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European Journal of Pharmacology 341 (1998) 65–71

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Glibenclamide inhibits thromboxane A_2-induced contraction in human internal mammary artery and saphenous vein

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Received 17 September 1997; revised 20 October 1997; accepted 24 October 1997

Abstract

Glibenclamide, like other hypoglycemic sulfonylurea derivatives, is a potent blocker of ATP-regulated K^+ channels. In addition, it is reported to inhibit prostanoid-induced contractions of isolated vascular smooth muscle from different animal species. We investigated the effect of glibenclamide on the thromboxane A_2-mimetic U-46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$)-induced contractions in human isolated internal mammary arteries and saphenous veins. In the two vascular preparations, glibenclamide (3, 10 and 30 μM) caused a concentration-dependent shift to the right of the U-46619 contraction–response curve with a reduction, at the highest concentrations, in the maximal responses. This inhibitory effect appears selective for thromboxane A_2-induced contractions since glibenclamide (30 μM) did not alter the contraction of internal mammary arteries in response to norepinephrine and of saphenous veins in response to 5-hydroxytryptamine (5-HT) and endothelin-1. However, glibenclamide reduced the endothelin-1-induced contraction in internal mammary arteries. The endothelin-1-induced contractions were similarly inhibited by GR 32191 ([1*R*-[1α(*Z*),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptonoic acid, a thromboxane A_2 receptor antagonist. These results suggest that glibenclamide also reduced the endothelin-1-induced contractions by inhibiting a thromboxane A_2 receptor-mediated component of the contraction elicited by this peptide. In conclusion, glibenclamide clearly appears to exert a specific inhibitory influence on prostanoid-induced contractions in human internal mammary arteries and saphenous veins. © 1998 Elsevier Science B.V.

Keywords: Glibenclamide; Internal mammary artery; (Human); Saphenous vein; Human; Thromboxane A_2

1. Introduction

Glibenclamide, a second generation sulfonylurea, is an oral hypoglycemic drug widely used for the treatment of diabetes mellitus. Glibenclamide acts by blocking the ATP-dependent K^+ channels (K_{ATP}) of the pancreatic β-cells (Schmid-Antomarchi et al., 1987), to trigger the release of insulin (Boyd et al., 1990). Glibenclamide also blocks K_{ATP} in cardiac myocytes (Escande et al., 1989) and in arterial smooth muscle (Standen et al., 1989). In addition, sulfonylurea derivatives exert an inhibitory effect on prostanoid-induced vasoconstrictions (Cocks et al., 1990; Nielsen-Kudsk and Thirstrup, 1991; Zhang et al., 1991; Zhang and Cook, 1994; Delaey and Van de Voorde, 1995, 1997; McPherson et al., 1997). This inhibitory effect appears to be specific for prostanoid-induced contractions since the sulfonylureas block contractions induced by the thromboxane A_2-mimetic, U-46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2 and prostaglandin E_2 but not the contractions elicited by norepinephrine, phenylephrine, 5-hydroxytryptamine (5-HT), endothelin-1 or KCl (Cocks et al., 1990; Zhang and Cook, 1994; Delaey and Van de Voorde, 1995, 1997). In addition, McPherson et al. (1997) found a significant correlation between the ability of sulfonylurea derivatives to inhibit vascular relaxation induced by a K_{ATP} opener and to inhibit vascular contraction induced by U-46619. These results obtained with pig coronary artery indicate that similar structure–activity relationships apply for the two activities, suggesting a similar site of action of sulfonylureas. Recent evidence indicates interspecies differences in the inhibitory influence of these compounds on prostanoid-induced smooth muscle contrac-

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tion, for example glibenclamide does not influence U-46619-induced contractions of human subcutaneous arteries (Delaey and Van de Voorde, 1997). In order to further characterize the effects of glibenclamide in human vascular tissues, we investigated its inhibitory effect on U-46619-induced contractions in human internal mammary artery and saphenous vein and whether this effect was specific for prostanoid-induced contractions.

