ELSEVIER

European Journal of Pharmacology 340 (1997) 187–193

ejp

Differential effects of glibenclamide on responses to thromboxane A2 mimic, U46619, in the pulmonary and hindquarters vascular beds of the cat

Alan D. Kaye [a,b,c,*], Bobby D. Nossaman [a,b,c], Jose A. Santiago [a,b,c], Bracken J. DeWitt [a,b,c], Ikhlass N. Ibrahim [a,b,c], Philip J. Kadowitz [a,b,c]

[a] *Department of Anesthesiology, Tulane University Medical Center, New Orleans, LA 70112-2699, USA*
[b] *Department of Pharmacology, Tulane University Medical Center, New Orleans, LA 70112-2699, USA*
[c] *Department of Anesthesiology and Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112, USA*

Received 3 July 1997; revised 6 October 1997; accepted 10 October 1997

Abstract

The inhibitory effects of the oral sulfonylurea, glibenclamide, on vasoconstrictor responses to the thromboxane A_2 mimic, U46619, were investigated in the pulmonary and hindquarters vascular beds of the cat under constant flow conditions. When lobar arterial tone was at resting conditions (14 ± 2 mm Hg), intralobar injections of U46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2, angiotensin II, norepinephrine, and BAY K 8644 caused dose-related increases in lobar arterial pressure without altering left atrial pressure. Following an intralobar infusion of glibenclamide (5 mg/kg), vasoconstrictor responses to U46619, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 were significantly reduced, whereas vasoconstrictor responses to norepinephrine and angiotensin II were not altered and responses to BAY K 8644 were significantly enhanced. When tone in the pulmonary vascular bed was raised to a high steady level (36 ± 3 mm Hg), glibenclamide in a dose of 5 mg/kg i.a. markedly attenuated responses to injections of U46619 and reduced the vasodilator responses to the K^+-ATP channel opener, levcromakalim, whereas responses to acetylcholine and *S*-nitroso-*N*-acetylpenicillamine (SNAP), a nitric oxide donor, were not changed. In the hindquarters vascular bed of the cat, administration of glibenclamide in a dose of 5 mg/kg i.a. had no significant effect on vasoconstrictor responses to U46619, norepinephrine or angiotensin II. Hindquarters vasodilator responses to levcromakalim, but not to nitric oxide, were decreased significantly following administration of glibenclamide. These data suggest that glibenclamide, in addition to inhibiting K^+-ATP channels, has thromboxane A_2 receptor blocking activity in the pulmonary vascular bed of the cat. These data also suggest that vasoconstrictor responses to U46619 may be mediated by different thromboxane A_2 receptors with different binding affinities in the pulmonary and in the hindquarters vascular beds of the cat.

Keywords: Glibenclamide; Thromboxane A_2 receptor; U46619; Prostaglandin $F_{2\alpha}$; Prostaglandin D_2; Pulmonary and hindquarters vascular bed; (Cat)

1. Introduction

Our understanding of the role of ATP-dependent K^+-channels has grown in the last decade. Since the original descriptions of membrane K^+-channels sensitive to intracellular ATP (K^+-ATP channels) levels in cardiac muscle (Noma, 1983), in arterial smooth muscle (Standen et al., 1989), in pancreatic B-cells (Ashcroft et al., 1984), and in skeletal muscle (Spruce et al., 1985), an entire class of agents interacting at these sites have been described. These agents have vasodilator activity in both the systemic and pulmonary vascular bed (Clapham and Buckingham, 1988; Buckingham et al., 1989; Minkes et al., 1991).

Glibenclamide, a hypoglycemic sulfonylurea, is an antagonist of the K^+-ATP channel. Glibenclamide stimulates the release of insulin from pancreatic acinar cells, probably by blocking K^+-ATP channels located in the plasma membrane (Schmid-Antomarchi et al., 1987). In addition, glibenclamide has been shown to block the effects of the K^+-ATP channel openers in the pulmonary vascular bed (Hood et al., 1991; Minkes et al., 1991), in cardiac tissue, and in smooth muscle (Clapham and Buckingham, 1988). Prostaglandin H_2 is the precursor for tromboxane A_2 and they appear to share the same pharmacological properties in virtually every tissue in which they have been studied (Halushka et al., 1989). Their major pharmacological ac-

* Corresponding author. Tel.: +1-504-5885903; fax: +1-504-5843501.

tions are constriction of vascular and bronchial smooth muscle and aggregation of platelets (Coleman et al., 1981; Mais et al., 1988). It is thought that tromboxane A_2 and prostaglandin H_2 share a common receptor and numerous receptor antagonists have supported this assumption (Lefer, 1985). Recent studies have failed to demonstrate an effect of a tromboxane A_2 mimetic on cAMP accumulation and several studies have demonstrated that tromboxane A_2/prostaglandin H_2 mimetics increase intracellular free calcium (Kawahara et al., 1983; Brass et al., 1987). Stimulation of the platelet tromboxane A_2/prostaglandin H_2 receptor results in action of phospholipase C and the subsequent formation of inositol triphosphate and diacylglycerol (Pollock et al., 1984). Stimulation of the prostaglandin $F_{2\alpha}$ receptors appear to be linked to activation of phospholipase C (Smith et al., 1988).

