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Prostanoid-induced contractions are blocked by sulfonylureas

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Abstract

The sulfonylureas glibenclamide and tolbutamide are blockers of ATP-regulated K^+ channels. The present study shows that these drugs also block contractions induced by prostaglandin $F_{2\alpha}$, prostaglandin E_2 and the thromboxane A_2 mimetic U-46619 on rat aorta. This effect of sulfonylureas is not related to the endothelium since it is also found in endothelium-denuded preparations. The blockade is specific for prostanoids since contractions with norepinephrine, phenylephrine, serotonin, endothelin-1 or K^+ (120 mM) are not or much less affected. On the other hand, contraction induced by activation of G-proteins with aluminium tetrafluoride anion (AlF_4^-) is significantly blocked by the sulfonylureas. Also on rat carotid artery the contraction of prostaglandin $F_{2\alpha}$ is importantly blocked by glibenclamide. It is concluded that the sulfonylureas glibenclamide and tolbutamide exert a specific inhibitory influence on prostanoid-induced contractions. This inhibition might be due to interference at the level of regulatory G-proteins, since the contractions induced by agonists that, like the prostanoids, activate phospholipase C (serotonin, phenylephrine, norepinephrine, endothelin) are not blocked.

Keywords: Aorta, rat; Sulfonylurea; Prostanoid; Prostaglandin $F_{2\alpha}$; Glibenclamide; Tolbutamide; Thromboxane A_2

1. Introduction

The sulfonylureas glibenclamide and tolbutamide are well known hypoglycemic drugs. They are believed to stimulate the release of insulin by blocking ATP-regulated K^+ channels in pancreatic β-cell membrane. In vascular smooth muscle cell membrane ATP-regulated K^+ channels are also present. Opening of these channels by drugs such as cromakalim or pinacidil hyperpolarizes vascular smooth muscle cell membrane and induces relaxations. These relaxations are blocked by the sulfonylureas glibenclamide and tolbutamide (Standen et al., 1989).

In 1991 Zhang et al. reported that glibenclamide relaxes rat aortic preparations pre-contracted with prostaglandin $F_{2\alpha}$ (Zhang et al., 1991). However, from a previous study we had no evidence for a potent relaxing influence of glibenclamide on aorta from rats (Van de Voorde et al., 1992). Moreover, a drug known as a blocker of K^+ channels is not expected to induce relaxation of smooth muscle cells, but rather contraction. Therefore we first investigated whether we could confirm the observations reported by Zhang et al. Then we investigated whether the inhibitory effect of glibenclamide was specific for prostaglandin $F_{2\alpha}$-induced contractions and whether another sulfonylurea, tolbutamide, would also exert this effect. We also looked whether the inhibitory influence was specific for aortic preparations and related to the endothelium. Lastly we also tried to analyse the mechanism of this enigmatic effect and to find out whether prostaglandin $F_{2\alpha}$ might influence the relaxation induced by K^+-channel openers.

2. Materials and methods

2.1. Tension measurements

The thoracic aorta and in some experiments the carotid artery were isolated from male Wistar rats (300 g) and dissected free of surrounding tissue. Ring segments (2–3 mm length) were prepared and mounted in muscle chambers (20 ml) containing a Krebs-Ringer bicarbonate solution (in mmol/l: NaCl, 135; KCl, 5; $NaHCO_3$, 20; glucose, 10; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; EDTA, 0.026) at 37°C, through which a mixture of 95% O_2-5% CO_2 was bubbled. The

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rings were suspended under a tension of 0.5 g and the isometric force of contraction was measured with a force-displacement transducer (UC-2 cell Gould-Statham, Oxnard, CA, USA). The preparations were equilibrated under tension for 1 h before the start of the measurements. Four adjacent ring preparations could be mounted in parallel. The endothelium was removed from some preparations by gently rubbing the intimal surface with a roughened polyethylene tube (PE 50). In all these preparations, removal of the endothelium was functionally confirmed by the absence of relaxation to acetylcholine (10 μM) after precontraction.

