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European Journal of Pharmacology 324 (1997) 193–200

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The thromboxane A_2 and K_{ATP} channel antagonist actions of a series of sulphonylurea derivatives in the pig coronary artery

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Received 9 December 1996; revised 21 January 1997; accepted 28 January 1997

Abstract

The ability of a series of sulphonylurea derivatives to antagonise the vasorelaxant actions of the ATP-dependent K^+ channel (K_{ATP}) opener, levcromakalim, and the vasoconstrictor responses of the thromboxane A_2 mimetic, U46619, were assessed in the pig coronary artery. The sulphonylurea derivatives of glibenclamide caused a rightward shift in the concentration-vasorelaxant response curve obtained to levcromakalim in arterial segments pre-constricted with acetylcholine (0.5 μM). From these shifts pK_B were calculated to estimate the potency of these compounds as levcromakalim antagonists. Similarly U46619 concentration-vasoconstrictor responses curves were constructed in the absence and in the presence of a sulphonylurea derivative and pK_B values calculated. Regression analysis of pK_B values showed that there was a significant correlation between the potency of these compounds in the two systems studied indicating similar structure-activity relationships apply in both cases. That sulphonylureas regulate K_{ATP} channel opening is well known and they do so through a specific receptor associated with the channel. The results obtained in this study may indicate that a sulphonylurea receptor may also be associated with thromboxane A_2 excitation-contraction coupling.

Keywords: Sulphonylurea; Levcromakalim; Thromboxane A_2; U46619; Structure-activity relationship; Coronary artery, pig

1. Introduction

Sulphonylureas, such as glibenclamide, inhibit the opening of ATP-dependent K^+ channels (K_{ATP}) in the pancreas and also in vascular and non-vascular smooth muscle (see Challinor-Rogers and McPherson, 1994). Because of its potent actions, glibenclamide has frequently been used in studies examining the role of K_{ATP} channels in regulating a number of physiological processes. It has been assumed that the ability of glibenclamide to selectively regulate K_{ATP} channels (either assessed directly or through its ability to antagonise the action of K_{ATP} channel openers such as levcromakalim; see Section 4) occurred over the concentration range of 0.3 to 3 μM. However in 1990, Thomas Cocks and colleagues (Cocks et al., 1990) showed that glibenclamide was also a potent competitive antagonist of the thromboxane A_2 mimetic, U46619, which causes vasoconstrictor responses in vascular smooth muscle. Concentrations of glibenclamide as low as 1 μM affected contractile responses to U46619 in the dog coronary artery. Thus there is clear overlap in the actions of glibenclamide against K_{ATP} channels and the thromboxane A_2 receptor. The authors (Cocks et al., 1990) highlighted the danger in interpreting results obtained with glibenclamide if active force was generated by exogenous thromboxane A_2 receptor agonists, or more importantly, the generation of endogenous thromboxane A_2 itself.

The relationship between the ability of the sulphonylurea to antagonise K_{ATP} channels and its ability to antagonise U46619-mediated vasoconstrictor responses in vascular smooth muscle is unclear at present. Recently we synthesised and tested a number of structurally related glibenclamide analogues for use in our work, examining the heterogeneous nature of sulphonylurea receptor/K_{ATP} channel interactions in vascular smooth muscle (Challinor-Rogers et al., 1995). In the present study we have used these compounds to determine whether the same structure-activity relationship holds for glibenclamide analogues antagonising U46619-mediated vasoconstrictor responses compared to that for the same compounds antagonising levcromakalim-mediated vasorelaxant responses. To

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PII S0014-2999(97)00086-1

this end we determined the antagonistic (U46619 and levcromakalim) potency of a number of glibenclamide derivatives in the pig coronary artery.

