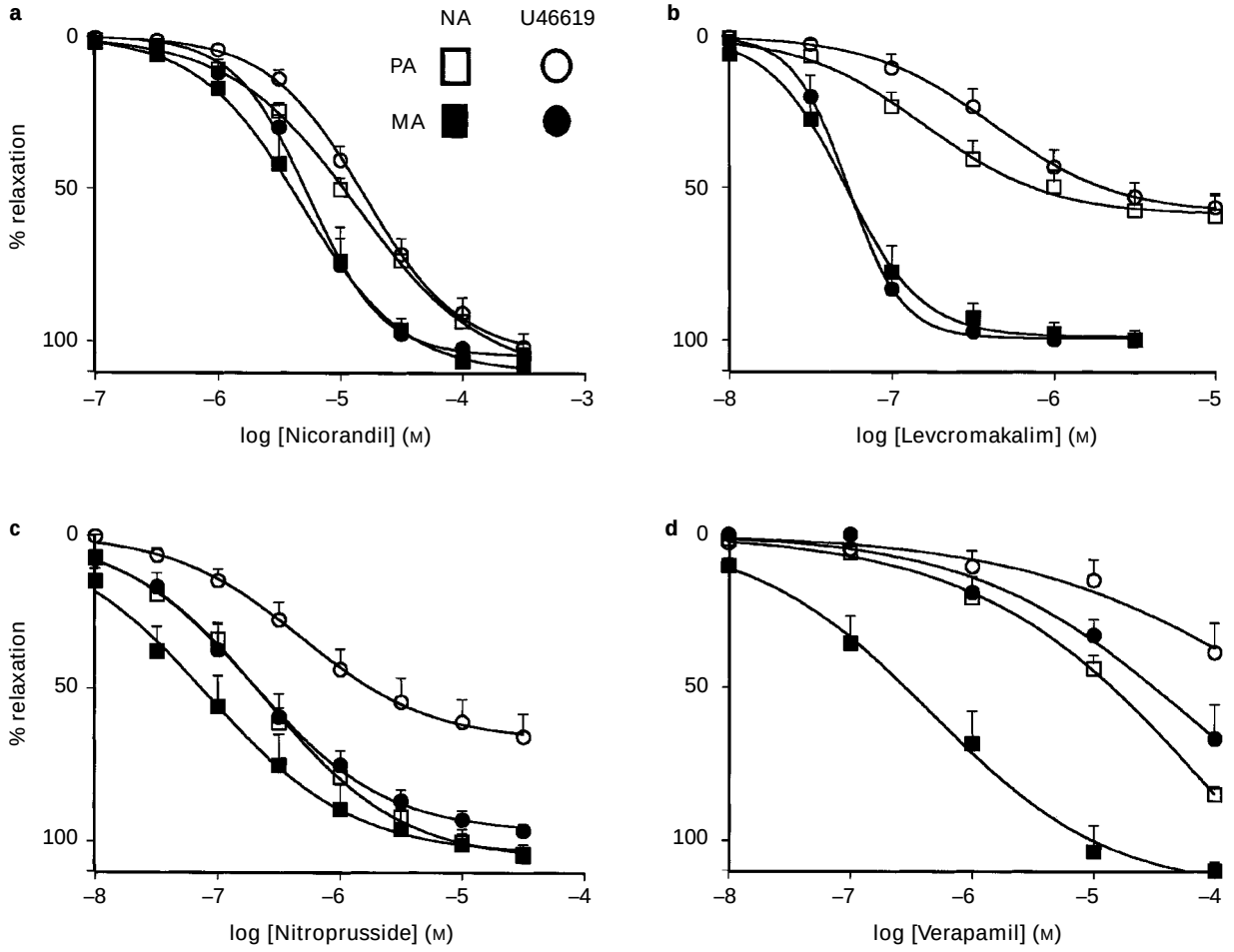

Figure 1 Concentration-response curves to nicorandil (a), levcromakalim (b), nitroprusside (c) and verapamil (d) in PA and MA pre-contracted by NA or U46619. Results are expressed as means with vertical lines showing s.e.mean. pD_2, E_{max} and *n* values are shown in Table 1.

