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Involvement of endothelium in relaxant action of glibenclamide on the rat mesenteric artery

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Abstract

The present report describes the complex effect of glibenclamide, an antidiabetic sulfonylurea agent, on the rat isolated mesenteric artery. Although glibenclamide concentration dependently reversed the relaxant effect of pinacidil, an activator of ATP-sensitive K^+ channels (the concentration for half-maximum reversal effect was 0.56 μM with endothelium and 0.17 μM without endothelium), in the artery precontracted with phenylephrine (1 μM), it relaxed phenylephrine-induced sustained contraction at higher concentrations (IC_{50}: 4.4 $\pm$ 1.1 μM with endothelium and 226.1 $\pm$ 44.2 μM without endothelium). The relaxant effect of glibenclamide was partially inhibited by pretreatment of the artery with either N^G-nitro-L-arginine (10–100 μM) or methylene blue (1 μM). Indomethacin (10 μM) had no effect. Moreover, glibenclamide also concentration dependently (3–500 μM) reduced the sustained contraction induced by 60 mM K^+ (IC_{50}: 99.5 $\pm$ 16.1 μM). The relaxation induced by glibenclamide was not affected by various putative K^+ channel blockers such as charybdotoxin (100 nM), tetraethylammonium ions (1 mM), apamin (100 nM) and 4-aminopyridine (1 mM). The results indicate an involvement of the endothelium, probably of nitric oxide, in the relaxation induced by glibenclamide in the endothelium-intact rat mesenteric arteries. The inhibitory effect of glibenclamide on the high-K^+-induced contraction suggests that glibenclamide may interfere with Ca^{2+} influx, which in turn affects intracellular Ca^{2+} levels in arterial smooth muscle, leading to reduction of muscle contractility. It is suggested that two distinct pharmacological effects induced by glibenclamide may be mediated through different glibenclamide binding sites, however, the data show an overlap of concentrations of glibenclamide for producing the two effects in rat isolated mesenteric arteries. © 1998 Elsevier Science B.V.

Keywords: Glibenclamide; Endothelium; Relaxation; K^+ channel, ATP-sensitive; Mesenteric artery; (Rat)

1. Introduction

Glibenclamide, an oral hypoglycemic drug, is a potent inhibitor of ATP-sensitive K^+ channels and of the effects of K^+ channel activators (Quast and Cook, 1989; Standen et al., 1989; Ashcroft and Ashcroft, 1990; Cook and Quast, 1990). It is well documented that gilbenclamide blocks ATP-sensitive K^+ channels in pancreatic β cells at nanomolar concentrations (Zünkler et al., 1988), thus leading to membrane depolarization and insulin secretion. In arterial smooth muscle, glibenclamide was shown to inhibit ATP-sensitive K^+ channels but not Ca^{2+}-activated K^+ channels at micromolar concentrations (Standen et al., 1989; Langton et al., 1991). Also, glibenclamide antagonized the effects of K^+ channel activators and other vasodilators on single channel activity, membrane potential and contractile force in isolated mesenteric arteries (Elitz, 1989; Standen et al., 1989; Nelson et al., 1990; Brayden et al., 1991). On the other hand, it was reported that glibenclamide at high concentrations relaxed the vascular smooth muscle contracted by a thromboxane A_2-mimetic, U46619 in dog coronary artery (Cocks et al., 1990) or by prostaglandin $F_{2\alpha}$ in rabbit and canine arteries (Nielsen-Kudsk and Thirstrup, 1991; Zhang et al., 1991). However, some contradicting results obtained with various arteries show the inability of glibenclamide to influence the basal tone or evoked tension (Buckingham et al., 1989; Zhang et al., 1991). It was suggested that glibenclamide is a competitive antagonist of vasoconstricting prostanoids since contractile responses to other agonists such as endothelin, noradrenaline and high K^+ were unaffected by glibenclamide in dog coronary artery (Cocks et al., 1990). Endothelium may not be involved in the vascular response to glibenclamide