2. Materials and methods

2.1. Isolation and study of pig coronary artery

The heart was obtained from freshly killed pigs at an abattoir. The right circumflex artery was rapidly removed and placed in ice-cold physiological Krebs' solution (composition in mM: NaCl 119, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.17, KH_2PO_4 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11). The artery was cut into 4 mm long segments. To avoid complications, the endothelium was removed from all preparations by gently rubbing the lumen with a wooden rod. Each segment was then suspended on two stainless steel wire hooks, 400 μm in diameter, in 25 ml jacketed glass organ baths. The lower hook was fixed to a support leg attached to a micrometer, while the upper wire hook was suspended from a Grass FT03C force transducer through which changes in isometric force were recorded. Force recordings were displayed on a 2-channel flat-bed chart recorder (Model 320, W + W Scientific Instruments, Switzerland). Vessels were left to equilibrate under zero force for 30 min at 37°C in Krebs' solution gassed with 5% CO_2 in O_2 and an initial force of 5 g was then applied. After another 30 min, the force was re-adjusted to 5 g and the tissues were left for a further 30 min. Subsequently, a K^+ depolarizing solution (KPSS, composition in mM: KCl 123, $MgSO_4 \cdot 7H_2O$ 1.17, KH_2PO_4 2.37, $CaCl_2$ 2.5, EDTA 0.026 and glucose 5.5) was added. This response was used to determine the maximal constrictor response of the tissue. After a plateau to KPSS was reached, vessels were washed twice and left until the response returned to the initial baseline, before commencing the experiment.

2.2. U46619 antagonist potency

After the equilibration period, an antagonist (or the vehicle control) was added 20 min prior to constructing a concentration-effect curve (0.5 log increments) to U46619 (0.1–3000 nM). Concentrations of U46619 were added when the response to the previous concentration had reached a plateau. Only one concentration–response curve was obtained on any one coronary artery ring. Preliminary experiments indicated that this incubation period with the sulphonylurea was sufficient for equilibrium antagonism to be produced. In these studies all glibenclamide analogues were tested at a final concentration of 30 μM.

In a separate series of experiments, the effect of tetraphenylphosphonium chloride (10 μM) on U46619-mediated vasoconstrictor responses was also assessed. The protocol used was identical to that used to assess the potency of the glibenclamide analogues. In addition, in this group of experiments the possible interaction between glibenclamide (10 μM) and tetraphenylphosphonium (10 μM) was assessed by co-incubating both agents.

2.3. Levcromakalim antagonist potency

Levcromakalim (0.01–30 μM) concentration-effect curves (0.5 log increments) were constructed in tissues precontracted with a submaximal concentration of acetylcholine (0.5 μM – which caused approximately 60–80% of the maximum tissue response to acetylcholine). The sulphonylurea derivative or vehicle was added 20 min prior to constricting the tissue with acetylcholine. Once a plateau response to acetylcholine had been reached, cumulative concentration-effect curves to levcromakalim were then constructed. Only one concentration-effect curve to levcromakalim was constructed on any ring segment. Preliminary experiments (see also Challinor-Rogers and McPherson, 1993) showed that a 20 min contact period for the sulphonylureas was sufficient to cause equilibrium blockade of levcromakalim-mediated responses. Sodium nitroprusside (100 μM) was added at the end of each curve to obtain maximal vessel relaxation. In these studies all compounds were tested at a final concentration of 30 μM with the exception of glibenclamide (studied at 1 μM) and I-glibenclamide (studied at 3 μM).

2.4. Data analysis

The concentration of U46619 required to cause 50% of the maximum vasoconstrictor responses, or the concentration of levcromakalim required to produce 50% relaxation of acetylcholine-precontracted tissue was calculated graphically and used for analysis.

Antagonist potency of the test compound was calculated using Eq. (1) which gives an estimate of antagonist potency based on a single concentration of antagonist (see Kenakin, 1987). Previous studies have shown that sulphonylurea compounds are classical competitive antagonists of levcromakalim (Challinor-Rogers and McPherson, 1993) and U46619 (Cocks et al., 1990) and consequently their calculated potency value (pK_B) would be independent of the concentration of antagonist used.

$$pK_B = -\log([\text{antagonist concentration, M}] / [\text{concentration ratio} - 1]) \quad (1)$$

For these calculations, a concentration ratio (EC_{50} for agonist in the presence of antagonist/EC_{50} in the absence of antagonist) of less than two (for any given concentration of antagonist) was set as the minimum requirement for activity. Thus, inactive compounds had a pK_B of < 4.52 when the antagonist concentration of 30 μM was used.

The line of best fit (line of symmetry) describing the relationship between pK_B U46619 and pK_B levcromakalim values of the sulphonylurea analogues was calcu-

lated using linear regression. Differences between two independent groups were assessed using a *t*-test (Sigma-Stat, Jandel). Results in the text are the mean ± S.E.M. for the specified number of experiments.