Table 1 Potency (pD_2) and efficacy (E_{max}) of levcromakalim, nitroprusside and nicorandil at relaxing PA and MA pre-contracted by NA or U46619 (calculated from data shown in Figure 1)

	PA						*MA*					
	NA (10^{-5} M)			*U46619* (10^{-7} M)			*NA* (10^{-6} M)			*U46619* (10^{-6} M)		
	n	pD_2	E_{max}	n	pD_2	E_{max}	n	pD_2	E_{max}	n	pD_2	E_{max}
Levcromakalim	6	6.8 ±0.1	59 ±7	6	6.5 ±0.2	58 ±5	6	7.3 ±0.1#	97 ±3#	5	7.3 ±0.1#	98 ±3#
Nitroprusside	13	6.6 ±0.1	104 ±2	12	6.2 ±0.1*	66 ±7*	7	6.8 ±0.2	109 ±4	6	6.7 ±0.2#	96 ±2*#
Nicorandil	7	4.8 ±0.1	112 ±4	6	4.8 ±0.1	104 ±4	6	5.4 ±0.2#	109 ±3	6	5.2 ±0.1#	106 ±2

*$P<0.05$ vs NA. #$P<0.05$ vs PA.

concentration-response curves to verapamil could not be calculated since, at the maximal concentration tested, the drug did not induce its maximal relaxant response.

Effects of glibenclamide and ODQ on the relaxant responses to nicorandil, levcromakalim and nitroprusside

Figure 2 shows the effects of 10^{-5} M glibenclamide and 10^{-6} M ODQ on the relaxant responses to levcromakalim and nitroprusside in PA precontracted with NA. The relaxant effect of levcromakalim was not significantly affected by ODQ, whereas pretreatment with glibenclamide produced a marked rightward shift of the concentration-response curve to levcromakalim (apparent $pK_B = 6.4$). In contrast, the relaxant effects of nitroprusside were unaffected by glibenclamide, but ODQ produced a rightward shift of the curve to nitroprusside (apparent $pK_B = 7.2$).

The effects of glibenclamide and ODQ on the relaxation induced by nicorandil in PA and MA are shown in Figure 3 and Table 2. The concentration-response curve to nicorandil in PA pre-contracted by NA (Figure 3a) was inhibited by 10^{-6} M ODQ (apparent $pK_B = 7.2$), but was unaffected by 10^{-5} M glibenclamide. Furthermore, the combination of glibenclamide plus ODQ did not produce a further inhibition of nicorandil-induced relaxation as compared with ODQ alone (apparent pK_B for ODQ in the presence of glibenclamide = 7.2). In contrast to PA pre-contracted by NA, in PA pre-contracted by U46619 (Figure 3b), both ODQ and glibenclamide produced a rightward shift of the concentration-response curves to nicorandil (apparent $pK_B = 6.8$ and 5.8, respectively). Moreover, the combination of glibenclamide plus ODQ produced a further inhibition of nicorandil-induced relaxation as compared with ODQ or glibenclamide alone (apparent pK_B for ODQ in the presence of glibenclamide = 7.0, apparent pK_B for glibenclamide in the presence of ODQ = 6.0).

In MA pre-contracted by NA (Figure 3c), ODQ produced a rightward shift (apparent $pK_B = 6.5$) of the concentration-response curve to nicorandil. Glibenclamide had no significant effect on nicorandil-induced relaxations but, in the presence of ODQ, it produced a marked rightward shift of the curve (apparent pK_B for ODQ in the presence of glibenclamide = 7.2, apparent pK_B for glibenclamide in the presence of ODQ = 5.7). In MA pre-contracted by U46619 (Figure 3d), both treatments produced a rightward shift of the curve to nicorandil (apparent $pK_B = 7.0$ and 5.3 for ODQ and glibenclamide, respectively), whereas the combination of both treatments further shifted the curve (apparent pK_B for ODQ in the presence of glibenclamide = 7.0, apparent pK_B for glibenclamide in the presence of ODQ = 5.3).