The second messenger system for the varied effects of prostaglandin D_2 in vascular and non-vascular smooth muscle is largely unknown. Recently, glibenclamide has also been shown to be a competitive antagonist of the stable thromboxane A_2 analog, U46619, in dog isolated coronary artery (Cocks et al., 1990). Although glibenclamide has been shown to relax isolated vascular smooth muscle constriction by prostaglandin $F_{2\alpha}$ in the canine cerebral arteries and the rat aorta (Zhang et al., 1991), little if anything is known about the interactions between U46619 and the prostaglandin vasoconstrictors $F_{2\alpha}$ and prostaglandin D_2 with glibenclamide in the pulmonary and regional circulations. The responses of glibenclamide on vasoconstrictor responses to U46619, prostaglandin D_2, prostaglandin $F_{2\alpha}$, BAY K 8644, angiotensin II and norepinephrine and on vasodilator responses to levcromakalim and *S*-nitroso-*N*-acetyl-penicillamine (SNAP) were investigated in the pulmonary vascular bed of the intact-chest cat under constant flow conditions. In addition, the influence of glibenclamide on vasoconstrictor responses to U46619, norepinephrine, BAY K 8644, angiotensin II and on vasodilator responses to levcromakalim and nitric oxide were also investigated in the hindquarters vascular bed under constant flow conditions. Therefore, the purpose of the present study was to investigate the effects of glibenclamide, an inhibitor of K^+-ATP channels, in the pulmonary and hindquarters vascular beds of the cat.

2. Methods and materials

Forty-one adult mongrel cats of either sex weighing 3.0–4.6 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). The animals were restrained in the supine position on a fluoroscopic table and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 100% oxygen. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from a femoral artery, and i.v. injections were made into a catheter positioned in the inferior vena cava from a femoral vein.

For perfusion of the left lower lung lobe, a triple-lumen 6F balloon perfusion catheter was passed under fluoroscopic guidance from an external jugular vein into the artery to the left lower lung lobe. After the animal had been heparinized, 1000 U/kg i.v., the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter, and the lobe was perfused with a Harvard model 1210 perfusion pump by way of the catheter lumen beyond the balloon cuff with blood withdrawn from a femoral artery. The perfusion rate was adjusted so that lobar arterial perfusion approximated mean pressure in the main pulmonary artery and was not changed thereafter. The flow rate ranged from 25–45 ml/min and left atrial pressure was measured with a radioopaque 5F single lumen or 6F double lumen catheter passed transseptally into the left atrium from an external jugular vein. All vascular pressures, measured with Spectromed DTX transducers, zeroed at right atrial level, were recorded on a Grass model 7 recorder.

For constant-flow perfusion of the hindquarters vascular bed, a 3–4 cm segment of distal aorta was exposed through a midline incision and was cleared of surrounding connective tissue. After administration of heparin sodium, 1,000 U/kg i.v., the aorta was ligated and catheters were inserted proximal and distal to the ligature. The inlet side of the perfusion circuit was connected to the proximal catheter and the outlet side to the distal aortic catheter. Blood flow to the hindquarters was maintained constant with a Sigmamotor pump model T-8, and perfusion pressure was monitored from a lateral tap in the perfusion circuit located between the pump and the distal catheter. In experiments in the hindquarters vascular bed, all vasoactive agents were injected directly into the perfusion circuit in volumes of 100 microliters or less. The pumping rate averaged 34 ± 2 ml/min in these studies, and the hindquarters vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia between L_3 and L_4. This procedure has been described previously (Minkes et al., 1991).

The present experiments were divided into 4 series. In the first series of experiments, the influence of glibenclamide on vasoconstrictor responses to U46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2, norepinephrine, angiotensin II, and BAY K 8644 was investigated in the intact-chest pulmonary vascular bed. In the second group of experiments, with tone in the pulmonary vascular bed raised to a high steady level with U46619, or in animals with spontaneously elevated tone, the influence of glibenclamide on vasodilator responses to levcromakalim, a K^+-ATP channel opener, acetylcholine, and SNAP, a nitric oxide donor, was investigated. Tone in the pulmonary

vascular bed was raised to a high steady level with U46619 (36 ± 3 mm Hg; infusion rate from 95 to 200 ng/min). Glibenclamide (5 mg/kg) was infused into the intralobar circuit for 30 min after terminating the infusion with U46619, the tromboxane A_2 mimic. Following the glibenclamide infusion, the U46619 was restarted and increases in the infusion rate with U46619 (280 ng/min) were observed to raise tone to control level.

