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Endothelium-mediated and N^{ω}-nitro-L-arginine methyl ester-sensitive responses to cromakalim and diazoxide in the rat mesenteric bed

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Abstract

The effects of two 'K^+ channel openers', (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-benzo[*b*]-pyran-3-ol (cromakalim) and 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (diazoxide), were studied on the rat isolated mesenteric bed. Differences in the perfusion pressure were measured as a parameter of vascular resistance. Cromakalim (0.1–700 μM) and diazoxide (1 μM–1 mM) reduced to 60% the contractions elicited by 10 μM noradrenaline and to 30% those evoked by 100 mM KCl. The relaxant effects of cromakalim and diazoxide on the noradrenaline-induced contractions were reduced by the K^+-ATP channel blocker, 5-chloro-*N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]-sulfonyl]phenyl]ethyl]-2-methoxybenzamide (glibenclamide, 0.01–0.3 μM), endothelium removal with 0.1% saponin and pretreatment with the nitric oxide synthesis inhibitor, *S*(±)-N^5-[imino(nitroamino)methyl]-L-ornithine methyl ester hydrochloride (L-NAME, 500 μM). Reductions in the relaxant responses after endothelium removal or L-NAME pretreatment were observed with 1–100 μM cromakalim and with 30 μM diazoxide but not with 100 and 300 μM diazoxide. Pretreatment with the inactive stereoisomer D-NAME as well as with the prostanoid synthesis inhibitor, 1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid (indomethacin, 10 μM), did not affect the reductions in contractile responses to noradrenaline caused by either cromakalim or diazoxide. It is concluded that the relaxant effects of cromakalim and diazoxide in the rat mesenteric bed are endothelium-mediated and L-NAME-sensitive and could at least partially involve the participation of nitric oxide.

Keywords: K^+ channel opener; Cromakalim; Diazoxide; Mesenteric bed, rat; Endothelium; Nitric oxide (NO)

1. Introduction

ATP-sensitive K^+ channels have been described in guinea pig and rabbit cardiac muscles (Noma, 1983), rat pancreatic β cells (Cook and Hales, 1984), frog skeletal muscle (Spruce et al., 1985), rat neuronal cells (Ashford et al., 1989) and rabbit as well as rat arteries (Standen et al., 1989). The opening of these ATP-sensitive K^+ channels in vascular smooth muscles leads to relaxation through the outward movement of K^+ from the cell, which causes an intracellular hyperpolarization (Hamilton et al., 1986) that, in turn, prevents Ca^{2+} entry through voltage-operated Ca^{2+} channels (Cook, 1988).

Compounds that produce opening of ATP-sensitive K^+ channels have been shown to act as vasodilators in several vascular smooth muscles, such as rat aortic, coronary and renal arteries (for review see Richer et al., 1990). Among others, cromakalim (Hamilton et al., 1986) and diazoxide (Newgreen et al., 1990) are standard representatives of this group of smooth muscle relaxant agents, known as the 'K^+ channels openers' (Weston and Abott, 1987), whose special characteristic is that they belong to a chemically heterogeneous group of compounds (Edwards and Weston, 1990). The pharmacological effects of the 'K^+ channel openers' show tissue selectivity. For instance, cromakalim dilates the rabbit coronary, gastrointestinal, and cerebral vessels but not those of the kidneys and skeletal muscle (Hof et al., 1988).

This tissue specificity is apparently linked to differences in ATP sensitivity and single channel conductance (Quast

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and Cook, 1989), and also probably to the existence of other factors that contribute to the vasodilatator activity of the K^+ channel openers (Hof et al., 1988). There are a number of substances, such as acetylcholine, bradykinin, substance P and ADP, that can produce vasodilation through a hyperpolarizing mechanism that is endothelium-dependent (for reviews, see Furchgott, 1984; Brayden et al., 1991).

Nevertheless, controversial evidence exists about the influence of endothelium-derived relaxing factors on the hyperpolarization-induced vasodilation produced by the 'K^+ channel openers'. For instance, endothelium dependence has been shown for cromakalim and pinacidil in the dog epicardial coronary artery (Drieu La Rochelle et al., 1992) and for LP-805 in the rat thoracic aorta (Kishii et al., 1992b) whereas endothelium independence has been reported for cromakalim in rat aorta and porcine coronary arteries (Bray and Quast, 1991). The aim of the present work was to study the effects of the 'K^+ channel openers' cromakalim and diazoxide on a peripheral resistance tissue such as the isolated mesenteric vascular bed of the rat and to analyze if these effects are dependent not only on the opening of the K^+-ATP channels, but also on the presence of endothelium-derived relaxing factors. The tissue was selected on the basis of its substantial contribution to the control of peripheral resistance (Christensen and Mulvany, 1993).

2. Materials and methods

2.1. Mesenteric vascular bed preparation

Male Wistar rats (200–250 g) were used. The animals were anesthetized with ether and the mesenteric vascular bed was isolated according to the method described by McGregor (1965). The abdominal cavity was opened and a polyethylene cannula was inserted into the superior mesenteric artery before the removal of the whole mesenteric bed, which was cut close to the intestinal border of the mesentery.