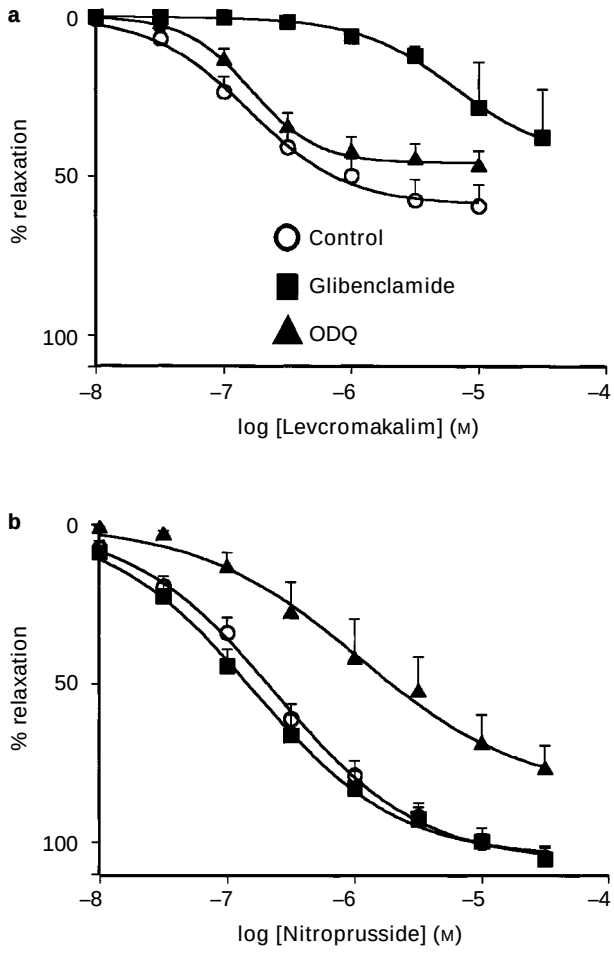

Figure 2 Concentration-response curves to levcromakalim (a) and nitroprusside (b) in PA precontracted by NA in the absence (control) or in the presence of 10^{-5} M glibenclamide or 10^{-6} M ODQ. Results are expressed as means with vertical lines showing s.e.mean ($n = 5–7$).

Effects of the combination of levcromakalim plus nitroprusside

We tested whether pretreatment with levcromakalim could modify the relaxant response to nitroprusside or, conversely, whether pretreatment with nitroprusside could modify the relaxant response to levcromakalim. The concentrations of the pretreatments were chosen to induce a 20–35% relaxation. The results were compared with (a) arteries pre-contracted by the same concentration of NA or U46619 (concentration control) or (b) arteries pre-contracted with a concentration of