2.2. Drugs and statistics

Norepinephrine bitartrate, endothelin-1, U-46619 (thromboxane A_2 mimetic), sodium fluoride (NaF), aluminium chloride ($AlCl_3$), L-phenylephrine hydrochloride, glibenclamide, tolbutamide, 5-hydroxytryptamine creatinine sulfate complex (serotonin) were obtained from Sigma (St. Louis, MO, USA). Prostaglandin E_2 (dinoproston; Prostin E_2) and prostaglandin $F_{2\alpha}$ (dinoprostum trometamolum; Dinolytic) were obtained from Upjohn (Puurs, Belgium). BRL 38227 was a gift from Beecham Pharmaceuticals, Essex, UK. Krebs-Ringer bicarbonate solution with 120 mM K^+ (K 120 mM) was prepared by adequate equimolar replacement of NaCl with KCl. Aluminium tetrafluoride anion (AlF_4^-) solution was prepared by adding 5 mM NaF and 10 μM $AlCl_3$ to the normal Krebs-Ringer bicarbonate solution.

All concentrations are expressed as final molar concentrations in the organ bath. Concentration-response curves were made by cumulative addition of a small volume (200 μl) in the experimental chamber (20 ml). All solutions were freshly prepared from appropriate stock solutions. Stock solutions were made in water except for glibenclamide, dissolved in dimethyl sulfoxide and tolbutamide, dissolved in ethanol. The final concentration of dimethyl sulfoxide and ethanol was 0.25%.

Statistical significance was evaluated using Student's t-test for unpaired observations. n indicates the number of preparations tested.

3. Results

3.1. Influence of glibenclamide on contractions induced by norepinephrine, serotonin and prostaglandin $F_{2\alpha}$

In a first series of experiments we investigated the contractile effects of increasing concentrations of prostaglandin $F_{2\alpha}$ (0.1–30 μM), norepinephrine (1 nM–10 μM) and serotonin (0.1 μM–0.1 mM) on control rat aortic rings and on rings incubated for 10 min with glibenclamide (1 and 10 μM) ($n = 5$–11). The results are presented in Fig. 1. They show that glibenclamide at a concentration of 10 μM almost completely blocks the contraction induced by prostaglandin $F_{2\alpha}$, while it has less pronounced effect on the contractions induced by norepinephrine and serotonin ($n = 5$–11). Additional experiments excluded the involvement of the solvent (0.25% dimethyl sulfoxide) ($n = 4$) in the inhibitory influence of glibenclamide on prostaglandin $F_{2\alpha}$-induced contractions and showed the reversibility of this inhibition after washout of the sulfonylureas ($n = 3$). In an additional series of experiments ($n = 4$) the influence of incubation with glibenclamide (10 μM) during 30 min was compared on contractions induced by norepinephrine and prostaglandin $F_{2\alpha}$. Similar results as after the 10 min incubation were obtained.

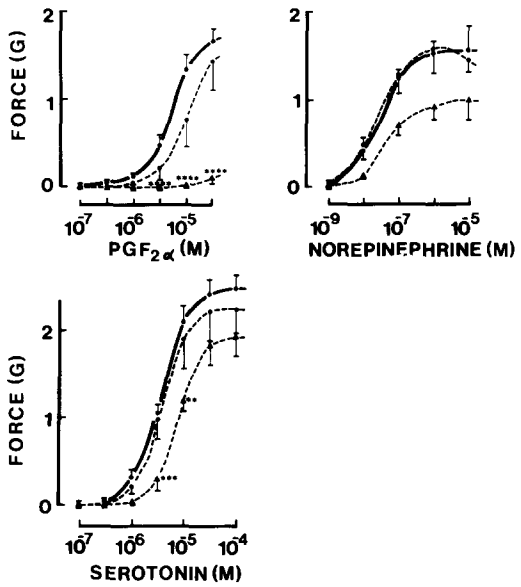

Fig. 1. Contraction (expressed as gram force) of rat aorta induced by increasing molar concentrations of prostaglandin $F_{2\alpha}$, norepinephrine and serotonin in the absence (●———●) and presence of glibenclamide 1 μM (●----●) or 10 μM (▲----▲) ($n = 5$–11) ($^{**}P < 0.02$; $^{***}P < 0.01$; $^{****}P < 0.001$).