The isolated preparation was perfused at a rate of 2 ml/min, using a peristaltic pump (Desaga, Heidelberg, Germany), with Krebs-Henseleit solution whose composition was (mM): NaCl 118; KCl 4.7; $MgSO_4$ 1.2; $NaHCO_3$ 25; NaH_2PO_4 1.0; $CaCl_2$ 2.6; glucose 11.1; EDTA disodium salt 0.004 and ascorbic acid 0.11. The solution was gassed with a 95% O_2/5% CO_2 mixture at 37°C. The final pH was 7.4.

2.2. Experimental protocol

After an equilibration period of 60 min at 37°C, the isolated mesenteric vascular bed was contracted with either 10 μM noradrenaline or 100 mM KCl infused for 15 min. At this time a plateau contractile response was reached and a non-cumulative concentration-response curve for either cromakalim (0.1–700 μM) or diazoxide (1 μM–1 mM) was started.

In other experiments, the preparation was contracted up to four times with a bolus injection of 30 nmol noradrenaline, 30 min apart. First and second contractions in response to noradrenaline were elicited in the presence of saline and were considered as the controls. The third contraction was preceded by a 15-min incubation with either 1 μM cromakalim or 30 μM diazoxide. For the subsequent fourth contraction either cromakalim or diazoxide was added simultaneously with one of the following drugs: glibenclamide (0.01, 0.1 and 0.3 μM) used as a K^+-ATP channel blocker (Schmid-Antomarchi et al., 1987; Fosset et al., 1988) and indomethacin (10 μM), used as a prostanoid synthesis inhibitor.

To evaluate the contribution of nitric oxide to the relaxation induced by the K^+ channel openers, three consecutive concentration-response curves in response to a bolus injection of noradrenaline (1, 2, 3, 10, 20 and 30 nmol) were recorded, 30 min apart. First curves were recorded in the presence of saline and considered as the controls. Second curves were recorded in the presence of either N^{ω}-nitro-L-arginine methyl ester (L-NAME 500 μM) or N^{ω}-nitro-D-arginine methyl ester (D-NAME 500 μM). Third curves were recorded under the simultaneous addition of the K^+ channel openers cromakalim and diazoxide and the nitric oxide synthase inhibitor L-NAME.

In other series of experiments second and third curves were obtained after a 45-s perfusion with 0.1% saponin either alone or simultaneously with the K^+ channel openers 1 μM cromakalim or 30 μM diazoxide. In some experiments non-cumulative concentration-responses curves for either cromakalim (1, 10 and 100 μM) or diazoxide (30, 100 and 300 μM) were evaluated on a unique response induced by a low (1 nmol) concentration of noradrenaline. Differences in perfusion pressure were measured as a parameter of vascular resistance and monitored by means of a Statham pressure transducer (P23AC) connected to a Grass polygraph (model 7 PCPA). The perfusion pressure, approximately 50 mmHg, remained constant during the equilibration period and it was taken as the basal value to assess changes in perfusion pressure induced by the different agents.

Results are expressed as percentage of relaxation, i.e. as percentage of the reduction of the maximal contractile responses elicited by either noradrenaline or KCl.

The endothelium-denuded preparations were prepared by perfusing them for 45 s with 0.1% saponin (Aristegui and Enero, 1990). To confirm that the removal of endothelial cells was complete, 0.1 μM acetylcholine was infused in the noradrenaline-contracted mesenteric preparations, and the absence of relaxation was taken as an index of the removal of the endothelium (Furchgott, 1984).

2.3. Drugs

The following drugs were used: (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-benzo-[*b*]-pyran-3-ol (cromakalim, Smith Kline Beecham), 5-chloro-*N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxybenzamide (glibenclamide, Roussell Uclaf), noradrenaline bitartrate, saponin, 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (diazoxide), 1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid (indomethacin), 2-(acetyloxy)-*N*,*N*,*N*-trimethylethanaminium iodide (acetylcholine iodide), pentakis(cyano-C)nitrosyl-ferrate(2-) disodium (sodium nitroprusside), *S*(+)-N^5-[imino(nitroamino)methyl]-L-ornithine methyl ester hydrochloride (L-NAME) and *R*(−)-N^5-[imino(nitroamino)methyl]-D-ornithine methyl ester hydrochloride (D-NAME) from Sigma. Stock solutions (10^{-2} M) of cromakalim, diazoxide and glibenclamide were prepared weekly in a 30% ethanol/70% distilled water mixture and the subsequent dilutions were made daily in Krebs solution. Saponin, L-NAME and D-NAME were dissolved daily in distilled water. Noradrenaline (10^{-2} M) was dissolved with 0.01 M HCl.