2.5. Drugs

Drugs used and their sources were: levcromakalim (Beecham, UK); glibenclamide, tetraphenylphosphonium chloride (Sigma, St. Louis, MO, USA); U46619 (Upjohn, Kalamazoo, MI, USA); sodium nitroprusside (David Bull, Melbourne, Australia). A number of novel analogues structurally related to glibenclamide were synthesised (as bases) at the Victorian College of Pharmacy (D. Kong, M. Pharm Thesis VCP, details of chemical synthesis available upon request). There were four separate groups of compound made and their structures and laboratory code numbers are listed in Figs. 1–3. Fig. 1 lists the amidoethylbenzenesulphonylurea, Fig. 2 the sulphonamide and Fig. 3 the benzamide and benzenesulphonylurea analogue(s).

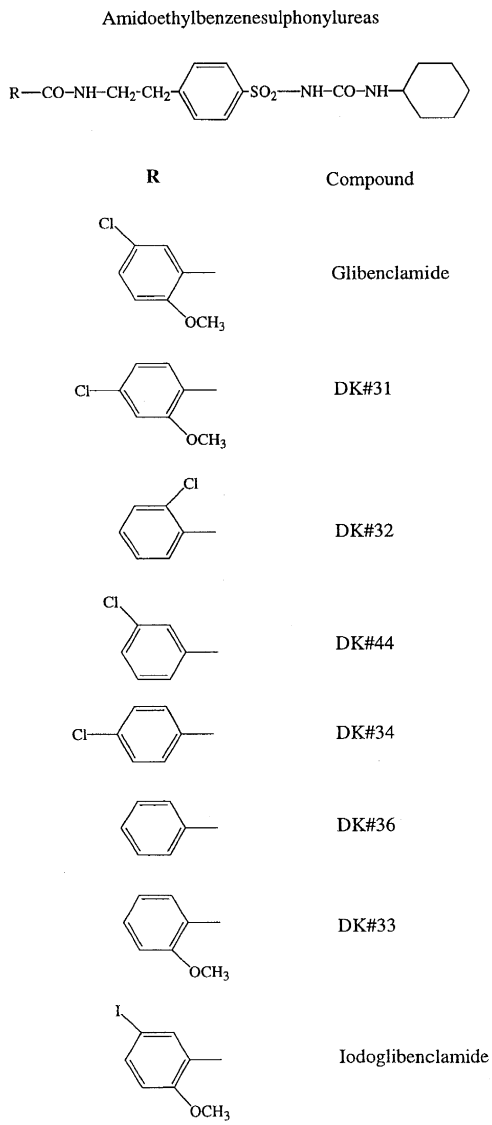

Fig. 1. The structures of the amidoethylbenzenesulphonylurea analogues investigated in the present study.

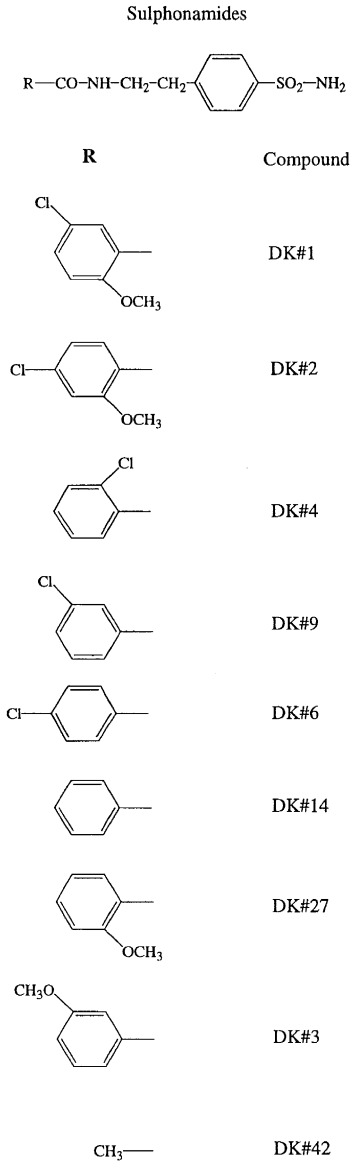

Fig. 2. The structures of the sulphonamide analogues investigated in the present study.