In animals with elevated resting tone, the vasodilators acetylcholine and levcromakalim were injected into the perfusion circuit before and after the glibenclamide was administered. In the third series of experiments, the influence of glibenclamide on vasoconstrictor responses to U46619, angiotensin II, BAY K 8644, and norepinephrine was investigated in the hindquarters vascular bed. In the last series of experiments the influence of glibenclamide on vasodilator responses to levcromakalim and to nitric oxide was investigated in the hindquarters vascular bed.

Stock solutions of U46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2 (Upjohn, Kalamazoo, MI) and levcromakalim (SmithKline Beecham, UK), were prepared in 100% ethanol at concentrations of 5–10 mg/ml and were stored in a freezer at -20°C. Working solutions were prepared on a frequent basis by diluting the stock solutions in 0.9% NaCl solution. *S*-nitroso-*N*-acetylpenicillamine (SNAP) was synthesized and kindly supplied by Dr. Louis J. Ignarro (UCLA School of Medicine, Los Angeles, CA) and dissolved in normal saline. Nitric oxide (NO) solutions were prepared in reagent grade methanol by first bubbling the methanol for 20 min with nitrogen in a gas-tight reaction vial to remove dissolved oxygen. The degassed methanol was then bubbled with NO gas (Hydrocarbon Technologies, Sulphur, LA) for 20 min and stored in a freezer in the gas-tight vial. The concentration of NO in the NO methanol solution was measured and ranged from 30 to 50 mM (Sievers Nitric Oxide Analyzer Model 270; Menon et al., 1989). The doses of NO administered are, therefore, expressed only as volume (μl) of NO solution injected. Injections of NO solution were made in small volumes (30–100 μl) directly into the perfusion circuit with a gas-tight syringe. The methanol vehicle for the NO solution had no significant effect on arterial pressure when injected into the hindlimb in volumes of 30 and 100 μl. The injection of 30 and 100 μl of 1 mM methyl nitrite (nitromethane; Aldrich) solution in methanol, a product that can be formed by the reaction of NO and methanol, had no consistent effect on arterial pressure in the cat. Norepinephrine hydrochloride (Sigma, St. Louis, MO) and acetylcholine chloride (Sigma, St. Louis, MO) were dissolved in 0.9% NaCl. BAY K8644 (Miles; West Haven, CT) was dissolved in a 1:4 solution of cremophor El and Tris (hydroxymethyl) aminomethane (Tris) and Tris hydrochloride, pH 7.4. The resulting suspension was warmed and polyethylene glycol and Tris, pH 7.4, were added to make a stock solution that was stored in a brown bottle in a freezer at -20°C. Working solutions were prepared in 0.9% NaCl, stored in brown bottles, and kept on crushed ice during an experiment. All agonists were injected directly into the lobar arterial or hindquarters perfusion circuit on a straight weight basis. Glibenclamide (Sigma, St. Louis, MO) was prepared in 4 ml propylene glycol and sonicated. To the resulting solution were added 300 microliter 1 N NaOH, 1.7 ml of 100% ethanol, and 4 ml of Tris (pH 8.4). Working solutions were prepared in a 1:1 (vol/vol) of the stock solution to Tris (pH 8.4) and used immediately. The solvents for these agents had no effect on any of the vascular parameters measured.

Arterial blood gas tensions and pH (Corning model 178, Corning Instruments) were measured at the beginning and during the experiments. Blood pH was maintained between 7.35 and 7.45 by adding small amounts of $NaHC0_3$ solution i.v. All agonists were injected directly into the lobar artery and hindquarters perfusion circuits in small volumes in a random sequence, and sufficient time was permitted between injections for pressure to return to baseline values. Since lobar and hindquarters vascular beds are perfused at constant flow, changes in pressure would represent changes in vascular resistance. All vascular pressures are expressed in absolute units (mm Hg) as means $\pm$ S.E.M. The data were analyzed using a paired *t*-test or using a one-way analysis of variance with post hoc Scheffe's *F*-test (StatView 4.01, Abascus Concepts, Berkeley, CA) completed on a Quadra 660AV. A P value of < 0.05 was used as the criterion for statistical significance.