2.4. Statistical analysis

The contractile effects evoked by the different treatments are expressed as percentages of the maximal responses to either noradrenaline or KCl. The IC_{50} and IC_{20} values correspond to the concentration of agonist producing either 50% or 20% reduction of the maximal response. Mean values ± S.E.M. were compared by means of either Student's *t*-test or two-way analysis of variance (ANOVA) followed by Scheffé's test. A P value < 0.05 was regarded as significant.

3. Results

3.1. Effects of cromakalim and diazoxide on noradrenaline- and KCl-induced contractions of the rat mesenteric bed

In the rat isolated mesenteric vascular bed perfusion of 10 μM noradrenaline for 15 min elicited a contractile response of 48.6 ± 7.1 mmHg ($n = 9$), which reached its maximum 2 min after the onset of the perfusion and remained stable up to the end of the perfusion period.

As shown in Fig. 1, both cromakalim (0.1–3 μM) and diazoxide (1–100 μM) produced a concentration-dependent relaxation of the contraction induced by 10 μM noradrenaline in the rat mesenteric bed. The reduction of the contractile response to noradrenaline reached up to 60% with either drug and the corresponding IC_{50} values were 0.60 ± 0.03 μM, $n = 9$, for cromakalim and 15.9 ± 3.9 μM, $n = 6$, for diazoxide.

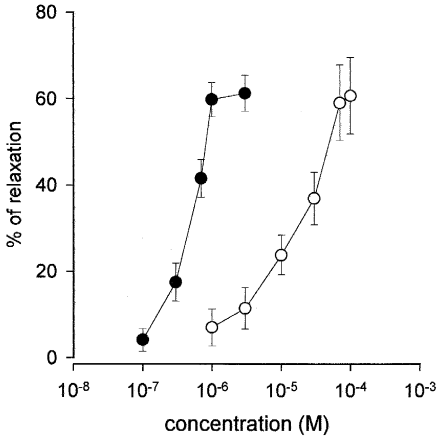

Fig. 1. Effects of cromakalim ($n = 9$, filled circles) and diazoxide ($n = 6$, open circles) on the noradrenaline-induced contraction of the rat isolated mesenteric vascular bed. Ordinate: percentage relaxation expressed as a reduction of the maximal contractile response elicited by 10 μM noradrenaline (48.6 ± 7.1 mmHg, $n = 9$).

Moreover, both cromakalim (1–700 μM) and diazoxide (10 μM–1 mM) also produced a concentration-dependent relaxation of the contraction elicited by 100 mM KCl in the rat mesenteric bed (Fig. 2). In this case, the maximal relaxant effect of both drugs was approximately 30% of the maximal contraction elicited by KCl, in spite of the fact that the maximal contractile responses induced by 100 mM KCl (55.0 ± 3.6 mmHg, $n = 6$) did not differ from those elicited by 10 μM noradrenaline (48.6 ± 7.1 mmHg, $n = 9$). The IC_{20} values for the relaxation induced by the K^+ channel openers of the KCl-induced contraction were 8.5 ± 2.1 μM, $n = 5$, for cromakalim and 316.0 ± 12.9 μM, $n = 4$, for diazoxide.

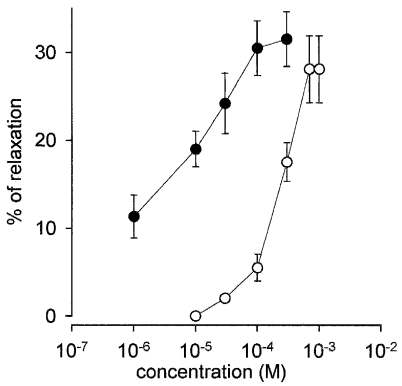

Fig. 2. Effects of cromakalim ($n = 5$, filled circles) and diazoxide ($n = 4$, open circles) on the KCl-induced contraction of the rat isolated mesenteric bed. Ordinate: percentage relaxation expressed as a reduction of the maximal contractile response induced by 100 mM KCl (55.0 ± 3.6 mmHg, $n = 6$).

3.2. Effects of glibenclamide on the reduction of noradrenaline-induced contractions caused by cromakalim and diazoxide

As shown in Fig. 3, the simultaneous addition of the ATP-sensitive K^+ channel blocker glibenclamide with either cromakalim or diazoxide prevented the reductions caused by these drugs of the contraction elicited by 10 μM noradrenaline, i.e., the relaxation elicited by cromakalim under control conditions was almost entirely abolished in the presence of 0.01 μM glibenclamide. In the case of diazoxide, the reduction elicited under control conditions was not prevented by 0.01 μM glibenclamide but it was reduced to about 50% by 0.1 μM glibenclamide, and it was almost completely abolished by 0.3 μM glibenclamide. Glibenclamide per se did not modify at any of the concentrations tested the basal perfusion pressure of the tissues.

3.3. Effects of endothelium removal and of nitric oxide synthesis inhibition on the contractions elicited by noradrenaline

The removal of the endothelium, by 45 s exposure to 0.1% saponin, caused a transient increase in the perfusion pressure of 40 ± 8.78 mmHg that returned to basal levels at the time that the concentration-response curve for noradrenaline was started, i.e., 45 min after the onset of the saponin perfusion. As shown in Fig. 4A, the contractile responses to noradrenaline were significantly increased in the de-endothelialized tissues compared to control values. This increase reached up to 300% of control responses with 1 nmol noradrenaline and was smaller at the higher concentrations of noradrenaline used.