3.2. Influence of tolbutamide on contractions induced by norepinephrine, serotonin and prostaglandin $F_{2\alpha}$

In a second series of experiments we investigated the contractile effects of increasing concentrations of prostaglandin $F_{2\alpha}$ (0.1–30 μM), norepinephrine (1 nM–10 μM) and serotonin (0.1 μM–0.1 mM) on control rat aortic rings and on rings incubated for 10 min with tolbutamide (0.1 and 1 mM). The results are presented in Fig. 2. Like glibenclamide (10 μM), tolbutamide (1 mM) almost completely blocks the contraction induced by prostaglandin $F_{2\alpha}$, while it has a much less pronounced influence on the contraction induced

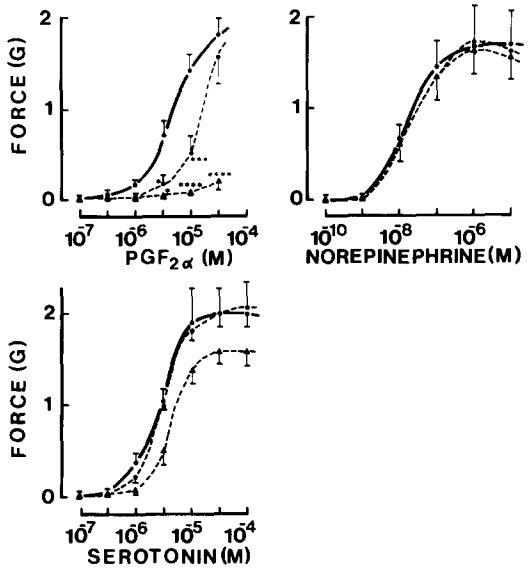

Fig. 2. Contraction (expressed as gram force) of rat aorta induced by increasing molar concentrations of prostaglandin $F_{2\alpha}$, norepinephrine and serotonin in the absence (●———●) and presence of tolbutamide 0.1 mM (●----●) or 1 mM (▲----▲) ($n = 5$) ($^{*}P < 0.05$; $^{****}P < 0.001$).

by serotonin and no effect on the contractions induced by norepinephrine ($n = 5$). Additional experiments excluded the involvement of the solvent (0.25% ethanol) ($n = 4$) in the inhibitory influence of tolbutamide on prostaglandin $F_{2\alpha}$-induced contractions and showed the reversibility of this inhibition ($n = 3$).

3.3. Influence of sulfonylureas on contractions induced by the thromboxane A_2 mimetic U-46619 and prostaglandin E_2

In another series of experiments the effect of increasing concentrations of the thromboxane A_2 mimetic U-46619 was tested on resting preparations in the presence and absence of different concentrations of glibenclamide or tolbutamide. The results, summarized in Fig. 3, clearly illustrate the pronounced inhibitory influence of tolbutamide and glibenclamide ($n = 5$–9). In addition the influence of 10 μM glibenclamide was tested on prostaglandin E_2-induced contractions. Also the contraction induced by prostaglandin E_2 was very significantly inhibited in the presence of glibenclamide ($n = 6$).

3.4. Role of endothelium

The inhibitory influence of glibenclamide (10 μM) on prostaglandin $F_{2\alpha}$-induced contraction (0.1 μM–30 mM) was also investigated on endothelium-denuded preparations. The absence of functional endothelium was assessed by the lack of relaxing influence of acetylcholine. The results are shown in Fig. 4, showing that the inhibitory influence of glibenclamide on prostaglandin $F_{2\alpha}$-induced contraction is also prominent in the absence of endothelium ($n = 6$).

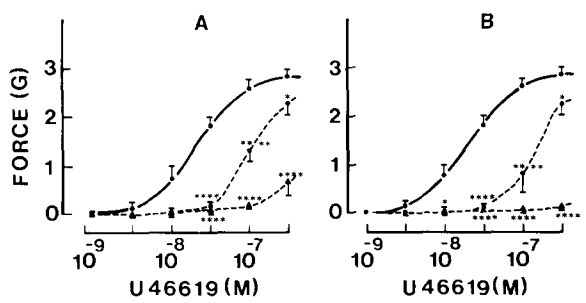

Fig. 3. Contraction (expressed as gram force) of rat aorta induced by increasing molar concentrations of the thromboxane A_2 mimetic U-46619 (A) in the absence (●———●) and presence of glibenclamide 1 μM (●----●) or 10 μM (▲----▲), (B) in the absence (●———●) and presence of tolbutamide 0.1 mM (●----●) or 1 mM (▲----▲) ($n = 5$–9) ($^{*}P < 0.05$; $^{****}P < 0.001$).