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(Zhang et al., 1991). Nevertheless, the mechanism by which glibenclamide causes vasorelaxation is still unknown. It is possible that glibenclamide could act through multiple sites in either endothelium or the underlying arterial smooth muscle and thus serve as a non-selective muscle relaxant at high concentrations. Regarding the importance of endothelium-derived relaxing factors and K^+ channels in the regulation of the membrane potential and muscle tone in blood vessels, the present study aimed to examine the possible involvement of endothelium in the glibenclamide-induced response in rat isolated mesenteric artery by using various pharmacological blockers, and to investigate the effect of glibenclamide on contractions induced by non-prostanoid vasoconstrictors.

2. Methods and materials

2.1. Tissue preparation

Male Sprague–Dawley rats of approximately 250 g were killed by cervical dislocation and bled. The main branch of the superior mesenteric artery was dissected out and the fatty and surrounding connective tissues were cleaned off. Arterial rings about 3 mm in length were prepared and mounted between two stainless steel hooks in 10 ml, water-jacketed, organ baths (37°C) containing Krebs–Henseleit solution of the following composition (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1; $NaHCO_3$, 25; KH_2PO_4, 1.2 and D-glucose, 11.1. The bath solution was constantly gassed with a mixture of 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate for 90 min under 0.5 g resting tension. The isometric tension was measured with myograph force-displacement transducers (Grass Instrument Co.).

2.2. Experimental protocol

30 min. after being set up in the organ baths, the preparations were first contracted with a single concentration of phenylephrine (1 μM) to test their contractile responses, after which they were washed several times in Krebs–Henseleit solution to restore the tension to its basal level. The concentration-dependent reversal effect of glibenclamide on the pinacidil (3 μM)-induced relaxation of the phenylephrine-precontracted artery was examined in the presence and absence of functional endothelium. In a second group of experiments, the effects of inhibitors of endothelium-derived factors were examined on the glibenclamide-induced relaxation. Rings of artery were incubated with each inhibitor (10–100 μM N^G-nitro-L-arginine, 3 μM methylene blue and 10 μM indomethacin) for 20 min before they were contracted with 1 μM phenylephrine to establish the sustained tone, then glibenclamide was added cumulatively (0.1–500 μM) to induce concentration-dependent relaxation.

In experiments using high-K^+ solution, Na^+ in the bathing medium was replaced by an equimolar concentration of K^+ to maintain a constant ion strength. The sustained contraction of the artery to 60 mm K^+ was obtained and glibenclamide was applied cumulatively (1–500 μM) to induce the concentration-dependent inhibition. In some experiments, the endothelial layer was mechanically removed by rubbing the lumen of the artery with plastic tubing. Successful removal of the endothelium was verified by the inability of the artery to relax in response to 1 μM acetylcholine at the beginning of each experiment.

2.3. Chemicals

The following compounds were used: phenylephrine hydrochloride, glibenclamide, charybdotoxin, (±)-pinacidil, 4-aminopyridine (Research Biochemicals International, Natick, MA), acetylcholine hydrochloride, methylene blue, N^G-nitro-L-arginine, sodium nitroprusside and indomethacin (Sigma, St. Louis, MO). Glibenclamide and pinacidil were dissolved in dimethyl sulfoxide. Dimethyl sulfoxide at a final concentration of 0.2% (v/v) did not induce muscle relaxation. Other chemicals were dissolved in Krebs–Henseleit solution.