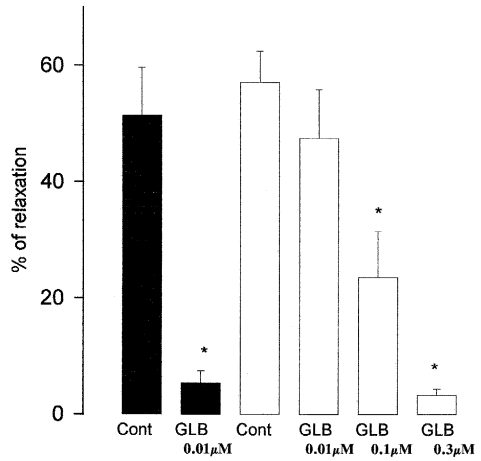

Fig. 3. Effect of glibenclamide (GLB) on the reductions of the contractile responses to 30 nmol noradrenaline induced by either 1 μM cromakalim (filled bars) or 30 μM diazoxide (open bars). Results are means ± S.E.M. of 5–10 experiments per group. * $P < 0.05$ compared to the corresponding control values. The absolute control values for the contractions elicited by 30 nmol noradrenaline were 62.8 ± 5.7 mmHg, $n = 7$, for the cromakalim group and 59.8 ± 4.1 mmHg, $n = 10$, for the diazoxide group.

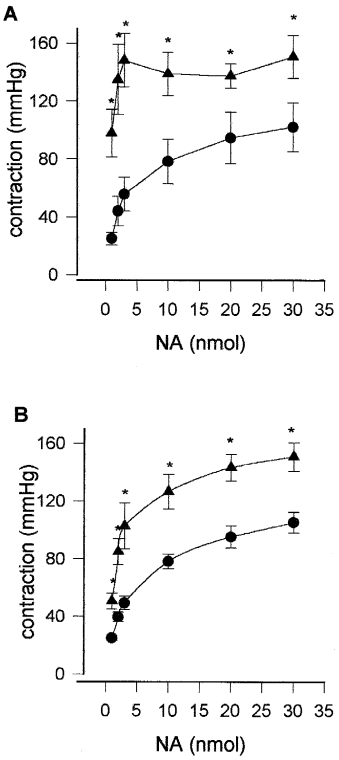

Fig. 4. Rat isolated mesenteric bed. Effects of endothelium removal with 0.1% saponin (panel A, filled triangles, $n = 7$) and of pretreatment with 500 μM L-NAME (panel B, filled triangles, $n = 8$) on the contractions elicited by different concentrations of noradrenaline (NA). The corresponding control values in A and in B are depicted by filled circles. Results are means ± S.E.M. * $P < 0.05$ compared to the correponding control values.

Different to the response observed with saponin, the inhibitor of nitric oxide synthesis L-NAME at 500 μM did not modify per se the basal perfusion pressure (data not shown). Nevertheless, the potentiation caused by L-NAME of the contractile response to noradrenaline was as high as that observed in the case of saponin (Fig. 4B).

3.4. Effects of indomethacin on the reduction of noradrenaline-induced contraction caused by cromakalim and diazoxide

As shown in Fig. 5, the simultaneous addition of 10 μM indomethacin, a prostanoid synthesis inhibitor, with either 1 μM cromakalim or 30 μM diazoxide did not modify the relaxation caused by these drugs of the contractions elicited by noradrenaline.

3.5. Effects of cromakalim and diazoxide on endothelium-denuded mesenteric bed

Fig. 6A and B shows the effects of a 15 min pretreatment with either 1 μM cromakalim or 30 μM diazoxide

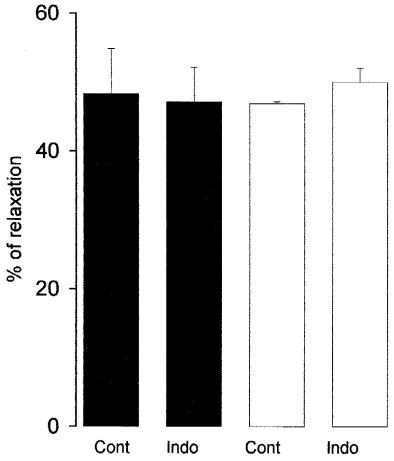

Fig. 5. Rat isolated mesenteric bed. Effects of 10 μM indomethacin (Indo) on the relaxation produced by either 1 μM cromakalim (filled bars) or 30 μM diazoxide (open bars) on the contraction elicited by 30 nmol noradrenaline. Results are means ± S.E.M. of 4–5 experiments per group. The absolute control values of noradrenaline contractions were 50.3 ± 3.8 mmHg, $n = 5$, for the cromakalim group and 45.5 ± 4.1 mmHg, $n = 4$, for the diazoxide group.

on the contractile responses elicited by different concentrations of noradrenaline in endothelium-denuded mesenteric beds. The removal of the endothelium reduced significantly ($P < 0.05$) the relaxation caused by either cromakalim or diazoxide of the contractile responses to all concentrations of noradrenaline tested. This reduction was between 32 and 46% in the case of cromakalim and between 20 and 36% in the case of diazoxide. The percentage reduction caused by endothelium removal at the different noradrenaline concentrations in the cromakalim as well as the diazoxide group did not show any significant difference when tested by one-way analysis of variance (ANOVA).