2. Materials and methods

2.1. Isolation and study of human internal mammary artery

Human internal mammary artery segments were collected from patients undergoing coronary artery bypass grafting. After sternotomy, the arteries were removed together with their pedicles. Any discarded segments were immediately placed in a container with oxygenated physiological salt solution (Krebs solution) maintained at 4°C, and then transferred to the laboratory. The Krebs solution had the following composition: NaCl (118 mM), KCl (4.7 mM), $CaCl_2$ (2.5 mM), $MgSO_4$ (1 mM), KH_2PO_4 (1 mM), $NaHCO_3$ (25 mM) and glucose (11 mM). The solution was aerated with a gas mixture of 5% CO_2 and 95% O_2. The internal mammary arteries were then carefully dissected free from the surrounding connective tissue and were precisely cut into 3 mm length segments; the number of rings obtained from each internal mammary artery varied from 2 to 4. The segments were suspended on wires in a 5 ml organ bath containing the Krebs solution at 37°C bubbled with the gas mixture; the lower wire was fixed to a micrometer (Mitutoyo, Japan) and the upper wire was attached to a force transducer (UF-1, Pioden, UK) through which changes in isometric force were displayed on recorders (Linseis 200, Bioblock, France). After mounting, the unstretched preparations were allowed to equilibrate for 30 min. A normalization procedure was then performed to standardize the baseline resting internal circumference (calculated from the distance between the wires) of each ring as previously reported (He et al., 1988, 1989). Briefly, the rings were stretched with the micrometer in progressive steps to determine the wall tension-internal circumference exponential curve for each ring. On the basis of this relationship, the distance between the wires was set at a normalized internal circumference corresponding to 90% of the internal circumference the vessel would have at a transmural pressure of 100 mm Hg. 112 rings of internal mammary artery obtained from 48 patients were used in this study. The average vessel internal diameter at an equivalent transmural pressure of 100 mm Hg was 1.62 ± 0.08 mm and the resting force was 5.42 ± 0.25 g. This degree of passive force was maintained throughout the experiment. Resting segments were allowed to equilibrate for 60 min, and then the rings were challenged once by the addition of KCl 90 mM. This initial maximal contraction was performed to stabilize the preparations and to ensure reproducible contractile responsiveness. During the following hour, the Krebs solution was changed every 15 min before the experiment was continued. In some preparations, the presence of the endothelium was functionally tested by assessing the relaxation elicited by acetylcholine (1 μM) after precontraction with KCl 30 mM. Relaxation in response to acetylcholine occurred in only 2 out of the 50 tested rings.

2.2. Isolation and study of human saphenous vein

Human saphenous veins were obtained from patients undergoing bypass surgery or surgical removal of varicose veins. The discarded saphenous vein segments were placed in oxygenated Krebs solution maintained at 4°C and transferred to the laboratory. The saphenous veins were cut into segments of about 5 to 6 mm length and suspended in a 5 ml organ bath containing Krebs solution at 37°C and continuously bubbled with 5% CO_2/95% O_2 gas mixture. At the beginning of the experiment, the rings were stretched to an initial tension of 2 g and allowed to equilibrate for 60 min in the Krebs solution, which was changed every 15 min. The saphenous vein rings were then challenged with 90 mM KCl to stabilize the preparations. As for the internal mammary artery segments, the preparations were allowed to equilibrate again for 60 min, with the Krebs solution being changed every 15 min before the experiment was continued. In some preparations, the presence of the endothelium was functionally tested by assessment of the relaxation in response to acetylcholine (1 μM) after precontraction with KCl 30 mM. None of the saphenous vein rings tested had a functional endothelium ($n = 40$).

2.3. Drugs

Glibenclamide, U-46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$), and 5-hydroxytryptamine hydrochloride were obtained from Sigma (L'isle d'Abeau); norepinephrine bitartrate was from Assistance Publique des Hôpitaux de Paris (France); endothelin-1 was from Euromedex (Souffelweyersheim); KCl Normapur was from Prolabo (Paris). GR 32191 ([1R-[1α(Z),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptonoic acid) was kindly provided by Glaxo Group Research. All drugs, except glibenclamide, were dissolved in distilled water. A stock solution of 3 mM glibenclamide was made in methanol. The maximal methanol concentration in the organ bath was 1%. Drugs were kept at −20°C, and were freshly dissolved in distilled water to the appropriate concentrations expressed as final molar concentrations in the organ bath.