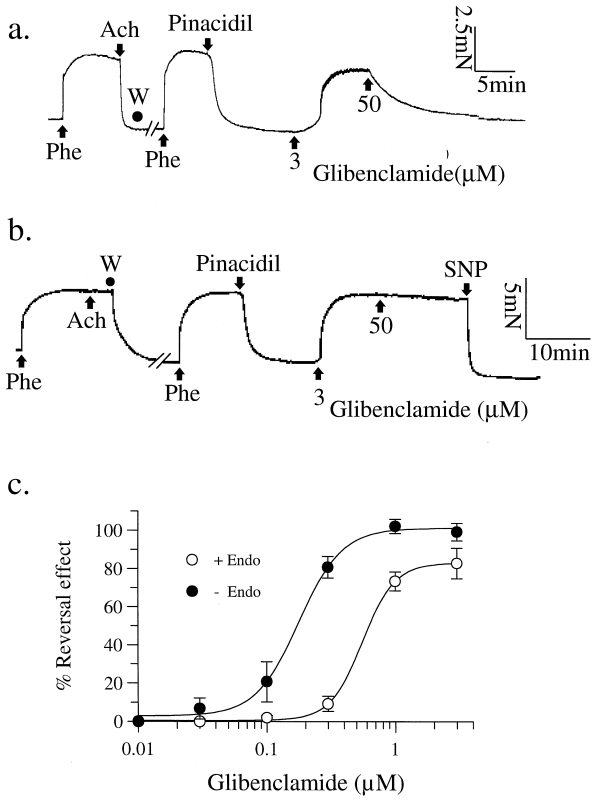

Fig. 1. Glibenclamide reversal of pinacidil-induced relaxation in phenylephrine-contracted arteries with (a) and without (b) endothelium. Further addition of glibenclamide (50 μM) induced greater reduction of the sustained tone in endothelium-intact artery than in endothelium-denuded artery. (c) Concentration–response curve for the reversal effect of glibenclamide on the pinacidil-induced relaxation in arteries with endothelium (○) and without endothelium (●). Results are means ± S.E.M. of seven experiments.

2.4. Statistical analysis

The effects of vasodilators on the sustained tone were expressed as percentages of the control value. Cumulative concentration–relaxation relationships were analyzed with non-linear curve fitting by means of a logistic equation (Grafit, Erithacus Software) and IC_{50} values were calculated as the drug concentration causing the half maximum relaxation. The data were presented as means ± S.E.M. from n experiments. A level of probability of less than 0.05 obtained from an unpaired Student's t-test was regarded as significant.

3. Results

3.1. Effect of glibenclamide on relaxation induced by pinacidil

Phenylephrine (1 μM) induced a sustained contraction in rat isolated mesenteric arteries (3.03 ± 0.14 mN, $n = 22$ in the presence of endothelium and 6.17 ± 0.34 mN, $n = 16$ in the absence of endothelium). Fig. 1a and b shows that pinacidil (3 μM) fully relaxed both endothelium-intact and -denuded arteries precontracted with phenylephrine and glibenclamide at 3 μM reversed the relaxant effect of pinacidil. However, glibenclamide at 50 μM caused an 87.3 ± 4.4% relaxation in endothelium-intact artery ($n = 5$, Fig. 1a) but had a small inhibitory effect (17.5 ± 4.7% relaxation) on the sustained tension in endothelium-denuded artery ($n = 6$, Fig. 1b). Fig. 1c shows the concentration–response curves for the reversal effect of glibenclamide on the relaxation induced by pinacidil (3 μM). The threshold concentration of glibenclamide to antagonize the effect of pinacidil was higher for arteries with endothelium (0.1 μM) than for those without endothelium (0.03 μM). The concentration at which glibenclamide produced the half-maximum reversal of the pinacidil-induced relaxation was 0.57 ± 0.03 μM ($n = 7$) and 0.17 ± 0.02 μM ($n = 7$), respectively, in the presence and absence of endothelium. Glibenclamide at 3 μM only caused 82.7 ± 7.9% reversal ($n = 7$) in arteries with endothelium while it caused complete reversal in the absence of endothelium. These results indicate that the endothelium-de-