3.6. Effects of L-NAME and D-NAME on the reduction of noradrenaline-induced contractions produced by cromakalim and diazoxide

As shown in Fig. 7, the pretreatment with 500 μM L-NAME, a nitric oxide synthesis inhibitor, reduced the relaxation caused by either 1 μM cromakalim or 30 μM diazoxide of the noradrenaline-induced contractions, i.e., the relaxations obtained under control conditions were significantly diminished to 40–55% in the case of cromakalim and to 14–28% in the case of diazoxide. This reduction was stereospecific, as shown by the lack of effect of 500 μM D-NAME on the relaxation caused by either cromakalim or diazoxide. Moreover, as observed for the removal of the endothelium, the percentage reduction

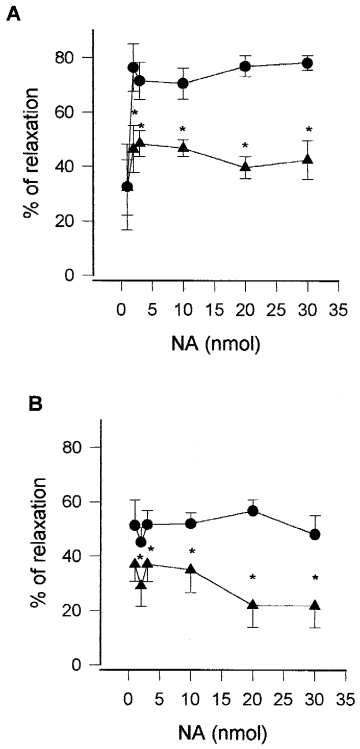

Fig. 6. Rat isolated mesenteric bed. Effects of the removal of endothelium with 0.1% saponin (filled triangles) on the reductions produced by either 1 μM cromakalim (panel A, $n = 8$) or 30 μM diazoxide (panel B, $n = 8$) on the contractions elicited by different concentrations of noradrenaline (NA). The corresponding control values in A and B are depicted by filled circles. Results are means ± S.E.M. * $P < 0.05$ compared to the corresponding control values.

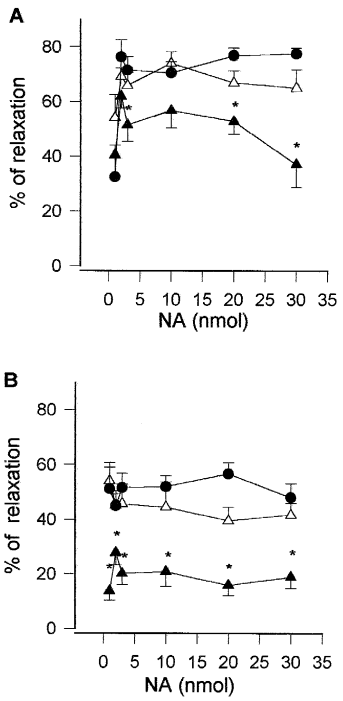

Fig. 7. Rat isolated mesenteric bed. Effects of 500 μM L-NAME (filled triangles) and 500 μM D-NAME (open triangles) on the percentage reduction produced by 1 μM cromakalim (panel A, $n = 5–6$) and by 30 μM diazoxide (panel B, $n = 5–6$) of the contractions elicited by different concentrations of noradrenaline (NA). The corresponding control values are depicted by filled circles. Results are means ± S.E.M. * $P < 0.05$ compared to the corresponding control values.

caused by L-NAME at the different concentrations of noradrenaline in either the cromakalim or the diazoxide group did not show any significant difference when tested by one-way analysis of variance (ANOVA).

3.7. Effects of different concentrations of cromakalim and diazoxide on the contractions elicited by low doses of noradrenaline either in the absence of the endothelium or in the presence of L-NAME

As already shown in Fig. 4, the removal of the endothelium with saponin as well as the inhibition of nitric oxide production by L-NAME markedly augmented the vasoconstrictor responses to noradrenaline. In order to preclude that the attenuation of the vasodilator effects of cromakalim and diazoxide (Figs. 6 and 7) could be a consequence of these higher responses to noradrenaline, concentration-response curves for cromakalim and diazoxide were made in preparations preconstricted to a similar degree by a low dose of noradrenaline (1 nmol). As shown in Figs. 8 and 9, the reductions in contractile responses to noradrenaline elicited by concentrations of cromakalim between 1 and 100 μM were again significantly impaired by endothelium removal and nitric oxide synthesis inhibition. In contrast, the reductions caused by diazoxide of the contractile responses to noradrenaline were reduced by endothelium removal and L-NAME pretreatment in the case of the lower (30 μM) but not of the higher (100 and 300 μM)