3. Results

The effects of glibenclamide on responses to tromboxane A_2 mimic, U46619, were investigated and compared in the pulmonary vascular bed under conditions of controlled blood flow. Vasoconstrictor responses to U46619, prostaglandin $F_{2\alpha}$, and prostaglandin D_2 were compared to angiotensin II, BAY K 8644, and norepinephrine. At resting pulmonary vascular tone (baseline 14 ± 2 mm Hg), intralobar injection of U46619, angiotensin II, prostaglandin $F_{2\alpha}$ and prostaglandin D_2, BAY K 8644, and norepinephrine caused dose-related increases in lobar arterial pressure without altering left atrial pressure (Fig. 1). In the pulmonary vascular bed of the cat, U46619 had approximately a three-fold greater effect than prostaglandin $F_{2\alpha}$ and had a thirty-fold greater effect than prostaglandin D_2. In the hindlimb vascular bed of the cat, U46619 had an approximately five-fold less effect than angiotensin II while the thromboxane mimic had a significantly greater effect than BAY K 8644 or norepinephrine. Following intralobar infusion of glibenclamide, increases in lobar arterial pressure in response to U46619, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 were attenuated significantly (Fig. 2), whereas responses to angiotensin II and to norepinephrine were not changed (Fig. 2 and Fig. 3). Responses to BAY K 8644 were significantly enhanced and these data

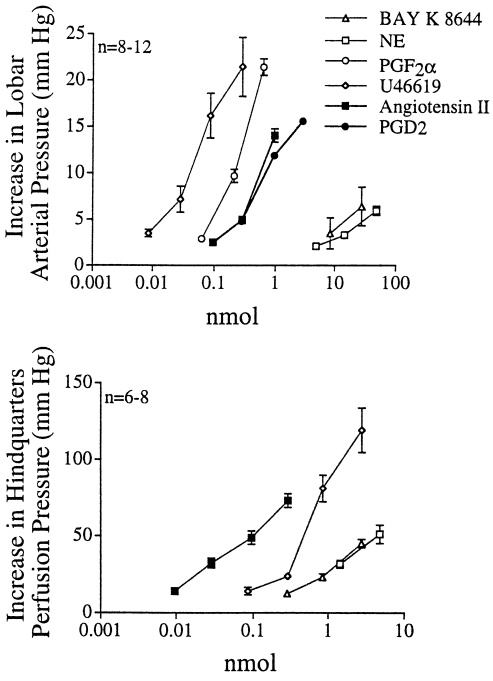

Fig. 1. Comparison of dose-response curves in the pulmonary vascular bed of the cat (upper panel) to BAY K 8644, norepinephrine, prostaglandin $F_{2\alpha}$, U46619, angiotensin II, and prostaglandin D_2. Comparison of dose-response curves in the feline hindlimb vascular bed to norepinephrine, U46619, angiotensin II, and BAY K 8644 (lower panel). Each vasoconstrictor agent is expressed in nmol equivalents. *n*, number of animals.

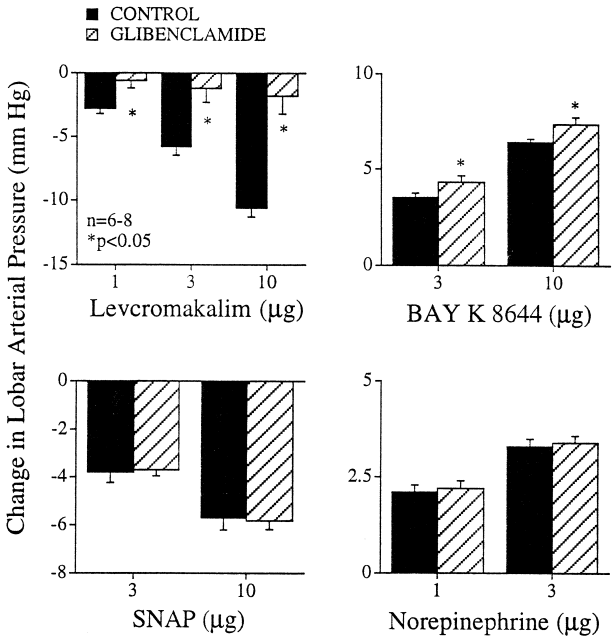

Fig. 3. Influence of glibenclamide, infused in a dose of 5 mg/kg i.a., on responses to levcromakalim, *S*-nitroso-*N*-acetylpenicillamine, norepinephrine, and BAY K 8644 in the pulmonary vascular bed of the cat. *n*, number of animals. * Significantly different from control.

are also summarized in Fig. 3. When tone in the pulmonary vascular bed was raised to a high steady level with U46619, intralobar injection of the K^+-ATP channel opener, levcromakalim, in doses of 1–10 μg caused dose-related decreases in lobar arterial pressure (Fig. 3). Following administration of glibenclamide, vasodilator responses