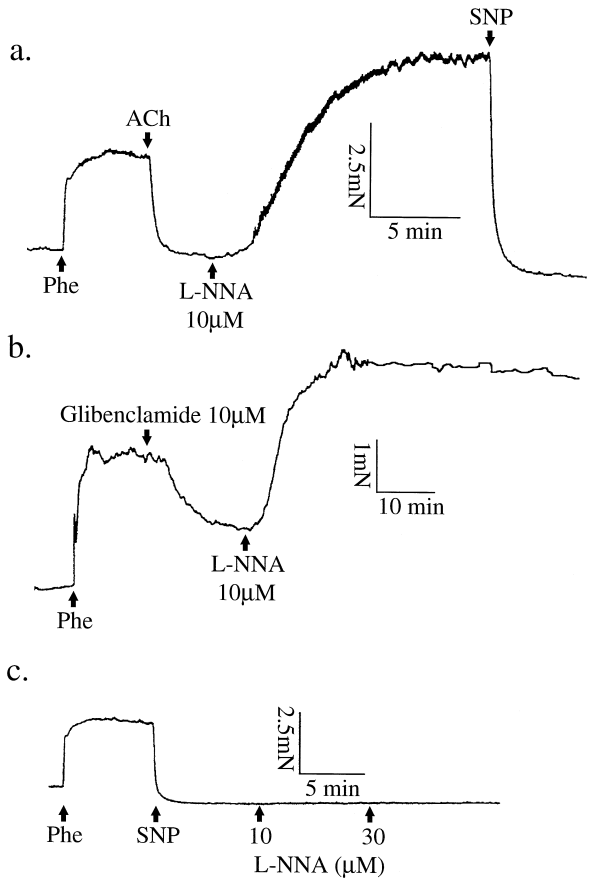

Fig. 2. Complete reversal by N^G-nitro-L-arginine (L-NNA, 10 μM) of the acetylcholine (ACh, 1 μM, a) and glibenclamide (10 μM, b)-induced relaxation in endothelium-intact arteries. Sodium nitroprusside (SNP, 1 μM) completely relaxed the arteries. (c) Lack of effect of L-NNA (10–30 μM) on the relaxation-induced by sodium nitroprusside (SNP, 1 μM). This series of experiments was performed with endothelium-intact rat mesenteric arteries.

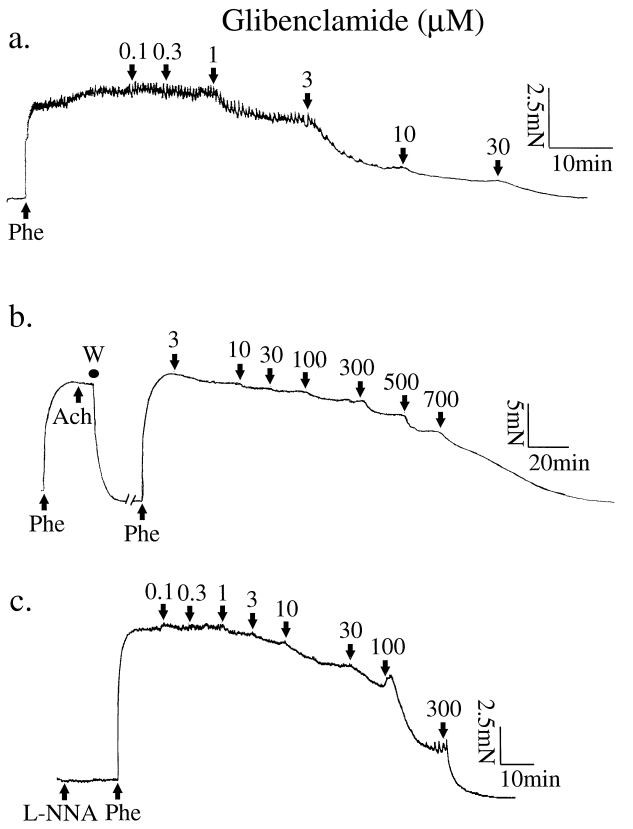

Fig. 3. The concentration-dependent relaxations induced by glibenclamide in phenylephrine-contracted arteries with endothelium (a) and without endothelium (b) and in the presence of endothelium plus 10 μM N^G-nitro-L-arginine (L-NNA, c). L-NNA was applied 20 min before addition of phenylephrine (1 μM).