3.5. Influence of glibenclamide (10 μM) and tolbutamide (1 mM) on contractions induced by phenylephrine, endothelin-1, K 120 and aluminium tetrafluoride anion (AlF_4^-)

All these experiments were performed on preparations without functional endothelium, evidenced by the lack of relaxing influence of acetylcholine. Fig. 5 shows the influence of glibenclamide ($n = 5$) and tolbutamide ($n = 4$) on contractions induced by the specific α_1-adrenoceptor agonist phenylephrine (1 nM–10 μM). While the sulfonylurea drugs almost completely block the contraction induced by prostaglandin $F_{2\alpha}$ on these preparations, phenylephrine-induced contractions are almost not influenced. On the same preparations, the effect of K 120 mM was assessed. It was found that the sulfonylurea drugs have no influence on the contraction induced by depolarization ($n = 4$–5).

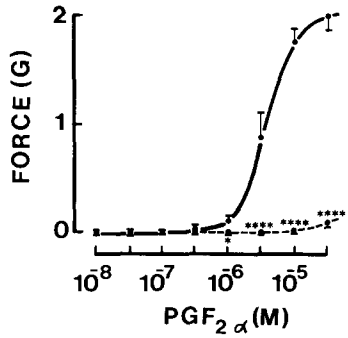

Fig. 4. Contraction (expressed as gram force) induced by increasing molar concentrations of prostaglandin $F_{2\alpha}$ on rat aorta without functional endothelium in the absence (●———●) and presence of glibenclamide 10 μM (●----●) ($n = 6$) ($^{*}P < 0.05$; $^{****}P < 0.001$).

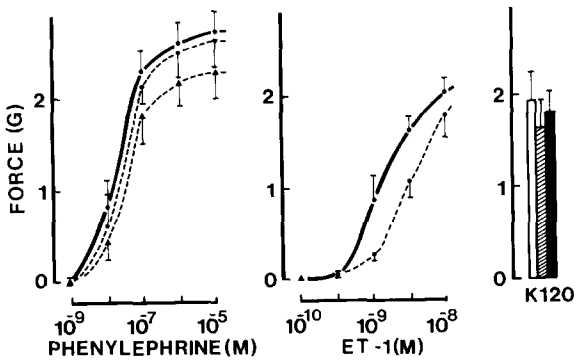

Fig. 5. Contraction (expressed as gram force) of rat aorta induced by increasing molar concentrations of phenylephrine, endothelin-1 and potassium 120 mM in the absence (●———●, open column) and presence of glibenclamide 10 μM (●----●, hatched column) or tolbutamide 1 mM (▲----▲, black column) ($n = 4$–5).

In another series of experiments we also investigated the influence of glibenclamide on endothelin-1 (0.1–10 nM) induced contractions. The contraction was slightly but not significantly inhibited, but the inhibition was not as pronounced as the inhibiton of prostaglandin $F_{2\alpha}$-induced contractions ($n = 4$) (Fig. 5).

In separate series of experiments the influence of glibenclamide ($n = 12$) and tolbutamide ($n = 8$) on contractions induced by aluminium tetrafluoride anion was investigated. Both sulfonylureas exert a pronounced inhibitory influence on this contraction induced by activation of G-proteins (Fig. 6).

3.6. Experiments on rat carotid arteries

The influence of glibenclamide (10 μM) on increasing concentrations of prostaglandin $F_{2\alpha}$ (0.1–30 μM) was also assessed on carotid artery preparations. Also on this preparation the response to prostaglandin $F_{2\alpha}$ is almost completely blocked in the presence of glibenclamide 10 μM ($n = 4$).

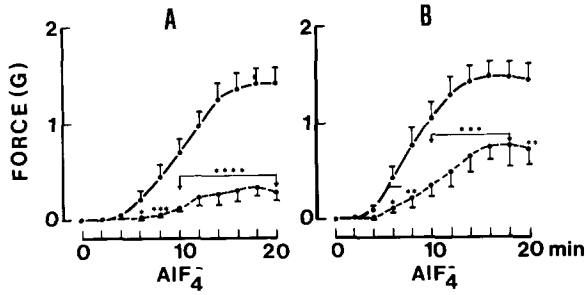

Fig. 6. Contraction of rat aorta (expressed as gram force) as function of time (minutes) induced by AlF_4^- (A) in the absence (●———●) and presence (●----●) of glibenclamide 10 μM ($n = 12$) and (B) in the absence (●———●) and presence (●----●) of tolbutamide 1 mM ($n = 8$) ($^*P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{****}P < 0.001$).