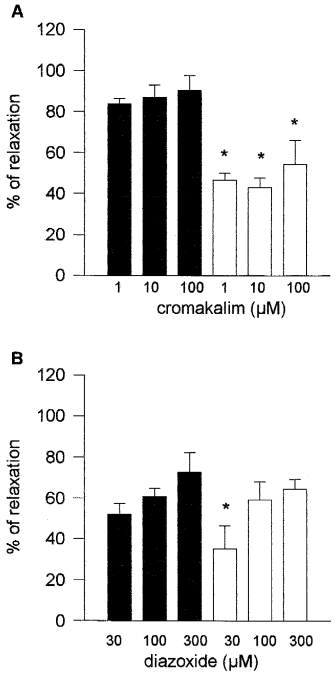

Fig. 8. Rat isolated mesenteric bed. Effects of the removal of the endothelium with saponin on the reductions produced by cromakalim (panel A, open bars, $n = 7$) and diazoxide (panel B, open bars, $n = 4$) of the contractile response to 1 nmol noradrenaline. The corresponding control values are depicted by filled bars. Results are means ± S.E.M. * $P < 0.05$ compared to the corresponding control values. The absolute magnitude of the contraction elicited by noradrenaline after saponin treatment was 52.00 ± 3.04 mmHg, $n = 7$, in the cromakalim group and 55.94 ± 14.37 mmHg, $n = 4$, in the diazoxide group.

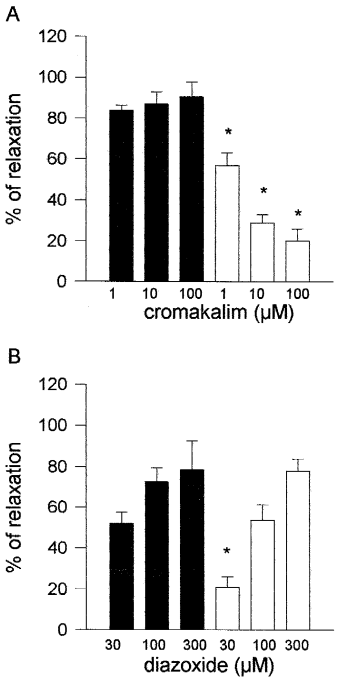

Fig. 9. Rat isolated mesenteric bed. Effects of 500 μM L-NAME on the reductions produced by cromakalim (panel A, open bars, $n = 6$) and diazoxide (panel B, open bars, $n = 8$) of the contractile responses to 1 nmol noradrenaline. The corresponding control values are depicted by filled bars. Results are means ± S.E.M. * $P < 0.05$ compared to the corresponding control values. The absolute magnitude of the contraction elicited by noradrenaline after L-NAME pretreatment was 53.75 ± 4.73 mmHg, $n = 6$, in the cromakalim group and 66.72 ± 8.32 mmHg, $n = 8$, in the diazoxide group.

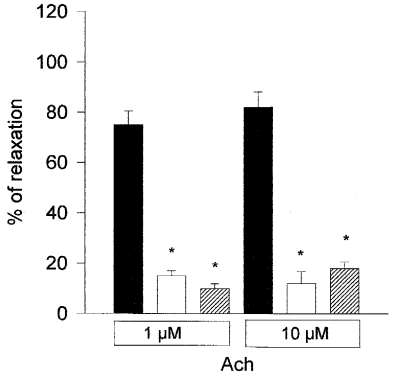

Fig. 10. Rat isolated mesenteric bed. Effects of the removal of the endothelium with saponin (open bars, $n = 5$) or of 500 μM L-NAME pretreatment (hatched bars, $n = 5$) on the reductions produced by acetylcholine (Ach) of the contractile responses to 1 nmol noradrenaline. The corresponding control values are depicted by filled bars. Results are means ± S.E.M. * $P < 0.05$ compared to the corresponding control values. The absolute magnitude of the contraction elicited by noradrenaline was 50.24 ± 3.54 mmHg, $n = 5$, in the case of saponin and 57.00 ± 5.40 mmHg, $n = 5$, in the case of L-NAME pretreatment.