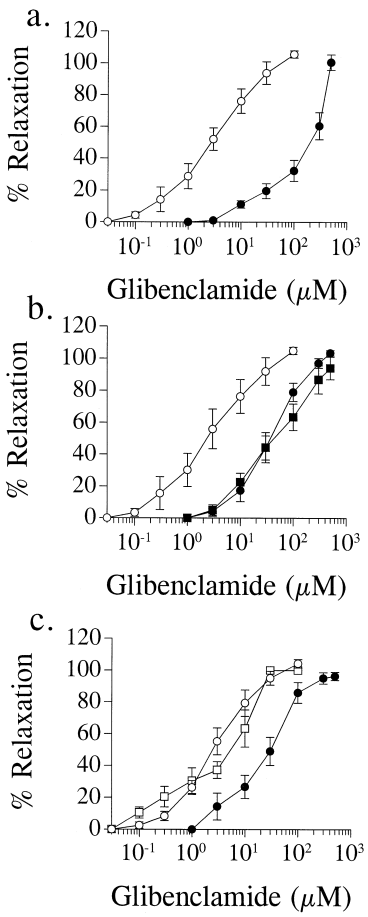

Fig. 4. (a) Concentration–response curves for the relaxation induced by glibenclamide in rat mesenteric arteries (○, $n=8$ with endothelium and ●, $n=7$ without endothelium. (b) Concentration–response curve for the relaxation induced by glibenclamide in the endothelium-intact arteries in the absence (○, $n=6$) and presence of N^G-nitro-L-arginine (L-NNA, ●, 10 μM, $n=10$; ■, 100 μM, $n=6$). (c) Concentration–response curves for the relaxation induced by glibenclamide in the control (○, $n=6$) and in the presence of 1 μM methylene blue (●, $n=6$) or 10 μM indomethacin (□, $n=4$). Each inhibitor was added to the bath for 20 min prior to application of phenylephrine. Curves were drawn by connecting adjacent data points. Results are means ± S.E.M. from n experiments.

rived factor may reduce the sensitivity to glibenclamide for inhibiting ATP-sensitive K^+ channels.

3.2. Relaxant effect of glibenclamide

The traces in Fig. 2a and b show that both acetylcholine (1 μM) and glibenclamide (10 μM) relaxed the phenylephrine-precontracted artery with endothelium, and N^G-nitro-L-arginine (10 μM) completely reversed the relaxations ($n=5$ in each case). Methylene blue (1 μM) also reversed the effect of 10 μM glibenclamide ($n=3$, data not shown). However, N^G-nitro-L-arginine (10–30 μM) did not affect the relaxation induced by 1 μM sodium nitroprusside in the same tissue ($n=4$, Fig. 2c). The traces in Fig. 3 show the concentration-dependent relaxation induced by glibenclamide in the presence (a) and absence (b) of intact endothelium and in tissues with intact endothelium in the presence of 10 μM N^G-nitro-L-arginine (c). In arteries precontracted by 1 μM phenylephrine, glibenclamide reduced the evoked tension in a concentration-dependent manner (IC_{50}: 4.4 ± 1.1 μM, $n=8$) and this effect was significantly attenuated upon removal of the endothelium (IC_{50}: 226.1 ± 44.2 μM, $n=7$, Fig. 4a). In another series of experiments, pretreatment of the tissue with N^G-nitro-L-arginine at two concentrations significantly inhibited the glibenclamide-induced relaxation in endothelium-intact arteries (IC_{50}: 4.69 ± 1.2 μM, $n=6$ in control; 52.7 ± 11.3 μM, $n=10$ in 10 μM N^G-nitro-L-arginine; 73.1 ± 21.2 μM, $n=6$ in 100 μM N^G-nitro-L-arginine; Fig. 4b). Fig. 4c shows that pretreatment with methylene blue also inhibited the relaxation induced by glibenclamide (IC_{50}: 4.8 ± 1.5 μM, $n=6$ in control; 38.1 ± 8.7 μM, $n=6$ in 1 μM methylene blue), but the cyclooxygenase inhibitor, indomethacin (10 μM), had no effect (IC_{50}: 5.6 ± 1.1 μM, $n=4$, Fig. 4c). In contrast, tolbutamide (1 mM), another sulfonylurea drug, did not relax phenylephrine-contracted arteries without endothelium but, instead, caused a 75 ± 7% increase of evoked tension ($n=3$).