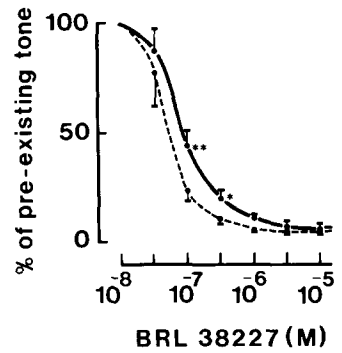

Fig. 7. Relaxation (expressed in percentage of pre-existing tone) in response to increasing molar concentrations of BRL 38227 on rat aortic rings precontracted with norepinephrine 0.1 μM (●———●) or prostaglandin $F_{2\alpha}$ 20 μM (●----●) ($n = 6$) ($^*P < 0.05$; $^{**}P < 0.02$).

3.7. Activation of ATP-regulated K^+ channels on prostaglandin $F_{2\alpha}$-induced contraction

These experiments were performed on preparations with endothelium present. In these experiments the effect of increasing concentrations of the opener of ATP-regulated K^+ channels, BRL 38227, was compared on preparations precontracted with norepinephrine (0.1 μM) or prostaglandin $F_{2\alpha}$ (20 μM). At these concentrations, these agonists elicit a comparable submaximal contraction on aorta preparations. Addition of increasing concentrations of BRL 38227 (10 nM–10 μM) elicits comparable relaxation responses, although the response on prostaglandin $F_{2\alpha}$-precontracted preparations seems to be somewhat stronger (significant at 0.1 and 0.3 μM) ($n = 6$) (Fig. 7).

4. Discussion

The results of the present study provide evidence that the sulfonylureas glibenclamide and tolbutamide have other effects besides their well-known blocking influence on ATP-regulated K^+ channels.

Our study supports the original observation reported by Zhang et al. (1991) that glibenclamide relaxes prostaglandin $F_{2\alpha}$-contracted rat aorta and extends this observation by showing that tolbutamide, another blocker of ATP-regulated K^+ channels, has a similar influence, although this requires a 100-fold higher concentration. This is, however, not surprising since tolbutamide is known to be less potent as a blocker of ATP-regulated K^+ channels (cf. Zhang et al., 1991). In preliminary experiments we also found that a similar higher concentration is required to block the relaxing effect of the K^+ channel opener BRL 38227. Notwithstanding the similarity of both sulfonylureas in their relative potency in blocking ATP-regulated K^+ channels and in blocking the prostaglandin

$F_{2\alpha}$ contraction, their influence on prostaglandin $F_{2\alpha}$ contraction is difficult to relate to interference with ATP-regulated K^+ channels. Inhibition of the efflux of K^+ would elicit an excitatory effect rather than an inhibitory effect.

It is well known that antagonists can have a partial agonistic effect. It thus could be argued that the inhibitory effect of the sulfonylureas might be due to an activation of K^+ channels leading to a relaxation which is not overcome by the addition of prostaglandin $F_{2\alpha}$. This hypothesis is, however, difficult to support since it is quite difficult to understand why the contractile effects of other agents are not blocked. One argument could be that a prostaglandin contraction is more sensitive to inhibition by K^+ channel opening than other contractions. Therefore we compared concentration-relaxation responses of the known opener of ATP-regulated K^+ channels, BRL 38227, on contractions induced by prostaglandin $F_{2\alpha}$ and norepinephrine. The results show that BRL 38227 elicits a similar response independent of the contractile agonist. There is thus no evidence for a higher sensitivity to an opener of K^+ channels in prostaglandin $F_{2\alpha}$- than in norepinephrine-contracted preparations.

In order to find out the possible mechanism responsible for the inhibitory influence of sulfonylureas on prostaglandin $F_{2\alpha}$ contraction, we also investigated whether this effect was specific for prostaglandin $F_{2\alpha}$-induced contraction. Therefore we compared the influence of both sulfonylureas on contractions induced by prostaglandin $F_{2\alpha}$, norepinephrine and serotonin. It was found that the sulfonylureas in a concentration that almost completely blocks the prostaglandin $F_{2\alpha}$-induced contractions, have no or only a limited influence on the contractions induced by serotonin or norepinephrine. These results provide evidence that the influence of the sulfonylureas cannot be explained by a non-specific effect on the contractile process. The observation that the difference in sensitivity is similar even after 30 min incubation of the preparations with glibenclamide illustrates that an incubation time of 10 min is sufficient to ensure maximal influence.