Levcromakalim was made up in 100% methanol as a stock solution of 10 mM. All dilutions were made in distilled water. Sodium nitroprusside was made up as a stock solution of 100 mM in distilled water, and all subsequent dilutions were made in distilled water. Tetraphenylphosphonium chloride (10 mM) was made in distilled water. Glibenclamide and related compounds were solubilized in either 100% methanol or 100% ethanol as stock solutions of 1–10 mM depending on the solubility of

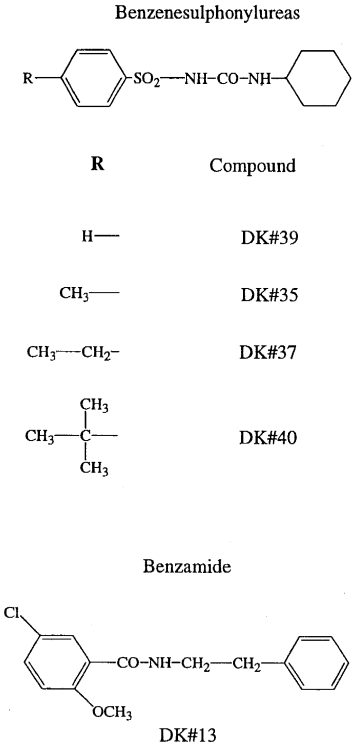

Fig. 3. The structure of the benzenesulphonylurea and benzamide analogue(s) investigated in the present study.

the compound. Sonication and warming were used if needed to aid dissolution.

3. Results

3.1. Antagonism of U46619 responses by glibenclamide analogues

U46619 caused concentration-dependent constriction of the pig coronary artery with a pD_2 value of 8.35 ± 0.07 ($n = 19$ rings taken from 19 separate pigs). At the highest concentration used (3 μM) U46619 caused a contractile response that was $90 \pm 3\%$ of the maximum constrictor response to KPSS.

Glibenclamide and related sulphonylureas (Figs. 1–3) were tested for their ability to antagonise U46619 vasoconstrictor responses in the pig coronary artery. The results from these studies are summarised in Table 1. All active compounds shifted the concentration–response curves to U46619 to the right in a parallel manner, with little change in maximum response (Fig. 4). Using the calculated pK_B values, the order of potency of the amidoethylbenzenesulphonylureas was determined to be I-glibenclamide (6.51), glibenclamide (6.34), DK#31 (6.21), DK#44 (5.89), DK#34 (5.87), DK#33 (5.62) and DK#36 (5.44). DK#32 was inactive at 30 μM. The benzenesulphonylureas (which lack the benzamido nucleus contained in the remaining sulphonylurea derivatives) also displayed activity: DK#40 (5.44), DK#35 (5.16) and DK#37 (4.88). DK#39 was inactive at 30 μM. Only two of the nine sulphonamides tested (DK#9 pK_B 5.17 and DK#42 pK_B 4.79) were active. It was found that none of the compounds significantly affected the maximum vasoconstrictor response to U46619 in the pig coronary artery (data not shown).

3.2. Antagonism of levcromakalim responses by glibenclamide analogues

Levcromakalim caused concentration-dependent relaxation responses of acetylcholine (0.5 μM) precontracted pig coronary artery with a pD_2 value of 6.47 ± 0.08 ($n = 15$ from rings taken from 15 separate pigs). At the highest concentration of levcromakalim used (30 μM) the relaxation was maximal and the remaining tone constituted less than 10% of the active tone induced by the acetylcholine.

Glibenclamide and related amidoethylbenzenesulphonylureas (Fig. 1) were tested for their ability to antagonise levcromakalim vasorelaxation responses. The results from these studies are summarised in Table 1. All active compounds shifted the concentration–response curves to lev-