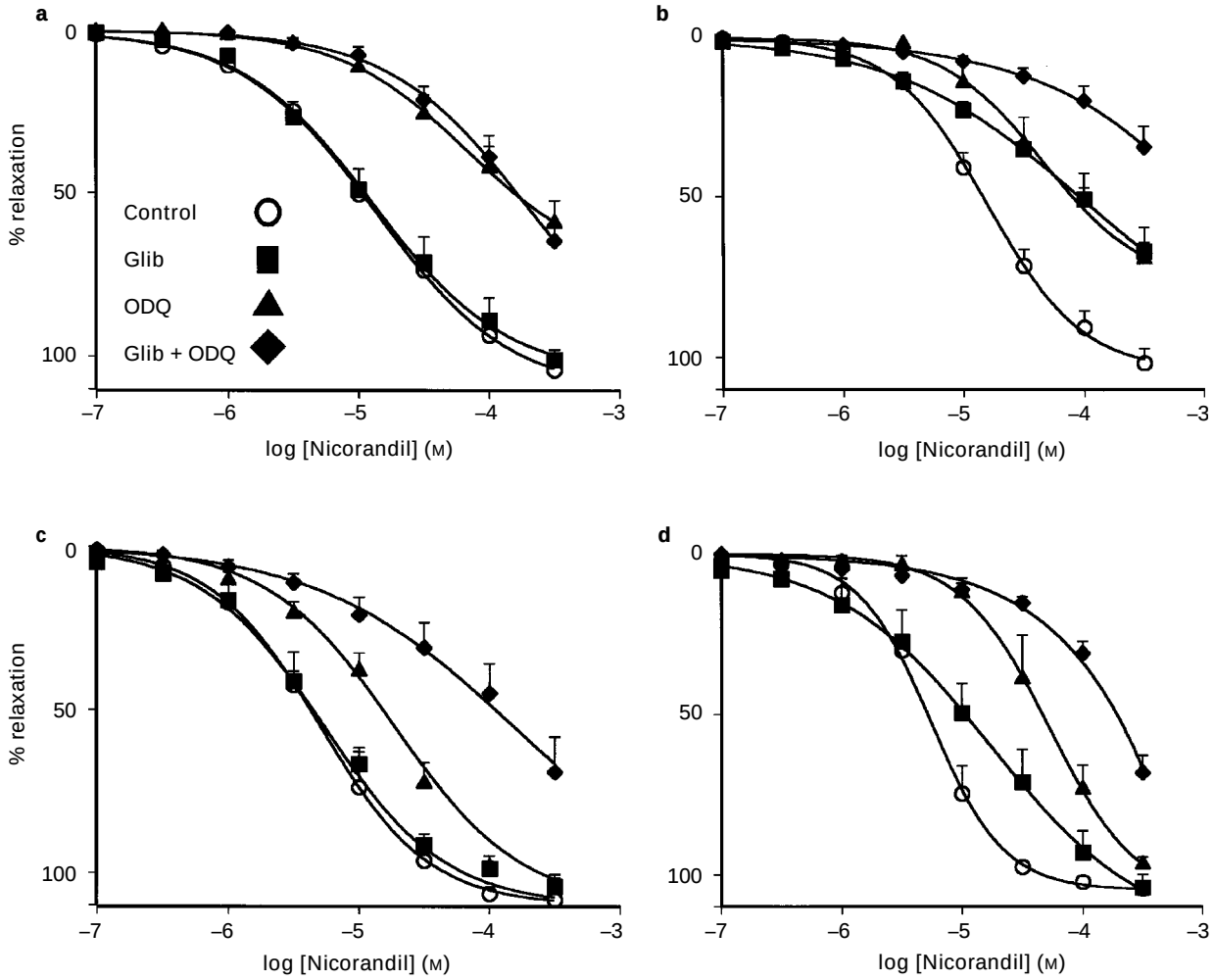

Figure 3 Concentration-response curves to nicorandil in PA (a and b) and MA (c and d) precontracted by NA (a and c) or U46619 (b and d) in the absence (control) or in the presence of 10^{-5} M glibenclamide (Glib), 10^{-6} M ODQ or both inhibitors. Results are expressed as means with vertical lines showing s.e.mean. Apparent pK_B and n values are shown in Table 2.