2.4. Analysis and expression of results

Concentration–responses curves were made by cumulative addition of the drugs at time intervals sufficient to

reach a stable response before the next addition was made. Only one concentration–contraction curve was made in each vascular ring. The contractions were expressed as grams isometric force developed by the rings. In some series of experiments, contractions were also expressed as percentages of the contraction induced by KCl 90 mM. The maximum effect (E_{max}) was the greatest response obtained with an agonist. EC_{50} was defined as the concentration of drug producing 50% of the maximal effect. The pD_2 value represents the negative logarithm of EC_{50} and was used to compare the shift of the concentration–response curves. The antagonist potency of glibenclamide was estimated by calculation of pK_B according to Kenakin (1993) for a non-competitive antagonist. Results are expressed as mean ± standard error of the mean (S.E.M.) for the specified number of preparations tested. Statistical analysis was performed by using the appropriate paired or unpaired Student's t test. $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of glibenclamide on the resting tone of internal mammary artery and saphenous vein

The addition of 30 μM of glibenclamide induced a slow decrease in the resting basal tone in both internal mammary artery and saphenous vein. This decrease in tone, measured after 45 min incubation, was 0.4 ± 0.1 g ($n = 8$) in internal mammary artery and 0.06 ± 0.03 g ($n = 4$) in saphenous vein and when expressed as a percentage of the maximal relaxation obtained with papaverine (0.3 mM) corresponded to 33 ± 5% in the internal mammary artery and 20 ± 12% in the saphenous vein. The vehicle used (methanol) was devoid of effect on the resting tone at the maximal concentration (1%) to which the tissues were exposed during the experiment ($n = 4$).

3.2. Effect of glibenclamide on contractions induced by U-46619

The effects of glibenclamide on contractions induced by cumulative addition of increasing concentrations (0.5-log increments) of U-46619 (0.1 nM–3 or 10 μM) were studied in internal mammary artery and saphenous vein segments incubated for 45 min with glibenclamide (3, 10 or 30 μM) or with the vehicle (control preparations). As far as possible, experiments were conducted on 4 rings (one ring serving as control) from the same vascular segment mounted in parallel in different organ baths.

Glibenclamide shifted the U-46619 concentration–contraction curves to the right in a parallel manner in internal mammary artery and in saphenous vein (Fig. 1), there being a decrease in the amplitude of contractions with 10 μM and 30 μM glibenclamide in internal mammary artery and with 30 μM glibenclamide in saphenous vein (Fig. 1 and Table 1). The calculated pK_B values were 6.3 ± 0.2 for 3 μM glibenclamide in internal mammary artery and 6.7 ± 0.2 and 6.6 ± 0.4 for glibenclamide (3 and 10 μM, respectively) in saphenous vein. Additional experiments excluded the involvement of the solvent (methanol 1%) in the inhibitory influence of glibenclamide on the thromboxane A_2-mimetic-induced contractions in internal mammary

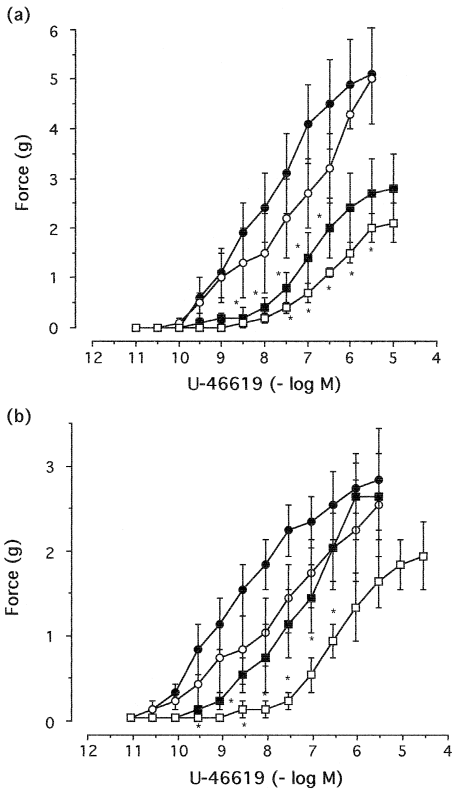

Fig. 1. Contraction (expressed as grams force) of human internal mammary artery (a) and of human saphenous vein (b) induced by cumulative addition of increasing concentrations of U-46619 in the absence (vehicle; ●) or the presence of glibenclamide 3 μM (○), 10 μM (■) and 30 μM (□). Values represent means of 6 to 11 experiments. The vertical bars are S.E.M. ($^*P < 0.05$).