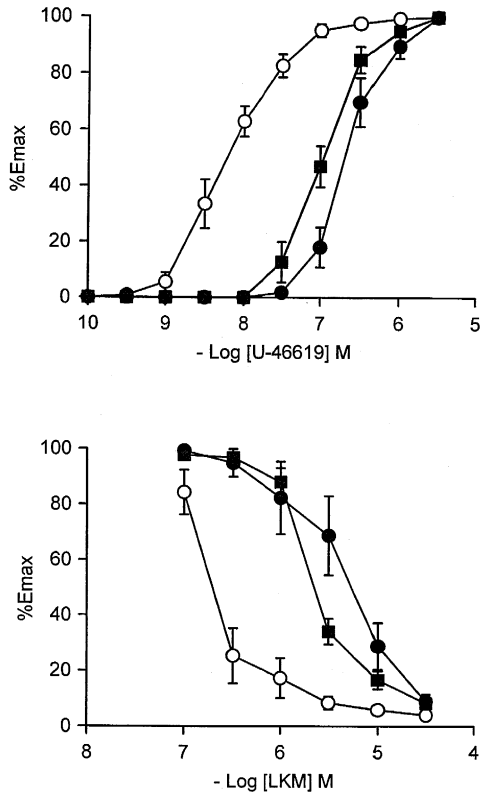

Fig. 4. Mean concentration-effect curves constructed to U46619 (top panel) and levcromakalim (bottom panel) in the pig coronary artery. Curves were obtained in the absence (control, ○) and presence of glibenclamide (■) and DK#44 (●). Top panel: Compounds tested at 30 μM. Bottom panel: Glibenclamide tested at 1 μM, DK#44 tested at 30 μM. Results are expressed as:

$$\frac{\text{U46619 – response to maximum concentration of U46619 used (= 100\%)}}{\text{levcromakalim – tone induced by acetylcholine (0.5 μM) (= 100\%)}}$$

cromakalim to the right in a parallel manner, with little change in maximum response (Fig. 4). Using the calculated pK_B values, the order of potency of the amidoethylbenzenesulphonylureas was determined to be glibenclamide (7.03), I-glibenclamide (6.82), DK#31 (6.39), DK#33 (6.18), DK#44 (5.95), DK#34 (5.50), DK#36 (5.17). DK#32 was inactive at the highest concentration tested.

3.3. Antagonism of U46619 responses by tetraphenylphosphonium.

In a separate series of experiments the ability of tetraphenylphosphonium to antagonise U46619-mediated vasoconstrictor responses was assessed. At a concentration of 10 μM, tetraphenylphosphonium failed to shift the U46619 concentration-effect curve (pD_2 control = 8.32 ± 0.1, pD_2 in the presence of tetraphenylphosphonium (10 μM) = 8.07 ± 0.16; $n = 4$). In addition tetraphenylphosphonium (10 μM) failed to affect the ability of glibenclamide (10 μM) to antagonise U46619-mediated contractile responses (pD_2 U46619 control = 8.32 ± 0.1, pD_2 in

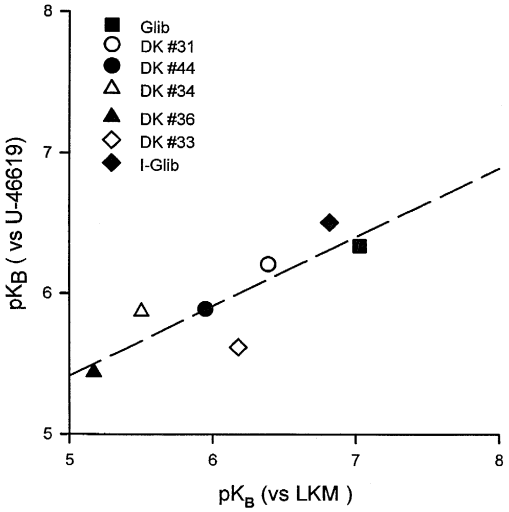

Fig. 5. Plot of the potency (pK_B) of the compounds as antagonists of U46619 vasoconstrictor responses vs. potency as antagonists of levcromakalim vasorelaxation responses in the pig coronary artery. The line of best fit was calculated via linear regression (see Section 2). There was a significant correlation ($P < 0.02$) between these two actions of the sulphonylureas.