Table 2 Apparent pK_B values of glibenclamide (Glib) and ODQ at inhibiting nicorandil-induced relaxation in PA and MA pre-contracted by NA or U46619

		PA				*MA*			
		NA		*U46619*		*NA*		*U46619*	
		n	pK_B	n	pK_B	n	pK_B	n	pK_B
Glib	−ODQ	9	< 5	6	5.8	5	< 5	4	5.3
	+ODQ	5	< 5	8	6.0	8	5.7	4	5.3
ODQ	−Glib	6	7.2	8	6.8	8	6.5	6	7.0
	+Glib	5	7.2	8	7.0	8	7.2	4	7.0

The results presented were calculated from data shown in Figure 3.

agonist which induced a similar tone to that induced by the combination of NA or U46619 plus nitroprusside or levcromakalim (tone control). Figure 4a shows that in arteries pre-contracted by U46619, levcromakalim enhanced the maximal relaxant response of nitroprusside ($E_{max} = 101 \pm 7\%$, $n = 6$, $P < 0.01$ vs $66 \pm 7\%$, $n = 12$ and $67 \pm 4\%$, $n = 14$, concentration control and tone control, respectively), but the pD_2 value of nitroprusside (6.0 ± 0.1) was not significantly different from that obtained in the absence of levcromakalim (5.9 ± 0.1 and 6.2 ± 0.1 concentration control and tone control, respectively). The relaxant responses to nitroprusside in MA pre-contracted by NA in the presence of levcromakalim were not significantly different from those in the absence of levcromakalim (Figure 4b). Similarly, in PA pre-contracted by NA, pretreatment with levcromakalim produced no change in the relaxant effects of nitroprusside (not shown). Pretreatment with nitroprusside (Figure 4c) enhanced the maximal relaxant response induced by levcromakalim in PA pre-contracted by U46619 ($E_{max} = 77 \pm 3\%$, $n = 4$, $P < 0.01$ vs $47 \pm 4\%$, $n = 5$ and $57 \pm 5\%$, $n = 6$, concentration control and tone control, respectively) but the pD_2 value of levcromakalim (6.7 ± 0.1) was not significantly different from that obtained in the absence of nitroprusside (6.7 ± 0.1 and 6.5 ± 0.1 concentration control and tone control, respectively). The relaxant responses to levcromakalim in MA pre-contracted by NA in the presence of nitroprusside were not significantly different from those in the absence of nitroprusside (Figure 4d).

Discussion

In this paper we have compared the vasorelaxant effects of nicorandil with those of levcromakalim, nitroprusside and verapamil in piglet endothelium-denuded PA and MA with a similar diameter pre-contracted by equieffective concentrations of NA or U46619. The results can be summarized as follows. (1) All these vasodilator drugs exhibited different potency and/