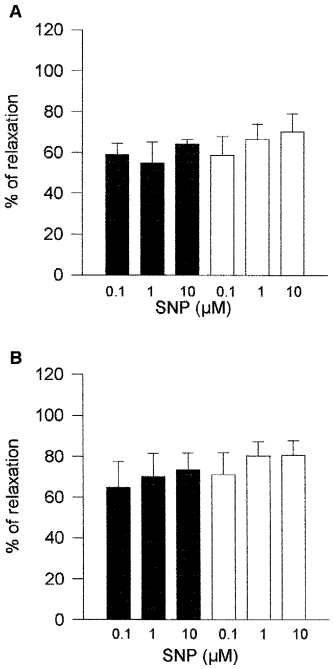

Fig. 11. Rat isolated mesenteric bed. Effects of the removal of the endothelium with saponin (panel A, open bars, $n=4$) or of 500 μM L-NAME pretreatment (panel B, open bars, $n=4$) on the reductions produced by sodium nitroprusside (SNP) of the contractile responses to 1 nmol noradrenaline. The corresponding control values are depicted by filled bars. Results are means ± S.E.M. The absolute magnitude of the contraction elicited by noradrenaline was 67.81 ± 8.42 mmHg, $n=4$, in the case of saponin and 67.81 ± 8.42 mmHg, $n=4$, in the case of L-NAME pretreatment.

diazoxide concentrations. It is of interest to note that the validity of our experimental method to evaluate the contribution of endothelium to contractile responses was provided by the results obtained with acetylcholine and sodium nitroprusside, substances that are known to be endothelium-dependent and endothelium-independent vasodilators, respectively. As shown in Figs. 10 and 11, whereas endothelium removal and L-NAME pretreatment significantly reduced the responses to 1 and 10 μM acetylcholine, they did not modify at all the relaxation evoked by 0.1, 1 and 10 μM sodium nitroprusside.

4. Discussion

The present results show that the K^+ channel openers cromakalim and diazoxide produced a concentration-dependent relaxation of the contractions elicited by 10 μM noradrenaline. The reduction of the contractile responses reached up to 60% of control values with both agents. The concentrations of cromakalim and diazoxide that were effective under our experimental conditions are similar to those for cromakalim in thoracic aortic rings (Buckingham et al., 1989) as well as for cromakalim and diazoxide in the rat portal vein (Newgreen et al., 1990). The observation that cromakalim was more potent than diazoxide to reduce the contractile responses induced by noradrenaline in the rat mesenteric bed agrees with the results obtained for the inhibition caused by these drugs of the spontaneous activity of rat aorta and portal vein (Quast and Cook, 1989; Newgreen et al., 1990) and suggests that, as already shown for the rat portal vein and bladder (Edwards et al., 1991), drugs that belong to the benzopyran group, such as cromakalim, are more potent than agents from other chemical groups of K^+ channel openers in relaxing smooth muscles. The observation that glibenclamide was without effect on the basal perfusion pressure of the rat mesenteric bed is likely to indicate that, as proposed for the rabbit aorta and rat portal vein (Quast and Cook, 1988), K^+-ATP channels are not normally involved in the maintenance of the resting tone in this vascular tissue.

Although glibenclamide almost totally abolished the relaxation caused by either cromakalim or diazoxide, the possibility exists that other mechanisms of relaxation were active in addition to the opening of K^+ channels. In support of this view, it has been reported that whereas glibenclamide inhibits the efflux of Rb^{86} as well as the vasorelaxation caused by cromakalim in the rat aorta, another K^+ channel blocker, tedisamil (Dukes and Morad, 1989; Pfünder and Kreye, 1991), interferes only with the release of Rb^{86} (Bray and Quast, 1992). Similarly, the non-selective K^+ channel blocker Ba^{2+} inhibits cromakalim-induced Rb^{86} efflux but has no effect on vasorelaxation (for review see Quast, 1993). Further support of this view is provided by the evidence (present results) that at least 10 times higher concentrations of cromakalim and diazoxide were required to produce a 30% reduction in the contractile responses to 100 mM KCl, compared to those effective to produce a 60% reduction in the contractile responses to 10 μM noradrenaline. This observation suggests that additional factors other than K^+ channel opening could contribute to produce relaxation of mesenteric arterial smooth muscles. As far as we know, this is the first evidence that the relatively pure K^+ channel opener cromakalim has additional mechanisms to produce relaxation, as proposed for the relaxation caused by diazoxide of the contraction elicited by 80 mM KCl in the rat aorta (Newgreen et al., 1990).

Hence, in the rat mesenteric bed vasodilation caused by cromakalim and diazoxide could involve the participation of processes other than the opening of K^+-ATP channels. Among these processes, the activation of K^+-ATP channels has been proposed to be linked to the activation of adenosine receptors (Merkel et al., 1991; Nakhostine and Lamontagne, 1993; Niiya et al., 1994), to the influx of Ca^{2+} and its mobilization from intracellular stores (Cook, 1988; Yanagisawa et al., 1990; Quast and Baumlim, 1991; Yamagishi et al., 1992), as well as to the effects of vasodilating prostanoids (Bouchard et al., 1994) and endothelial factors (Feletou and Vanhoutte, 1988; Tare et al., 1990; Garland and McPherson, 1992).

Although the participation of adenosine or the interference with Ca^{2+} movements cannot be precluded in the

present experiments, the results obtained with indomethacin, a prostaglandin synthesis inhibitor, suggest that the latter substances are not involved in the vasodilator effects of K^+ channel openers in the rat mesenteric bed. This observation agrees with the inability of the blockade of the synthesis of vasodilator prostaglandins to modify the hypotensive effects produced by cromakalim in anesthetized rats (for review see Richer et al., 1990).