Table 1
Effect of glibenclamide (3, 10 and 30 μM) on contractions induced by U-46619 in internal mammary artery and saphenous vein

	Internal mammary artery			Saphenous vein		
	pD_2	E_{max} (g)	n	pD_2	E_{max} (g)	n
Control (vehicle)	8.0 ± 0.3	5.1 ± 1.0	11	8.6 ± 0.2	2.8 ± 0.6	11
Glibenclamide						
3 μM	7.4 ± 0.4	5.0 ± 1.2	8	8.0 ± 0.4	2.5 ± 0.6	10
10 μM	7.0 ± 0.3[a]	2.8 ± 0.7	7	7.5 ± 0.5[a]	2.6 ± 0.9	11
30 μM	6.5 ± 0.3[a]	2.1 ± 0.4[a]	7	6.4 ± 0.2[a]	1.9 ± 1.1	6

The responses to U-46619 obtained in absence (control) and in presence of glibenclamide are expressed in pD_2 value and E_{max} (gram force). Results are expressed as means ± S.E.M., n represents the number of rings tested.
[a] $P < 0.05$, control vs. glibenclamide.

artery and saphenous vein ($n = 7$ for the two preparations, data not shown).

3.3. Effect of glibenclamide on the contractions induced by norepinephrine, 5-HT and endothelin-1

In a second series of experiments, cumulative concentration–contraction curves for norepinephrine (1 nM–0.1 mM) and endothelin-1 (1 nM–0.3 μM) in internal mammary artery and for 5-HT (1 nM–30 μM) and endothelin-1 in saphenous vein were recorded in the presence or absence of glibenclamide 30 μM.

Glibenclamide did not significantly alter the contractions induced by norepinephrine in internal mammary artery (Fig. 2a) and by 5-HT in saphenous vein (Fig. 2b). In addition, when norepinephrine- and 5-HT-induced contractions were normalized to the KCl-induced contractions (data expressed as percentages of the maximal contraction elicited by KCl 90 mM), the differences between the tissues in the presence or absence of glibenclamide were even reduced. However, glibenclamide decreased the amplitude of the endothelin-1-induced contraction in internal mammary artery (Fig. 3a) but did not alter the sensitivity of the tissue (no shift of the concentration–response curve). In contrast, glibenclamide had no influence on the endothelin-1-induced contraction in saphenous vein (Fig. 3b). The E_{max} and pD_2 values for the three agonists in the absence or in the presence of glibenclamide 30 μM are shown in Table 2.

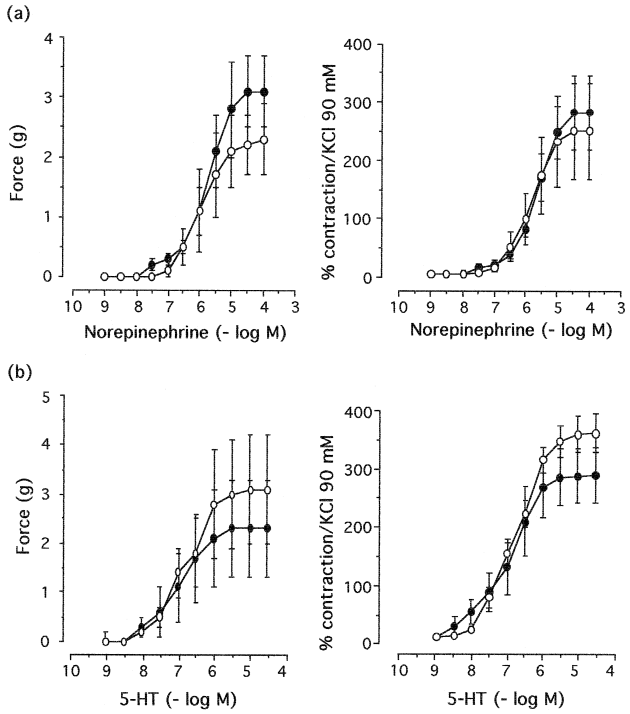

Fig. 2. Contraction (expressed as grams force or as a percentage of the maximal contraction elicited by KCl) induced by cumulative addition of increasing concentrations of norepinephrine in human internal mammary artery (a) and of 5-HT in human saphenous vein (b) in the absence (vehicle; ●) or the presence of glibenclamide 30 μM (○). Values represent means of 6 paired experiments. The vertical bars are S.E.M.