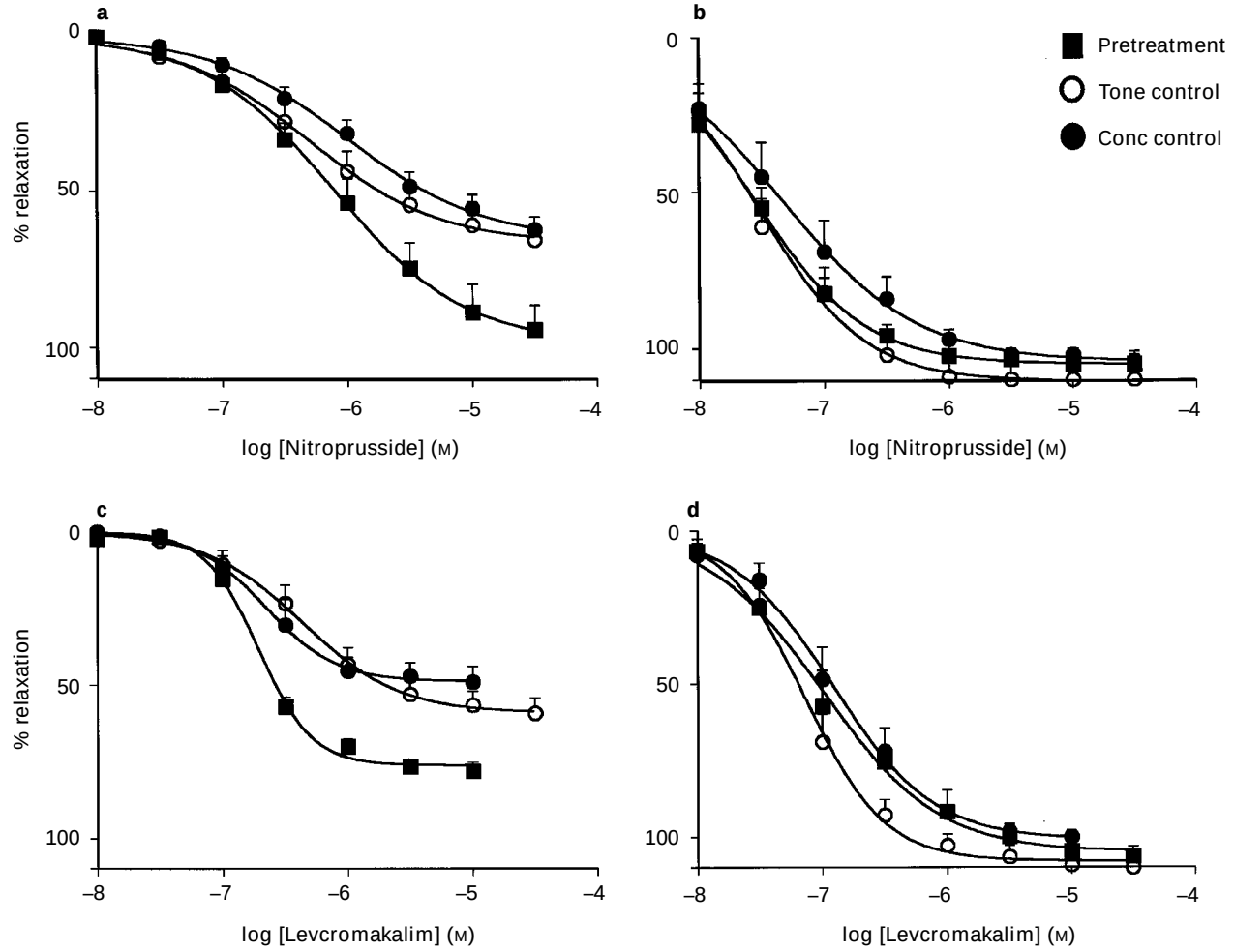

Figure 4 Effects of the combination of nitroprusside and levcromakalim. Concentration-response curves to nitroprusside in the presence or absence of levcromakalim (a and b) and concentration-response curves to levcromakalim in the presence or absence of nitroprusside (c and d) were carried out in PA pre-contracted by U46619 (a and c) and MA pre-contracted by NA (b and d). The concentrations of levcromakalim and nitroprusside as pretreatments were chosen to relax the artery to 20–35% of the initial contraction. Two types of control responses are shown: arteries contracted by the same concentration of vasoconstrictor as in the presence of pretreatment (Conc control) or arteries contracted with a concentration of vasoconstrictor that produced a contraction similar to the final tone induced by the vasoconstrictor plus pretreatment (Tone control). See Methods for details of concentrations of vasoconstrictors and pretreatments. Results are expressed as means with vertical lines showing s.e.mean ($n=4-14$).

or different efficacy depending on the artery and/or the agonist used to pre-contract the artery. (2) Despite nitroprusside and levcromakalim alone having low efficacy at relaxing PA pre-contracted by U46619, the combination of both drugs or the dual drug nicorandil was able to induce full vasodilatation. However, pretreatment with nitroprusside did not modify the pD_2 values for levcromakalim or vice versa. (3) ODQ inhibited the relaxation induced by nicorandil in all experimental conditions. Glibenclamide inhibited nicorandil-induced relaxation in PA and MA pre-contracted by U46619, but had no effect in PA or MA pre-contracted by NA, even though in MA pre-contracted by NA it potentiated the inhibitory effect of ODQ.