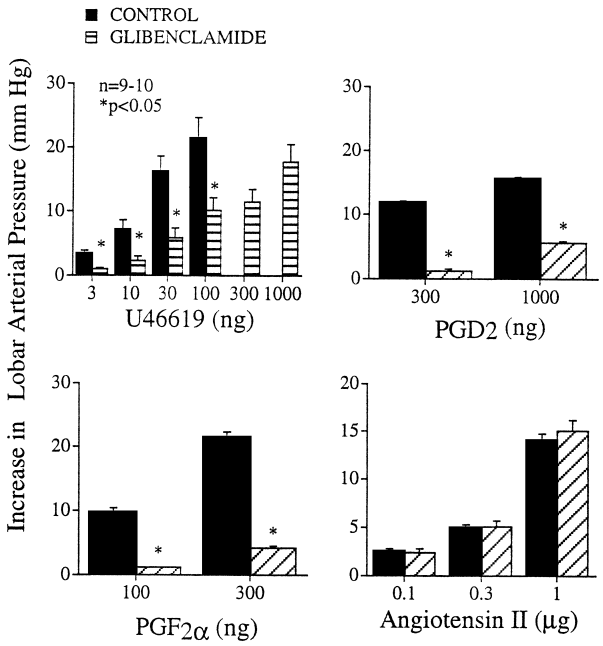

Fig. 2. Influence of glibenclamide, infused in a dose of 5 mg/kg i.a. on vasoconstrictor responses to U46619, prostaglandin $F_{2\alpha}$, angiotensin II, and prostaglandin D_2 in the pulmonary vascular bed of the cat. *n*, number of animals. * Significantly different from control.

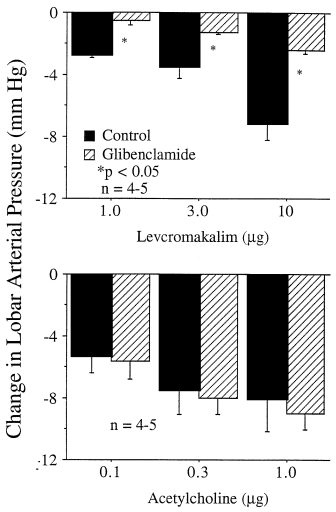

Fig. 4. Influence of glibenclamide, infused in a dose of 5 mg/kg i.a., on responses to levcromakalim and acetylcholine in the pulmonary vascular bed of the cat. *n*, number of animals. * Significantly different from control.

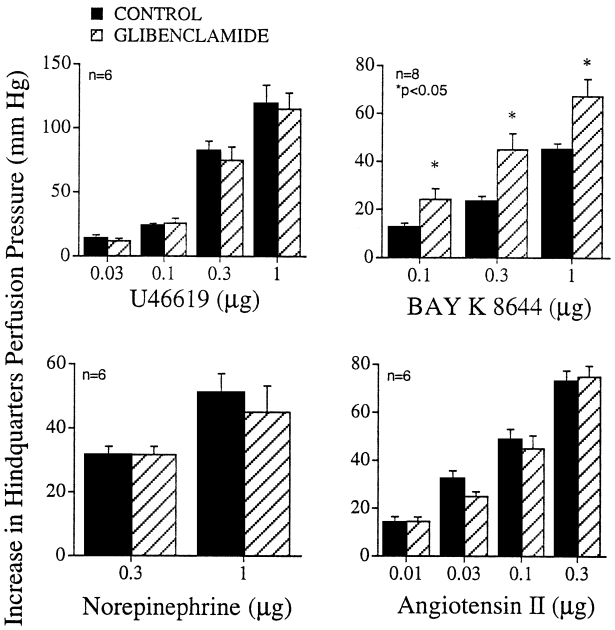

Fig. 5. Influence of glibenclamide, infused in a dose of 5 mg/kg i.a., on responses to U46619, norepinephrine, BAY K 8644, and angiotensin II in the hindlimb vascular bed of the cat. *n*, number of animals. * Significantly different from control.

to levcromakalim were significantly attenuated, whereas responses to SNAP, a nitric oxide donor, were not altered (Fig. 3). When tone in the pulmonary vascular bed was naturally elevated without infusion of U46619, vasodilator responses to levcromakalim were significantly attenuated, whereas responses to acetylcholine were not altered (Fig. 4).