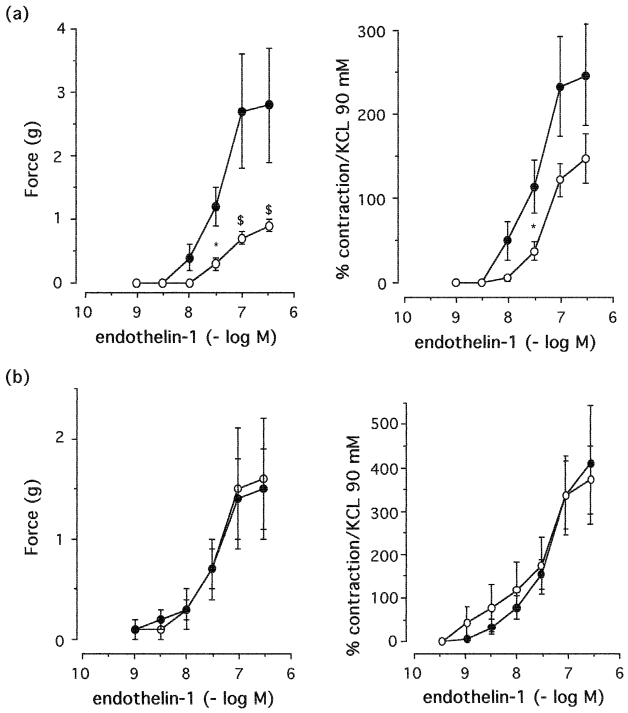

Fig. 3. Contraction (expressed as grams force or as a percentage of the maximal contraction elicited by KCl) of human internal mammary artery (a) and of human saphenous vein (b) induced by cumulative addition of increasing concentrations of endothelin-1 in the absence (vehicle; ●) or the presence of glibenclamide 30 μM (○). Values represent means of 9 paired experiments. The vertical bars are S.E.M. ($^{*}P < 0.05$; $^{\$}P = 0.07$).

3.4. Effect of a thromboxane A_2 receptor antagonist on the endothelin-1 induced contraction in internal mammary artery

Cumulative concentration–contraction curves for endothelin-1 were made in the absence (control) or in the

Table 2
Effect of glibenclamide (30 μM) on the contractions induced by norepinephrine and endothelin-1 in internal mammary artery, and on the contractions induced by 5-HT and endothelin-1 in saphenous vein

Agonists	Internal mammary artery		Saphenous vein	
	control	glibenclamide	control	glibenclamide
5-HT				
pD_2	n.d.	n.d.	6.7 ± 0.4	6.7 ± 0.1
E_{max}	n.d.	n.d.	2.7 ± 1.1	3.7 ± 1.2
Endothelin-1				
pD_2	7.5 ± 0.1	7.2 ± 0.1	7.6 ± 0.2	7.5 ± 0.1
E_{max}	2.9 ± 0.9	0.9 ± 0.1[a]	1.9 ± 0.6	1.6 ± 0.3
Norepinephrine				
pD_2	6.0 ± 0.2	5.8 ± 0.2	n.d.	n.d.
E_{max}	3.2 ± 0.5	2.2 ± 0.5	n.d.	n.d.

Results are expressed as means ± S.E.M. of 6 to 10 experiments. E_{max} are expressed as grams force (control = vehicle; n.d.: not determined)
[a] $P < 0.05$, control vs. glibenclamide.

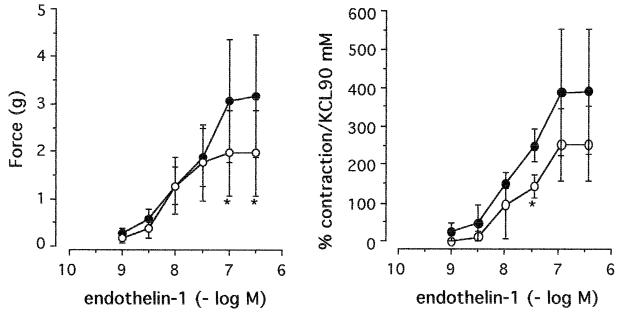

Fig. 4. Contraction (expressed as grams force or as a percentage of the maximal contraction elicited by KCl) of human internal mammary artery induced by cumulative addition of increasing concentrations of endothelin-1 in the absence (vehicle; ●) or the presence of the thromboxane A_2 antagonist, GR 32191 at 0.3 μM (○). Values represent means of 9 paired experiments. The vertical bars are S.E.M. ($^*P < 0.05$).

presence of a potent thromboxane A_2 receptor antagonist, GR 32191, at 0.3 μM (He and Yang, 1995). The incubation time was 45 min and the experiments were conducted in organ baths covered with aluminium foil because of the instability of GR 32191 on exposure to light.