The effects of levcromakalim were markedly dependent on the artery, being 3–6 fold more potent and more effective in MA than in PA. In rabbit arteries, the K_{ATP} channel opener aprikalim was also about 10 fold more potent at relaxing MA than PA although a similar efficacy was found for both tissues (Magnon *et al*., 1994). Similarly, the vasorelaxant effects of verapamil (present results) and nifedipine (Pérez-Vizcaíno *et al*., 1996) were more potent in MA than in PA from neonatal piglets. However, in contrast to levcromakalim, verapamil and nifedipine were more potent relaxing arteries pre-contracted by NA than by U46619. These results suggest that U46619-induced contractions are less dependent on Ca^{2+} entry through L-type channels than NA-induced contractions. This is consistent with previous evidence indicating that in rabbit PA U46619 induces a greater sensitization of contractile filaments to Ca^{2+} than the α-adrenoceptor agonist phenylephrine (Himpens *et al*., 1990). Furthermore, the different profile of inhibition of levcromakalim and verapamil suggests that the relaxant effect of levcromakalim cannot be accounted for solely by its inhibitory action on Ca^{2+} entry and, therefore, other mechanisms must also be involved (Quast, 1993).

Nitroprusside fully relaxed MA pre-contracted by either U46619 or NA and PA precontracted by NA with a similar potency, while it induced only a partial and less potent relaxation in PA pre-contracted by U46619. Furthermore, other drugs acting through stimulation of the guanosine 3′:5′-cyclic monophosphate (cyclic GMP) pathway, such as atrial natriuretic peptide or 8-bromo-cyclic GMP, were also unable to induce a full relaxant response in U46619 pre-contracted PA (Pérez-Vizcaíno *et al*., 1997a).

Interestingly, the E_{max} to levcromakalim and nitroprusside in PA pre-contracted with U46619 was only about 60%,

whereas nicorandil induced a full relaxant response. In addition, nitroprusside induced a full relaxant response in PA when they were previously partially relaxed by levcromakalim or, conversely, the maximal relaxation of levcromakalim was increased by pretreatment with nitroprusside. Thus, the combination of both mechanisms, either by nicorandil alone or by the association of levcromakalim plus nitroprusside, abolishes the component of contraction in PA which is resistant to dilatation by one of the mechanisms alone, indicating that the mechanisms responsible for the resistance of U46619-induced contraction to dilate in response to nitroprusside are different from those to levcromakalim.

Nicorandil induces vasodilatation via two main mechanisms of action, namely stimulation of guanylate cyclase through its nitric oxide donor activity (sharing this effect with nitroprusside) and activation of K_{ATP} channels (sharing this effect with levcromakalim). Whether one of these two mechanism prevails or both coexist is dependent on the vascular preparation investigated and on the nature of the agonist employed to pre-contract the artery (Borg *et al*., 1991; Magnon *et al*., 1994; Akai *et al*., 1995). Magnon *et al*. (1994) found differences in the mechanism of nicorandil-induced relaxation in arteries pre-contracted by KCl and by NA. In our study, we found differences in the mode of nicorandil-induced relaxation even in arteries pre-contracted by two vasoconstrictors (NA and U46619) acting through stimulation of G-protein coupled receptors. In PA pre-contracted by NA, the main mechanism of action of nicorandil appears to be due to stimulation of guanylate cyclase, since its vasorelaxant effects were inhibited by ODQ, but not by glibenclamide, alone or in combination with ODQ. In MA pre-contracted by NA, ODQ also inhibited nicorandil-induced relaxation but glibenclamide alone had no effect. However, when given in combination with ODQ, it strongly shifted the concentration-response curve to nicorandil, indicating that both mechanisms are involved in the relaxant effect. Similar results have been obtained in rabbit MA and PA pre-contracted by NA (Magnon *et al*., 1994). In contrast, in PA and MA precontracted by U46619, both ODQ and glibenclamide inhibited nicorandil-induced relaxation, indicating that both activation of guanylate cyclase and K_{ATP} channel opening participate in the relaxant effects of nicorandil. Thus, in the present study activation of guanylate cyclase seemed to be a mechanism involved in the relaxant effects of nicorandil in all the experimental conditions, whereas the contribution of K_{ATP} was more variable. Furthermore, in PA precontracted by NA the inhibitory potency of ODQ on nicorandil-induced relaxations was similar to that observed on nitroprusside-induced relaxations, whereas glibenclamide was less potent at inhibiting nicorandil- than levcromakalim-induced relaxations.