In spite of the fact that L-NAME did not affect the resting tone of the mesenteric bed, the functional role of the endothelium in this tissue is suggested by the potentiation of the noradrenaline-induced contractions caused by either endothelium removal by saponin pretreatment or inhibition of nitric oxide synthesis by L-NAME exposure.

The lack of importance of basal release of nitric oxide compared to that of stimulation with noradrenaline has previously been reported for the contractile responses of the rat mesenteric bed in the presence of either L-NAME or L-nitroarginine used as inhibitors of nitric oxide synthesis (Parsons et al., 1994). Moreover, potentiation of contractile responses to noradrenaline after nitric oxide synthesis inhibition has also been reported for the rat aorta (Martin et al., 1986) as well as for the rat mesenteric bed and rabbit aorta (Moore et al., 1990). In addition, since noradrenaline is an agonist for both α- and β_1-adrenoceptors, the possibility exists that the potentiation of the contractions elicited by noradrenaline after either endothelium removal or L-NAME pretreatment could be due to a reduction of the vasodilation normally caused by β_1-adrenoceptor activation. This is because vasorelaxation induced by stimulation of β_1-adrenoceptors has been shown to be linked to the release of nitric oxide from the endothelium of rat mesenteric arteries (Graves and Poston, 1993).

However, the observation that either the removal of endothelium or pretreatment with the nitric oxide synthesis inhibitor, 500 μM L-NAME, reduced the relaxation caused by cromakalim and diazoxide of the contractile responses to different concentrations of noradrenaline, is likely to indicate that an endothelium-derived relaxing factor, probably nitric oxide, could contribute to the vasodilator effects of these drugs. In support of this view, the contribution of the endothelium to the vasodilating effects of potassium channel openers has been reported for cromakalim and pinacidil in the large epicardial coronary arteries of the dog (Drieu La Rochelle et al., 1992) as well as for LP-805 in the rat thoracic aorta (Kishii et al., 1992a) and in the rabbit femoral arteries (Ushio-Fukai et al., 1994).

The possibility exists that the reduction of the vasorelaxant effects of cromakalim and diazoxide after either endothelium removal or L-NAME pretreatment could have resulted from the relative ineffectiveness of the K^+ channel openers against the larger contractions to noradrenaline obtained under the latter conditions. Nevertheless, the observation that when a low (1 nmol) concentration of noradrenaline was tested (Figs. 8 and 9), the relaxations caused by 1–100 μM cromakalim were indeed reduced after either endothelium removal or L-NAME pretreatment gives further support to the view that an endothelium-derived relaxing factor participates in the effects of cromakalim. Regarding the mechanism involved in the diazoxide-induced relaxation, the observation that the effect of 30 μM but not of 100 and 300 μM diazoxide was attenuated by either endothelium removal or L-NAME pretreatment is likely to indicate that the participation of the endothelial factor in the effects of diazoxide is absent when a relatively high concentration of diazoxide is used. Hence, it appears that tissue differences as well as drug structure and drug concentration should be considered when trying to understand the vasodilation caused by the K^+ channel openers. Moreover, recent evidence indicates that hyperpolarization caused by exogenous nitric oxide in the rabbit mesenteric arteries is sensitive to glibenclamide and is probably due to the activation of K^+-ATP channels (Murphy and Brayden, 1995).

It is of interest to note that McCulloch and Randall (1996) have reported that a potentiation, rather than an inhibition, of the relaxant effect of nmol concentrations of the K^+ channel openers levcromakalim and pinacidil is produced by endothelium removal or L-NAME pretreatment in the rat mesenteric bed precontracted with methoxamine. Since the latter agent is a selective α-adrenoceptor agonist, the difference with our present results could be because under our experimental conditions contraction of the mesenteric bed was elicited with noradrenaline. This agonist, which stimulates both α- and β_1-adrenoceptors, could induce the β_1-adrenoceptor-mediated release of nitric oxide, as reported for the rat isolated mesenteric arteries (Graves and Poston, 1993).

It is concluded that the relaxant effects of cromakalim and diazoxide in the rat mesenteric bed are endothelium-mediated and L-NAME-sensitive and could at least partially involve the participation of nitric oxide. Nevertheless, in addition to nitric oxide, an endothelium-derived hyperpolarizing agent (EDHF) has been proposed to contribute to vasodilation in several tissues (Taylor and Weston, 1988; Kilpatrick and Cocks, 1994). In this regard, both nitric oxide and EDHF have been reported to hyperpolarize vascular smooth muscles by activating K^+-ATP channels (Quayle and Standen, 1994). Hence, the possibility exists that cromakalim and diazoxide interacted with both nitric oxide and EDHF, as has been proposed for several endothelium-dependent vasodilators (Nagao and Vanhoutte, 1993; Waldron and Garland, 1994; Zygmunt et al., 1994). This latter possibility cannot be ruled out on the basis of the present experiments, and it is a topic of work that is being performed at our laboratory.

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