In a further series of experiments, we investigated the possible influence of the sulfonylureas on contractions induced by other prostanoids. It was found that the contractions induced by prostaglandin E_2 and the thromboxane A_2 mimetic U-46619 are also blocked. An inhibitory influence of glibenclamide on thromboxane A_2-induced contraction in dog coronary artery has previously been reported by Cocks et al. (1990). The sulfonylureas thus seem to interfere with different prostanoid-induced contractions.

The vascular endothelium is considered as a potent modulator of vascular tone, mainly by releasing NO (Moncada et al., 1991). The release of this relaxing substance is stimulated by a variety of agonists. Therefore we investigated whether the endothelium could be involved in the inhibitory effect of sulfonylureas on prostanoid-induced contractions. Since similar effects were observed on endothelium-denuded as on endothelium-intact preparations, this hypothesis can be excluded. The influence thus should be situated at the level of the smooth muscle cells.

Contraction of smooth muscle cells relies on the increase in cytosolic calcium as a result either from influx through the cell membrane, or from release from the endoplasmic reticulum. Influx of calcium occurs when voltage-operated calcium channels are opened by depolarization of the cell membrane (Nelson et al., 1990). This mechanism is responsible for the contraction elicited by K 120 mM. From the fact that this contraction is not blocked by glibenclamide it can be concluded that the sulfonylureas do not interfere with the voltage-operated calcium channels.

The action of prostanoids has been associated with different receptor subtypes and different transduction systems, mainly stimulation of adenylyl cyclase, increasing cAMP levels and stimulation of phospholipase C, resulting in inositol 1,4,5-trisphosphate and diacylglycerol formation (Coleman et al., 1994). Since cAMP decreases tone in rat aortic smooth muscles, this mechanism is not involved in the prostaglandin-induced contractions. On the other hand, stimulation of phosphoinositol turnover results in contraction of rat aorta. This mechanism is considered as the transduction mechanism after stimulation of FP- and TP-prostanoid receptor subtypes (Coleman et al., 1994) and thus is very likely to be responsible for the prostanoid contractions in our experiments.

In a further series of experiments we therefore investigated whether the sulfonylureas would interfere with the pathway stimulated by activation of phospholipase C. It is accepted that this pathway is activated after stimulation of α_1-adrenoceptors (Minneman, 1988) and endothelin-1 (Masaki et al., 1994) receptors. Therefore we studied the influence of glibenclamide on contractions induced by the specific α_1-adrenoceptor agonist phenylephrine and by endothelin-1. To exclude possible interference from the endothelium, these experiments were performed on endothelium-denuded preparations. From the fact that these contractions are not blocked, it can be concluded that the influence of the sulfonylurea compounds cannot be explained by interference within the pathway following phospholipase C activation.

The observations with endothelin-1 and phenylephrine indicate that the inhibitory influence of sulfonylureas on prostanoid-induced contractions must be explained by interference with either the prostanoid receptors or the G-proteins coupling the receptor to phospholipase C. In order to locate the level of sulfonylurea interaction we investigated their influence on

contractions induced by aluminium tetrafluoride anion. This molecule moiety has a similar structure to PO_4^{3-} and is able to interact with guanosine 5'-diphosphate situated on the α-subunit of the G-proteins, resulting in activation by mimicking GTP at this binding site (Wu et al., 1992). The observation that the contraction induced by aluminium tetrafluoride anion is significantly blocked by the sulfonylureas indicates that their inhibitory influence on prostanoid contraction might be situated in the signal transduction system(s), possibly certain G-proteins, linking rat aortic prostanoid receptors to the increase in phosphoinositol turnover (Coleman et al., 1994). A possible interference with prostanoid receptors can, however, not be excluded, considering that glibenclamide was reported to have thromboxane A_2 receptor antagonistic properties (Cocks et al., 1990).

In summary, we can conclude that the sulfonylureas glibenclamide and tolbutamide are not only blockers of ATP-dependent K^+ channels, but that they also have an important inhibitory influence on prostanoid-induced vasoconstriction. This influence is not limited to the aorta of the rat, but is also observed in the rat carotid artery. Whether this influence is also present in smaller blood vessels and other smooth muscle preparations must further be investigated.

Acknowledgements

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