Although the explanation for the varying contribution of each mechanism, depending on the vessel and agonist, to nicorandil-induced relaxation remains unclear, two hypothesis can be proposed. First, if in a given condition, one mechanism acts more effectively than the other, one could expect that nicorandil would act preferably through that mechanism. This might explain why: (a) in PA pre-contracted by NA, which show lower E_{max} values to levcromakalim than to nitroprusside, the effects of nicorandil were insensitive to glibenclamide but sensitive to ODQ; and (b) in PA and MA pre-contracted by U46619, which showed similar E_{max} values to levcromakalim and nitroprusside, nicorandil-induced relaxations were sensitive to both glibenclamide and ODQ. However, the small differences in E_{max} values to levcromakalim and nitroprusside found in MA pre-contracted by NA do not appear to explain the insensitivity of nicorandil-induced relaxations to glibenclamide.

A second hypothesis is the possible interactions between the two vasodilator pathways. In MA pre-contracted by NA, the potency of glibenclamide and ODQ increased (higher apparent pK_B values for both inhibitors) when used in combination. A similar increase of the effects of glibenclamide on nicorandil-induced relaxation has been found by the inhibition of the cyclic GMP pathway with methylene blue (Kreye *et al*., 1992) or after the induction of nitrate tolerance in porcine coronary arteries (O'Rourke, 1996). If negative interactions are assumed between both pathways, glibenclamide might reduce the potency of nicorandil as a K^+ channel opener but, as a consequence, it might remove the negative influence of K^+ channel opening on cyclic GMP-induced relaxation and, thus, it could increase the potency of nicorandil as a guanylate cyclase stimulant. Negative interactions between K_{ATP} channel openers, and basal nitric oxide have been observed in two recent studies in anaesthetized pigs (Herity *et al*., 1994) and in rat isolated mesenteric bed (McCulloch & Randall, 1996) but not in conscious rats (Gardiner *et al*., 1991). However, in the present study, levcromakalim did not modify the pD_2 values of nitroprusside and, conversely, nitroprusside did not modify the pD_2 values of levcromakalim. Therefore, under the present experimental conditions, there are not apparent interactions between the two mechanisms. Thus, the variable contribution of K_{ATP} channel opening and stimulation of guanylate cyclase on nicorandil-induced relaxation depending on vessel and agonist, cannot be accounted for by different interactions between both pathways.

In conclusion, K^+ channel opening and guanylate cyclase stimulation are independent pathways that induce vasorelaxation in piglet PA and MA. These two mechanisms are additive, since even under conditions were maximal activation of any of them produce only partial relaxation, the combination of both (either by combining levcromakalim plus nitroprusside or by the dual effects of nicorandil) is able to relax fully agonist-induced vasoconstriction. Moreover, the mechanism of nicorandil is dependent on the artery and the agonist employed to pre-contract the artery. The relative efficacy of K^+ channel opening vs guanylate cyclase stimulation may partially explain the preferential contribution of each mechanism to the relaxant effects of nicorandil. However, we failed, to demonstrate that interactions between the two pathways could be responsible for the varying contribution of each pathway to the relaxant effects of nicorandil.

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