As with glibenclamide, incubation for 45 min with GR 32191 induced a slow decrease in the basal tone of the internal mammary artery as well as the saphenous vein (data not shown). The decrease in internal mammary artery tone (0.2 ± 0.1 g), expressed as a percentage of the maximal relaxation elicited by papaverine, was $34 \pm 14\%$ ($n = 6$). In addition, GR 32191 significantly decreased the endothelin-1-induced contraction (Fig. 4).

4. Discussion

The results of the present study provide evidence that glibenclamide, in addition to causing blockade of K_{ATP}, is a potent inhibitor of thromboxane A_2-induced contraction in human internal mammary artery and saphenous vein.

Our study extends the original observation made by Cocks et al. (1990), who reported that glibenclamide was an antagonist of U-46619-induced contractions in dog coronary artery. Glibenclamide has since been shown to relax prostaglandin $F_{2\alpha}$-precontracted isolated rabbit coronary artery (Nielsen-Kudsk and Thirstrup, 1991) and prostaglandin $F_{2\alpha}$-precontracted rat aorta as well as different precontracted dog arteries (Zhang et al., 1991). In addition, glibenclamide has been reported to inhibit the contraction evoked by prostaglandin $F_{2\alpha}$, prostaglandin D_2 and prostaglandin E_2 in rat aorta and in various types of dog arteries (Zhang and Cook, 1994). Furthermore, Delaey and Van de Voorde (1995, 1997) reported the inhibitory influence of glibenclamide on U-46619-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-induced contractions in rat aorta and on U-46619- and prostaglandin $F_{2\alpha}$-induced contractions in various types of rat and bovine arteries. However, in aorta and carotid arteries from guinea pigs and in human subcutaneous arteries, glibenclamide (10 μM) did not inhibit prostaglandin $F_{2\alpha}$- and U-46619-induced contractions (Delaey and Van de Voorde, 1997) which suggest that there are interspecies differences in the ability of sulfonylureas to block prostanoid-induced contractions.

In contrast to the results for human subcutaneous arteries, we report that glibenclamide (3 to 30 μM) exerted an inhibitory influence on U-46619-induced contractions in human internal mammary artery and saphenous vein. We focused our work on contractions induced by U-46619 since this compound is a highly potent agonist at the thromboxane A_2/endoperoxide receptor and since U-46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2 and prostaglandin E_2 produce vascular contractions that can be blocked by a thromboxane A_2 receptor antagonist, which suggests that these prostanoids mainly act through the stimulation of thromboxane A_2 receptors (Lumley et al., 1989; Norel et al., 1991; Coleman et al., 1994; He and Yang, 1995).

In human internal mammary artery and saphenous vein, glibenclamide shifted the U-46619 concentration–response curves to the right in a parallel manner and, at the highest concentrations (10 and 30 μM), caused a decrease in the maximal contractile responses. A decrease in the maximal effect of U-46619 by glibenclamide has been also reported in different types of rat arteries (Delaey and Van de Voorde, 1995, 1997) and does not therefore support a competitive interaction. However, glibenclamide behaves as a competitive antagonist of U-46619 in dog coronary artery (Cocks et al., 1990) and appears also to behave in that way in pig coronary artery (McPherson et al., 1997) because, in both preparations, glibenclamide shifted the curves for U-46619 to the right in a parallel manner without changing the maximal responses. The potency of glibenclamide to antagonize U-46619 constrictor responses in pig coronary artery ($pK_B = 6.3 \pm 0.1$) and in dog coronary artery ($pK_B = 6.2$) is very close to its potency in human internal mammary artery and saphenous vein. The discrepancies in terms of effect of glibenclamide on the maximal response elicited by U-46619 may reflect some differences within thromboxane A_2-receptors among species or tissues (reviewed by Armstrong and Wilson, 1995). However, Delaey and Van de Voorde (1995) reported in rat aorta that the sulfonylureas glibenclamide and tolbutamide powerfully inhibited the contractions induced by aluminium tetrafluoride anion, which acts by activating G-proteins. These results suggest that sulfonylureas may exert their inhibitory influence at the level of the regulatory G-proteins. Such an interference in the signal transduction system is consistent with a non-competitive interaction between glibenclamide and U-46619 but does not exclude possible competitive interactions at the thromboxane A_2 receptor binding level.