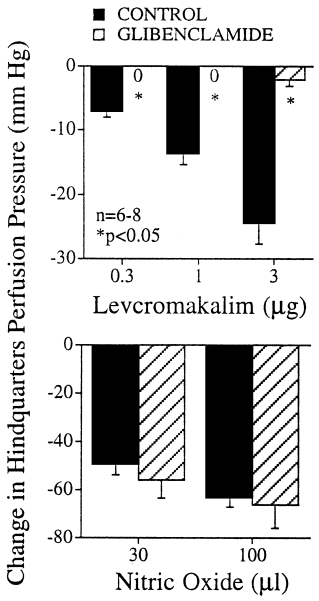

Fig. 6. Influence of glibenclamide, infused in a dose of 5 mg/kg i.a., on responses to levcromakalim, and nitric oxide in the hindquarters vascular bed of the cat. *n*, number of animals. * Significantly different from control.

Table 1
Effect of glibenclamide ($n = 12$) on left atrial pressure (LAP), aortic pressure (AP), and lobar arterial pressure (PAP)

Parameter	Control	Glibenclamide
AP (mm Hg)	120 ± 8	122 ± 9
LAP (mm Hg)	5.7 ± 0.5	6 ± 0.6
PAP (mm Hg)	19 ± 3	20.4 ± 3.4

Values represent means and are followed by the standard error. No significant change was observed ($P < 0.05$).

In the hindquarters vascular bed of the cat under conditions of constant blood flow, the influence of glibenclamide on responses to U46619, BAY K 8644, norepinephrine, angiotensin II, levcromakalim and nitric oxide were investigated and these data are summarized in Figs. 5 and 6. At normal resting tone (150 ± 10 mm Hg), intraarterial injections of U46619 (3–100 ng), norepinephrine (0.3–1 μg), BAY K 8644 (0.1–1.0 μg), and angiotensin II (0.01–0.3 μg) caused dose-related increases in hindquarters perfusion pressure (Fig. 5). Following administration of glibenclamide in a dose of 5 mg/kg i.a., vasoconstrictor responses to U46619 as well as responses to norepinephrine and angiotensin II were not changed (Fig. 5). Responses to the calcium channel opener BAY K 8644 were significantly enhanced after administration of glibenclamide. Under similar tone conditions, intraarterial injections of the K^+-ATP channel opener, levcromakalim, also caused dose-dependent decreases in hindquarter perfusion pressure (Fig. 6). Following an intraarterial injection of glibenclamide, decreases in hindquarters perfusion pressure in response to levcromakalim were reduced significantly, whereas responses to nitric oxide were not altered (Fig. 6). Table 1 shows baseline hemodynamic parameters before and after administration of glibenclamide. Injection of glibenclamide in a dose of 5 mg/kg i.a. produced transient increases in aortic pressure (AP), lobar arterial (PAP), and left atrial (LAP) within 3–5 min after administration of the K^+-ATP channel blocking agent. These parameters returned toward control value and were not significantly different from control value 10–20 min after the administration of the K^+-ATP channel blocking agent. Infusion of the K^+-ATP channel blocking agent over 30 min caused no significant difference in AP, PAP, and LAP.

4. Discussion

The results of the present study demonstrate that U46619, prostaglandin $F_{2\alpha}$, prostaglandin D_2, angiotensin II, norepinephrine, and BAY K 8644 caused dose-related increases in lobar arterial pressure in the intact-chest cat. Inasmuch as pulmonary blood flow and left atrial pressure were maintained constant, increases in lobar arterial pressure in response to these agents reflect increases in pul-

monary lobar arterial resistance. Glibenclamide has been reported to be one of the most potent inhibitors of K^+-ATP channels in vivo and in vitro experiments (Nelson et al., 1990). These data show that following intralobar infusion of glibenclamide, increases in lobar arterial pressure in response to U46619, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 were significantly attenuated. U46619 is a stable endoperoxide analog, and it has been shown that U46619 has a spectrum of activity similar to tromboxane A_2 on isolated smooth muscle preparations. It has been suggested that tromboxane A_2 and prostaglandin H_2 share the same type of tromboxane A_2 receptor in the pulmonary vascular bed (Lefer, 1985; Harris et al., 1989). U46619 and the prostanoids vasoconstrictor responses are reduced in the pulmonary vascular bed by glibenclamide and this may suggest that the K^+-ATP channel blocking agent has tromboxane A_2 receptor blocking activity in this vascular bed. These findings are in agreement with previous studies in the isolated canine coronary artery and in the canine cerebral arteries (Cocks et al., 1990). Glibenclamide appeared to have no effect on vasoconstrictor responses to norepinephrine or to angiotensin II and significantly enhanced responses to the calcium entry enhancing agent, BAY K 8644. When tone in the pulmonary vascular bed was raised to a high steady level, glibenclamide in a dose that markedly attenuated responses to U46619 and the prostanoids, prostaglandin $F_{2\alpha}$ and prostaglandin D_2, significantly reduced the vasodilator responses to K^+-ATP channel openers, levcromakalim, and had no significant effects on SNAP and acetylcholine responses. These findings suggest that blockade from glibenclamide on responses to U46619 and the prostanoids prostaglandin $F_{2\alpha}$ and prostaglandin D_2 is selective in the pulmonary vascular bed.