Other putative blockers of K^+ channels such as charybdotoxin (100 nM), tetraethylammonium ions (1 nM), apamin (100 nM) and 4-aminopyridine (1 mM) did not

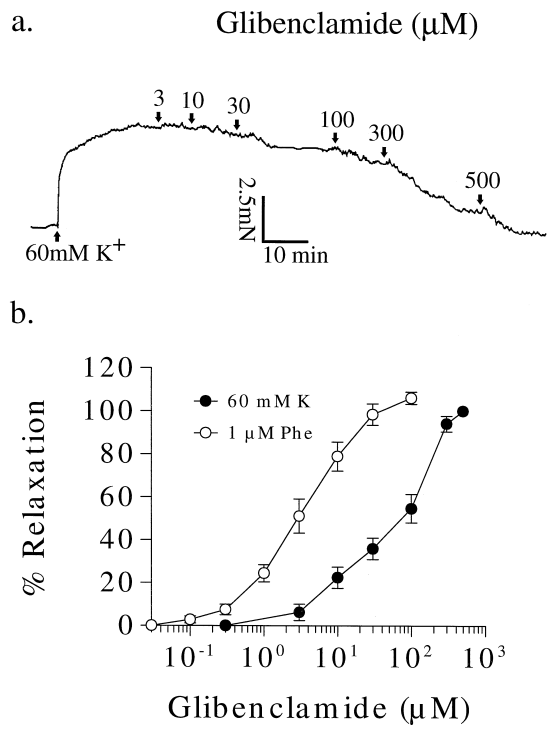

Fig. 5. (a) Trace showing the concentration-dependent relaxation induced by glibenclamide in arteries contracted with 60 mM K^+. (b) Concentration–response curves for the relaxation induced by glibenclamide in endothelium-intact arteries precontracted with 1 μM phenylephrine (○, $n=6$) or with 60 mM K^+ (●, $n=6$). Curves were drawn by connecting adjacent data points. Results were means ± S.E.M. from n experiments.

reverse the relaxant effect of glibenclamide ($n = 4$ in each case, data not shown).

3.3. Effect of glibenclamide on high K^+-induced contraction

To study the possible inhibitory action of glibenclamide on Ca^{2+} influx, its effect on contractions induced by 60 mM extracellular K^+ was examined. The trace in Fig. 5a shows that, in the 60 mM K^+-contracted artery, glibenclamide reduced the sustained contraction in a concentration-dependent manner. The IC_{50} was 99.5 ± 16.1 μM ($n = 6$) for the effect of glibenclamide on the high-K^+ response (Fig. 5b), a value 23-fold greater than that for the relaxant effect on phenylephrine-induced contraction (IC_{50} of 4.2 ± 1.0 μM, $n = 6$, Fig. 5b). The high-K^+-induced tension was unaffected by prazosin (1 μM), indicating that noradrenergic nerve terminals were not involved in the responses.