Whatever the nature of the interactions between glibenclamide and U-46619, glibenclamide and several other sulfonylureas appear to exert a specific inhibitory influ-

ence on prostanoid-induced contractions in vascular preparations isolated from different animal species (Cocks et al., 1990; Zhang et al., 1991; Zhang and Cook, 1994; Delaey and Van de Voorde, 1995, 1997). We also provide evidence that glibenclamide exerts a specific inhibition of U-46619-induced contractions in two human vascular preparations since, at the highest concentration (30 μM), it did not alter the contractions elicited by norepinephrine in internal mammary artery and by 5-HT and endothelin-1 in saphenous vein. The weak and non-significant alterations in the maximal effect of norepinephrine in internal mammary artery and of 5-HT in saphenous vein were even weaker when the data were normalized to the KCl-induced contractions in order to reduce inter- and intra-series variability in force of contraction.

However, glibenclamide significantly decreased the endothelin-1-induced contraction in internal mammary artery. In dog coronary artery, glibenclamide (30 μM) has no influence on endothelin-1-induced contraction (Cocks et al., 1990) whereas in rat aorta, glibenclamide (10 μM) slightly but not significantly alters endothelin-1-induced contractions (Delaey and Van de Voorde, 1995). Endothelin-1 has been reported to induce the release of prostanoids, including thromboxane A_2, from isolated airway and vascular preparations (Resink et al., 1989; Takayasu-Okishio et al., 1990; Reynolds and Mok, 1990; Sokolovsky, 1992; Hay et al., 1993). Therefore, we investigated whether the endothelin-1-induced contraction could be altered by GR 32191, a specific and potent thromboxane A_2 receptor antagonist (Lumley et al., 1989; He and Yang, 1995). GR 32191 inhibited the contraction elicited by endothelin-1 in internal mammary artery, suggesting that the response to endothelin-1 involves at least in part a thromboxane A_2 receptor-mediated response through the release of thromboxane A_2 or prostaglandins. This contractile component is not related to the release of prostanoids from the endothelium since most internal mammary artery preparations were devoid of a functional endothelium. A thromboxane A_2 receptor-mediated pathway appears also involved in the control of the basal tone since GR 32191 decreased the basal tone in internal mammary artery and saphenous vein. In the two preparations, glibenclamide also caused a fall in basal tone similar to that elicited by GR 32191. Since the blockade of K_{ATP} is not expected to induce relaxation but rather contraction, this result suggests that glibenclamide exerts this effect by inhibiting the prostanoid component of basal tone. A decrease in basal tone after incubation with glibenclamide has been previously reported in dog coronary artery (Cocks et al., 1990) and in dog cerebral artery (Zhang et al., 1991). However, this decrease in basal tone could not explain the decrease in maximal responses to endothelin-1 in internal mammary artery since no change in maximal responses occurred with endothelin-1 and 5-HT in saphenous vein and with norepinephrine in internal mammary artery. These results collectively suggest that glibenclamide alters endothelin-1-induced contractions in internal mammary artery by inhibiting the thromboxane A_2 receptor-mediated component of the contractile response to this peptide.

In conclusion, glibenclamide clearly appears to exert a specific inhibitory influence on prostanoid-induced contractions in human internal mammary artery and saphenous vein. Besides taking this activity into consideration for in vitro studies on K_{ATP} in smooth muscles, the inhibitory effect of sulfonylureas on prostanoid-mediated vasoconstriction may be of therapeutic relevance since a reduction of prostaglandin I_2 formation in the vessel walls of diabetic patients has been reported, which results in an imbalance between prostaglandin I_2 and thromboxane A_2 (Johnson et al., 1979; Silverbauer et al., 1979).

Acknowledgements

This work was supported by a grant from the Clinical Research Regional Direction, Grenoble, France. The authors thank F. Caron for the helpful technical assistance.

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