The influence of glibenclamide on responses to BAY K 8644, a nifedipine analogue that promotes calcium entry, was greater in the hindquarters compared with the pulmonary vascular bed studied, and responses to the calcium agonist were increased in magnitude and in duration after administration of the K^+-ATP channel blocker. These data suggest glibenclamide does not interfere with calcium entry through dihydropyridine voltage-dependent channels in the hindquarters and pulmonary vascular beds. The mechanism by which glibenclamide enhanced responses to BAY K 8644 is uncertain, but may be related to an interaction between K^+-ATP channels and voltage-dependent calcium channels.

In contrast to the inhibitory effects of glibenclamide on responses to U46619 in the pulmonary vascular bed, hindlimb vasoconstrictor responses to U46619, angiotensin II and norepinephrine were not affected, whereas the vasodilator responses to levcromakalim were reduced significantly by intraarterial administration of the K^+-ATP channel blocking agent. Glibenclamide had no significant effect on responses to nitric oxide in the hindlimb or to SNAP and acetylcholine in the pulmonary vascular bed. These data suggest that in both the hindquarters and the pulmonary vascular beds, glibenclamide did not interfere with NO-induced relaxation.

The data of the present study suggest that responses to U46619 in the hindquarters vascular bed are mediated by tromboxane A_2 receptors with different binding affinity for U46619 when compared with the tromboxane A_2 receptor in the pulmonary vascular bed. Since the prostanoids prostaglandin $F_{2\alpha}$ and prostaglandin D_2 have very weak activity in the hindlimb, the effects of glibenclamide were not studied. The difference in results obtained in the pulmonary and hindquarters vascular beds of the cat suggests a difference in the nature of tromboxane A_2 receptors in different organ systems.

Previous studies have demonstrated that inhibitory effects of glibenclamide on responses to prostanoids differ in canine cerebral artery and in isolated rat aorta, suggesting that inhibition from the blocking agent is dependent upon species. In isolated canine cerebral arteries, it has been reported that glibenclamide relaxes prostaglandin $F_{2\alpha}$-induced contraction, suggesting that glibenclamide may have relaxant activity in the isolated cerebral arteries (Zhang et al., 1991). However, increases in resting tone after administration of glibenclamide (5 mg/kg i.a.) was transiently observed in the pulmonary and hindquarters vascular beds. The difference in the isolated canine cerebral arteries and in pulmonary and hindquarters vascular beds is uncertain. The increases in resting tone by glibenclamide may be due to inhibition of activation of K^+-ATP channels in the dilated state in the pulmonary and hindlimb vascular beds, and relaxation of precontracted with prostaglandin $F_{2\alpha}$ by glibenclamide in isolated canine cerebral arteries may be attributed to stimulation of release of prostaglandin I_2 (Zhang et al., 1991).

In our laboratory, glibenclamide has been used to investigate the vasodilator responses mediated by activation of K^+-ATP channels in the pulmonary and hindquarter vascular beds. In order to express vasodilator responsiveness, tone in the pulmonary vascular bed was elevated to 35 ± 2 mm Hg in most experiments and an infusion rate of U46619 at ranges from 38 ± 8 ng/min to 200 ± 35 ng/min was given. Certain animals had elevated tone in the pulmonary vascular bed and were not given U46619 infusion. These animals also had decreases in vasodilator responses to levcromakalim after glibenclamide infusion. In most animals, resting tone was low and large amounts of U46619 were required to infuse into the lobar artery circuit to raise the tone before and following administration of glibenclamide (5 mg/kg i.a.). It is, however, unlikely that the tromboxane A_2 receptor blocking property of glibenclamide should interfere with the interpretation of the results in our laboratory, since tone in the pulmonary vascular bed was raised to a similar level before and after the K^+-ATP channels were administered with the blocking agent. Further, similar results were obtained with or without infusion of the thromboxane

mimic. The role of glibenclamide in mediated or modulated responses further downstream from the receptor site cannot be excluded and further studies are warranted.

In conclusion, results of the present study suggest that glibenclamide selectively reduces vasoconstrictor responses to U46619 and prostanoids in the pulmonary vascular bed, but has no effect on responses to tromboxane A_2 mimic in the hindquarters vascular bed. The data suggest that the K^+-ATP channel blocking agent glibenclamide may have tromboxane A_2 receptor blocking activity and responses to tromboxane A_2 may be mediated by tromboxane A_2 receptors with different binding affinity or differences in sensitivity in the pulmonary and hindquarters vascular beds.