4. Discussion

The present results showed that the hypoglycemic agent, glibenclamide, concentration dependently reversed the relaxation induced by the K^+ channel opener, pinacidil, in rat isolated mesenteric arteries. The potency of glibenclamide was related to the presence of endothelium. Glibenclamide reversed the pinacidil-induced relaxation more effectively in endothelium-denuded arteries than in endothelium-intact arteries. These findings suggest that factors from endothelium may reduce the antagonizing effect of glibenclamide in ATP-sensitive K^+ channels in arterial smooth muscle cells. When its concentration was increased, glibenclamide (1–100 μM) relaxed the phenylephrine-precontracted arteries in a concentration-dependent fashion. The vasorelaxant action of glibenclamide and other antidiabetic sulfonylureas was previously shown in other blood vessels (Cocks et al., 1990; Nielsen-Kudsk and Thirstrup, 1991; Zhang et al., 1991). In the canine coronary artery, glibenclamide (1–30 μM) was found to competitively inhibit the contractile response to a thromboxane A_2 analogue, U46619, whilst glibenclamide at 30 μM had no effect on either the EC_{50} value or maximal contraction in response to endothelin 1, noradrenaline or high K^+ (Cocks et al., 1990). In contrast, glibenclamide could inhibit the high-K^+-induced contraction in rabbit coronary arteries with IC_{50} of 114 μM (Nielsen-Kudsk and Thirstrup, 1991). In rat aorta, however, the noradrenaline-induced contractile response was unaffected by glibenclamide (1–10 μM) (Buckingham et al., 1989). Our results showed the inhibition of contractile responses to both phenylephrine and high K^+ by glibenclamide in rat mesenteric artery. In addition, glibenclamide was reported to suppress contractions of the rat uterus in response to oxytocin, acetylcholine and KCl (Villar et al., 1986) and contractions in response to a number of different spasmogens in airway smooth muscle (Nielsen-Kudsk and Thirstrup, 1993). These results suggest that glibenclamide at high concentrations may be a general smooth muscle relaxant, and that its effect is unlikely to be confined to the contractions induced by vasoconstricting prostanoids as proposed by Cocks et al. (1990). It is apparent that the observed inhibitory effect of glibenclamide on muscle contractility is a property additional to its pharmacological action as a selective blocker of ATP-sensitive K^+ channels. The expected consequence of inhibition of ATP-sensitive K^+ channels would be depolarization of the cell membrane, activation of voltage-sensitive Ca^{2+} channels and an increase in vascular tone. Indeed, in the present work (with pinacidil), as in many other earlier studies, the vascular relaxation induced by a K^+ channel activator was antagonized by glibenclamide at a low concentration. In fact, glibenclamide is one of the most effective blockers of ATP-sensitive K^+ channels in vascular smooth muscle (Standen et al., 1989) and it inhibits levcromakalim-induced relaxation of rat aorta with a K_i of 365 nM (Challinor-Rogers et al., 1995). It is probable, therefore, that the vasorelaxant action of glibenclamide is mediated by a mechanism independent of the involvement of ATP-sensitive K^+ channels. The present results do not indicate that glibenclamide relaxes arterial smooth muscle through activation of other types of K^+ channels since its effect was unaffected by charybdotoxin, tetraethylammonium ions, apamin and 4-aminopyridine, blockers for large- and small-conductance Ca^{2+}-activated K^+ channels, and voltage-sensitive K^+ channels, respectively.