Table 1
Table summarising the actions of glibenclamide and a series of analogues as antagonists of the thromboxane A_2 agonist, U46619 (left columns), and as antagonists of the K_{ATP} channel opener levcromakalim (right columns), in pig coronary artery

Compound	U46619		Levcromakalim		Selectivity index
	pK_B	Relative potency	pK_B	Relative potency	
Amidoethylbenzene-sulphonylureas					
Glibenclamide	6.34 ± 0.13	1	7.03 ± 0.07 [a]	1	4.9
DK#31	6.21 ± 0.12	1.3	6.39 ± 0.08	4.3	1.5
DK#32	< 4.52		< 4.52		
DK#44	5.89 ± 0.11	2.8	5.95 ± 0.04	12	1.1
DK#34	5.87 ± 0.09	2.9	5.50 ± 0.05 [a]	34	0.43
DK#36	5.44 ± 0.12	7.9	5.17 ± 0.11	72	0.54
DK#33	5.62 ± 0.24	5.2	6.18 ± 0.08 [a]	7	3.6
I-Glibenclamide	6.51 ± 0.08	0.7	6.82 ± 0.08	1.6	2
Sulphonamides					
DK#1	< 4.52		–		
DK#2	< 4.52		–		
DK#4	< 4.52		–		
DK#9	5.17 ± 0.05	15	–		
DK#6	< 4.52		–		
DK#14	< 4.52		–		
DK#27	< 4.52		–		
DK#3	< 4.52		–		
DK#42	4.79 ± 0.25	35	–		
Benzene-sulphonylureas					
DK#39	< 4.52		–		
DK#35	5.16 ± 0.09	15	–		
DK#37	4.88 ± 0.17	29	–		
DK#40	5.44 ± 0.10	8	–		
Benzamides					
DK#13	5.27 ± 0.10	12	–		

Values are the mean ± S.E.M. for 3–6 separate determinations. Relative potency: antilog(pK_B glibenclamide − pK_B analogue). Values give the number of fold less potent the analogue is than glibenclamide. Selectivity index: antilog[analogue pK_B levcromakalim − analogue pK_B U46619].
[a] Significantly different from pK_B U46619 ($P < 0.05$, *t*-test). (–) not calculated.

the presence of glibenclamide (10 μM) = 7.28 ± 0.16, pD_2 in the presence of glibenclamide (10 μM) and tetraphenylphosphonium (10 μM) = 7.47 ± 0.3; $n = 4$).

3.4. Relationship between U46619 and levcromakalim antagonism by glibenclamide analogues

There was a significant relationship ($P = 0.02$, pK_B U46619 = pK_B levcromakalim × 0.49 + 2.95, $r^2 = 0.73$) between the ability of the amidoethylbenzenesulphonylureas to antagonise U46619- and levcromakalim-mediated actions in the pig coronary artery (Fig. 5). Thus structural modifications that led to losses in potency of the compounds as levcromakalim antagonists also led to a loss of potency as U46619 antagonists although this was not a one-to-one relationship. This was most obvious in the case of compound DK#34 which was only 8 times less potent than glibenclamide as a U46619 antagonist but 70 times less potent than glibenclamide as a levcromakalim antagonist (Table 1). Glibenclamide and DK#33 were significantly more potent as levcromakalim antagonists than as U46619 antagonists. Conversely DK#34 was approximately twice as potent as a U46619 antagonist than as a levcromakalim antagonist (Table 1).

4. Discussion

The main finding from this study is that a number of compounds related to glibenclamide are potent antagonists of the stable thromboxane A_2 agonist, U46619, and that there is a similarity in the structure–activity relationship for this action compared to their ability to antagonise the K_{ATP} channel agonist actions of levcromakalim. For the series of amidoethylbenzenesulphonylureas (exemplified by glibenclamide) tested in this study, there was a significant correlation ($P = 0.02$) between potencies of the compounds for the two systems studied. However, the relationship between the two groups was not unity (slope of regression line was 0.5) suggesting there may be quantitative but not qualitative differences between the activity of the sulphonylurea derivatives in the two systems.

We also showed that the ability of sulphonylureas to antagonise U46619-mediated contractile responses was not shared by other potent antagonists of levcromakalim-mediated vasorelaxant (K_{ATP} opening) responses. Thus tetraphenylphosphonium (10 μM), a compound with approximately the same potency of glibenclamide as a levcromakalim antagonist ($pK_B = 7.2$; McPherson and Piekarska, 1994) failed to affect the U46619 concentration-effect curve in the pig coronary artery. In addition tetraphenylphosphonium (10 μM) failed to affect the antagonism displayed by glibenclamide (10 μM) when both agents were added to the preparation simultaneously. This finding rules out the possibility that glibenclamide is behaving as a K_{ATP} channel opener (rather than its usual activity of an antagonist) in tissues activated by U46619 since tetraphenylphosphonium should then inhibit the antagonistic action of glibenclamide. Tetraphenylphosphonium is thought to block the K_{ATP} channel pore directly and thus has a different mechanism of action to glibenclamide in antagonising the action of levcromakalim (McPherson and Piekarska, 1994).