References

Ashcroft, F.M., Harrison, D.E., Ashcroft, S.J., 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. Nature 312, 446.

Brass, L.F., Shaller, C.C., Belmonte, E.J., 1987. Inositol 1,4,5-trisphosphate-induced granule secretion in platelets. Evidence that the activation of phospho-lipase C mediated by platelet thromboxane receptors involves a guanine nucleotide binding protein-dependent mechanism distinct from that of thrombin. J. Clin. Invest. 79, 1269.

Buckingham, R.E., Hamilton, T.C., Howlett, D.R., Mootoo, S., Wilson, C., 1989. Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. Br. J. Pharmacol. 97, 57.

Clapham, J.C., Buckingham, R.E., 1988. The hemodynamic profile of cromakalim in the cat. J. Cardiovasc. Pharmacol. 12, 555.

Cocks, T.M., King, S.J., Angus, J.A., 1990. Glibenclamide is a competitive antagonist of the thromboxane A_2 receptor in dog coronary artery in vitro. Br. J. Pharmacol. 100, 375.

Coleman, R., Humphrey, P.P., Kennedy, I., Levy, G.P., Lumley, P., 1981. Comparisons of the actions of U46619, a prostaglandin H_2 analog, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. Br. J. Pharmacol. 73, 773.

Halushka, P.V., Mais, D.E., Mayeux, P.R., Morinelli, T.A., 1989. Thromboxane, prostaglandin and leukotriene receptors. Annu. Rev. Pharm. Tox. 10, 213.

Harris, D.N., Hall, S.E., Hedberg, A., Ogletree, M.L., 1989. 7-Oxabicycloheptane analogs: modulators of the arachidonate cascade. Drugs Future 13, 153.

Hood, J.S., McMahon, T.J., Kadowitz, P.J., 1991. Pulmonary vasodilator responses to RP 52891 are mediated by activation of a glibenclamide-sensitive K+ ATP channel. Eur. J. of Pharmacol. 202, 121.

Kawahara, Y., Yamanishi, J., Furuta, Y., Kaibuchi, K., Takai, Y. et al., 1983. Elevation of cytoplasmic free calcium concentration by stable thromboxane A_2 analog in human platelets. Biochem. Biophys. Res. Commun. 117, 663.

Lefer, A.M., 1985. Comparison of the actions of thromboxane receptor antagonists in biological systems. Drugs Today 21, 283.

Mais, D.E., Halushka, P.V., Saussy, D.L., Jr., et al., 1988. Platelet and vascular smooth muscle thromboxane A_2/prostaglandin H_2 receptors, Eicosanoids in the Cardiovascular and Renal Systems, MTP Press, Lancaster, UK, p. 210.

Menon, N.K., Wolf, A., Zehetgruber, M., Bing, R.J., 1989. An improved chemiluminescence assay suggest non nitric oxide-mediated action of lysophosphatidylcholine and acetylcholine. Proc. Soc. Exp. Biol. Med. 191, 316.

Minkes, R.K., Kvamme, P., Higuera, T.R. .No, Nossaman, B.D., Kadowitz, P.J., 1991. Analysis of pulmonary and systemic vascular responses to cromakalim, an activator of K^+ ATP channels. Am. J. Physiol. 260, H957.

Nelson, J., Patlak, B., Worley, J.F., Standen, N.B., 1990. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am. J. Physiol. 259, 3.

Noma, A., 1983. ATP-regulated K^+ channels in cardiac muscle. Nature 305, 147.

Pollock, W.K., Armstrong, R.A., Branden, L.J., Jones, J.L., MacIntyre, D.E., 1984. Thromboxane induced phosphatidate formation in human platelets. Biochem. J. 219, 833.

Schmid-Antomarchi, H., De Weille, J., Fosset, M., Lazdunski, M., 1987. The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. J. Biol. Chem. 262, 15840.

Smith, W.L., Sonnenbur, W.K., Watanabe, Y., Umegaki, T. et al., 1988. Mechanisms of action of prostaglandin E_2 and prostaglandin $F_{2\alpha}$, Eicosanoids in the Cardiovascular and Renal Systems, MTP Press, Lancaster, UK, p. 232.

Spruce, A.E., Standen, N.B., Stanfield, P.R., 1985. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. Nature 316, 736.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177.

Zhang, H., Stockbridge, N., Weir, B., Krueger, C., Cook, D., 1991. Glibenclamide relaxes vascular smooth muscle constriction produced by prostaglandin $F_{2\alpha}$. Eur. J. Pharmacol. 195, 27.