The present results point to involvement of the endothelium, probably of nitric oxide, in the relaxation caused by glibenclamide. Removal of the endothelium markedly attenuated the relaxation induced by glibenclamide. In addition, the concentration–relaxation response curve for glibenclamide was displaced to the right by the endothelial nitric oxide synthase inhibitor, N^G-nitro-L-arginine, and by methylene blue, an inhibitor of soluble guanylate cyclase. On the other hand, indomethacin, the cyclooxgenase inhibitor had no effect. It is unlikely that the effect of N^G-nitro-L-arginine is non-specific since this agent reversed the relaxation induced by glibenclamide as well as by the endothelium-dependent vasodilator acetylcholine but it did not affect the relaxant effect of sodium nitroprusside, an exogenous nitric oxide donor. These findings were in contrast to the earlier report concerning canine middle cerebral artery in which neither methylene blue (10 μM) nor removal of endothelium affected the vasorelaxation induced by glibenclamide (Zhang et al., 1991), a discrepancy which may be due to the use of different arteries from different species. Our data show that the relaxation induced by glibenclamide was attenuated to a greater degree in endothelium-denuded arteries than in endothelium-intact arteries pretreated with the maximally effective concentration of N^G-nitro-L-arginine. It is therefore possi-

ble that other undefined factors from the endothelium may also be involved.

The inhibitory effect of glibenclamide on the high-K^+-induced contraction indicates that glibenclamide may interfere with Ca^{2+} influx or exert some other non-selective action. Yoshitake et al. (1991) previously demonstrated that glibenclamide inhibited the increase in cytosolic Ca^{2+} concentration and force generation induced in rabbit aorta by noradrenaline in the presence and absence of extracellular Ca^{2+} without an effect on the $[Ca^{2+}]_i$-force relationship. These data indicate that this inhibitory effect of glibenclamide on contraction is not due to an effect on the Ca^{2+}-sensitivity of the intracellular contractile proteins, but is probably related to initial steps involved in the contraction. It is yet to be determined whether glibenclamide at high concentrations could affect plasma membrane Ca^{2+} channels in arterial smooth muscle.

The reversal of the pinacidil-induced relaxation in endothelium-intact arteries was observed with 0.03 to 1 μM glibenclamide (the concentration for the half maximum reversal effect was 0.56 μM), while the inhibition of the phenylephrine-induced contraction was observed with 0.3 to 100 μM glibenclamide (the concentration for the half maximum inhibition was 4.4 μM). The difference in concentration range between the two effects of glibenclamide would suggest that glibenclamide may act at two distinct sites. In endothelium-denuded rings of rat aorta, glibenclamide inhibited the specific binding of [^{3}H]P1075, an analogue of pinacidil, with a pK_i of 6.4, a value which correlates well with the corresponding pD_2 value of 7.0 calculated for the inhibition of P1075-evoked $^{86}Rb^+$ efflux from rat aorta (Bray and Quast, 1992). However, with [^{3}H]glibenclamide used as the radiolabel in membrane preparations from guinea pig ileum smooth muscle, pK_d values of 9.4 (high affinity component) and 7.1 (low affinity component) were obtained for glibenclamide (Zini et al., 1991). It appears that the glibenclamide binding site which inhibits the relaxant effect of cromakalim in guinea pig ileum may be related to the high-affinity glibenclamide receptors (Zini et al., 1991). Nevertheless, it remains to be determined whether the relaxant effect of glibenclamide is associated with the low-affinity binding site in rat mesenteric arteries.

In summary, glibenclamide within a low concentration range (0.03–1 μM) antagonized the relaxation induced by K^+ channel activators mainly through blockade of ATP-sensitive K^+ channels in arterial smooth muscle, while within a higher concentration range (0.3–100 μM), it relaxed the rat isolated mesenteric artery partially through the increased release or/and synthesis of nitric oxide in endothelium and partially through inhibition of Ca^{2+} influx. The present results indicate that glibenclamide may have two sites of action but show an apparent overlap in the concentrations of glibenclamide that produce two distinct pharmacological effects, antagonism of the effect of the K^+ channel activator and vasorelaxation. The results also suggest that the widespread use of glibenclamide as a potent and selective blocker of ATP-sensitive K^+ channels may have caused underestimation of the role of these channels in the regulation of membrane potential, muscle contractility and vascular tone under physiological conditions.

Acknowledgements

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