Previous studies in our laboratory (Challinor-Rogers and McPherson, 1993) have shown that glibenclamide is a classical competitive antagonist of levcromakalim-mediated vasorelaxant responses in vascular preparations, with a pA_2 value in the rat thoracic aorta of approximately 7 and slope value near unity. A similar pK_B value ($pK_B = 7$) was calculated using the pig coronary artery in this study based on a single concentration of antagonist. Similarly the calculated pK_B for glibenclamide as a U46619 antagonist obtained in this study ($pK_B = 6.3$) is similar to the pA_2 value calculated in dog coronary artery (6.2; Cocks et al., 1990) where glibenclamide is a classical competitive antagonist (slope value of unity). That sulphonylureas display the same type of competitive antagonism in both systems may again suggest a common site of action.

While K_{ATP} channels in a number of tissues show some similarities in their ATP, calcium and voltage dependence (Ashcroft and Ashcroft, 1990), there appear to be two concentration ranges over which sulphonylurea-based compounds are active in regulating their function. In the β-cell of the pancreas, glibenclamide modulation of insulin release occurs at nanomolar concentrations (Ashcroft and Ashcroft, 1990). In the brain [^{3}H]glibenclamide binds with similar potency to its activity in the pancreas (see Challinor-Rogers et al., 1995). However, in vascular smooth muscle and various cardiac preparations (see Challinor-Rogers and McPherson, 1994) micromolar concentrations are required. Despite these quantitative differences we recently showed (Challinor-Rogers et al., 1995), using many of the same compounds used in the present study, that there was a strong correlation between the potency of the glibenclamide analogues at high affinity sulphonylurea receptors in the brain (assessed by calculating their pK_i value against [^{3}H]glibenclamide binding) and the potency of the same compounds as levcromakalim antagonists in vascular smooth muscle from the rat aorta. Regression of the pK_i (Challinor-Rogers et al., 1995) data obtained previously against the pK_B data obtained in the present study for the same compounds in the pig coronary artery as levcromakalim antagonists (Fig. 6, top panel) shows there is a highly significant correlation between the activity of the amidoethylbenzenesulphonylureas tested in each study ($P < 0.0001$, $r^2 = 0.98$). The receptor/process mediating the effects of these compounds against U46619 appears to be similar (Fig. 6, bottom panel), but not identical to, the sulphonylurea receptor characterized in the pig coronary artery and rat brain. Thus there is a significant correlation ($P = 0.0007$, $r^2 = 0.74$) in the activity of these sulphonylurea derivatives as U46619 antagonists and

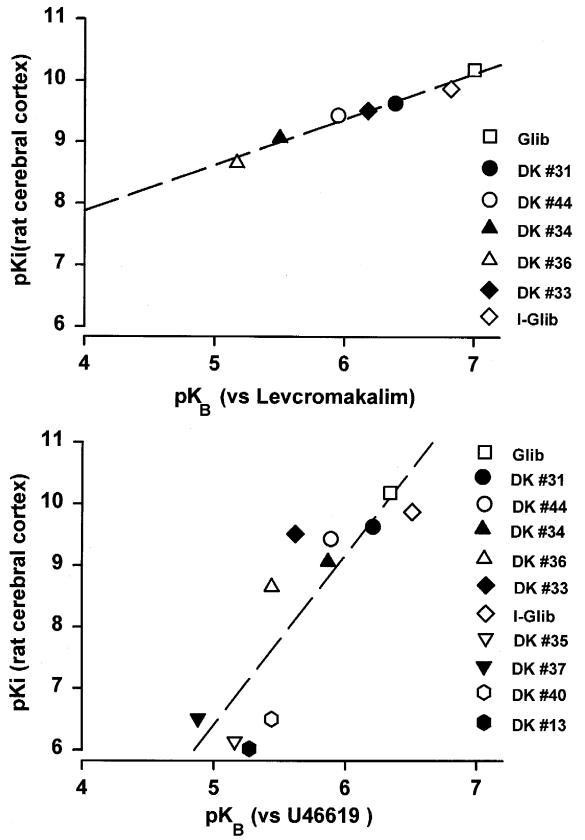

Fig. 6. Top panel: Plot of the potency (pK_B) of the compounds as antagonists of levcromakalim vasorelaxation responses in the pig coronary artery vs. the ability of the compounds to displace [^{3}H]glibenclamide binding rat cerebral cortex (pK_i) calculated in a previous study (Challinor-Rogers et al., 1995). Bottom panel: Plot of the potency (pK_B) of the compounds as antagonists of U46619 vasoconstrictor responses vs. the ability of the compounds to displace [^{3}H]glibenclamide binding rat cerebral cortex (pK_i) calculated in a previous study (Challinor-Rogers et al., 1995).

their ability to displace [^{3}H]glibenclamide binding in the rat brain.

These results suggest that there may be a common link in the receptor excitation–response pathway for both levcromakalim-mediated relaxation responses and U46619 contractile responses that involves a type of sulphonylurea receptor. In the β-cell the sulphonylurea receptor is thought to combine with a member of the inward rectifying K^+ channel to form the K_{ATP} channel (Iwasaki et al., 1996; Aguilar-Bryan et al., 1995). Sulphonylureas such as glibenclamide interact with K_{ATP} channel openers at the level of the K_{ATP} channel to prevent K_{ATP} channel opening. Conversely U46619 activates the thromboxane A_2 receptor, a member of the 7 membrane-spanning G-protein-coupled superfamily. What site, if any, would allow the sulphonylureas to interact with both systems? Recently the sulphonylurea receptor has been cloned (Aguilar-Bryan et al., 1995) and shown to be a multiple membrane-spanning protein of approximately 1600 amino acid sequences of the ATP binding cassette superfamily. Conversely, U46619 activates a receptor of approximately 350 amino acid sequences that belongs to the 7 membrane-spanning G-protein-coupled superfamily (Coleman et al., 1994). We performed homology analysis using BIOScan (University of North Carolina, USA) and found that there were no structural similarities between the sulphonylurea receptor and the thromboxane A_2 receptor based upon amino acid sequence analysis suggesting that the receptor site is unlikely to be the common site of action. A recent work (Delaey and Van de Voorde, 1995) showed that sulphonylureas were antagonists of not only thromboxane-mediated contractile responses in vascular smooth muscle, but also other prostanoids such as prostaglandin $F_{2\alpha}$ and prostaglandin E_2. Their work provided evidence that the site of the prostanoid antagonism may be at the level of the regulatory G-proteins since glibenclamide also antagonised constrictor responses to AlF_4, an agent which is thought to act by activating the G_α component which then acts on phospholipase C to cause phosphoinositide breakdown. Coincidentally, the K_{ATP} channel also has a G-protein regulatory site, the activation of which leads to channel opening (Birnbaumer, 1992). One possibility is that both phospholipase C and the K_{ATP} channel possess a sulphonylurea binding site, the occupation of which blocks the action of the activated G_α component on its regulator site in both systems. Obviously this hypothesis needs to be tested directly.

In conclusion the main finding from this study is that there is a significant correlation between the ability of some sulphonylurea-based compounds to antagonise thromboxane-mediated vasoconstrictor responses in the pig coronary artery and the ability of the same compounds to antagonise K_{ATP} opener-mediated vasorelaxant responses in the same tissue. Other potent K_{ATP} channel antagonists, such as tetraphenylphosphonium, failed to affect U46619-mediated responses indicating that the antagonism is specific to the sulphonylureas. This raises the possibility that there is a common site of action of the compounds in the two systems that can be regulated by sulphonylurea receptor binding. Based on previous work it is possible that this site is the G-protein-mediated regulation of the effector system.

Acknowledgements

We would like to thank Simon Keily for his contribution to this project. G.A.M. is a Senior Research fellow of the NH&MRC. This work was supported by a project grant from the NH&MRC of Australia. D.C.M.K. is grateful to the Australian International Development Assistance Bureau and Monash University for Financial support.

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