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Structure–activity relationship of quaternary ion antagonism of levcromakalim-induced relaxation in pig coronary artery

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Abstract

The aim of this study was to investigate the interaction between the K^+ channel opener levcromakalim and several quaternary ions. Cumulative vasorelaxant-response curves to levcromakalim were constructed in the absence and in the presence of the quaternary ions, in the pig coronary artery. The most potent compounds (based on 'apparent pK_B' values) were: propyltriphenylphosphonium (7.33), butyltriphenylphosphonium (7.04), tetraphenylarsonium (6.86), tetraphenylphosphonium (6.81), ethyltriphenylphosphonium (6.70), and hexyltriphenylphosphonium (6.63). Tetrabutylphosphonium (6.06), tetrabutylammonium (5.12), methyltriphenylphosphonium (5.25), clofilium (5.66) and guanethidine (5.61) were significantly less potent. Tetrapropylammonium, tetrapentyltin and tetraphenylboron were inactive at the maximum concentrations used (30 μM). Tetraphenylboron (10–100 μM) fully reversed tetraphenylphosphonium, tetraphenylarsonium (both at 3 μM), tetrabutylammonium (30 μM) and clofilium (10 μM) and partially reversed guanethidine (10 μM) antagonism of levcromakalim responses indicating a similarity in the mechanism of action of these chemically distinct compounds. The results show that quaternary ions similar in structure to tetraphenylphosphonium, i.e., containing phosphonium ion centre and phenyl side chains, are the most potent antagonists of levcromakalim, in pig coronary artery. It is also apparent that marked changes can be made in the substitution on the phosphonium ion (ethyl to hexyl) with little or no effect on their potency.

Keywords: Levcromakalim; Quaternary ion; K^+ channel antagonist; Circumflex artery, right, pig; Tetraphenylphosphonium; Triphenylphosphonium compound

1. Introduction

Levcromakalim is representative of a class of smooth muscle relaxants (Edwards and Weston, 1993), which are thought to open ATP-sensitive K^+ (K_{ATP}) channels in a range of different tissues (see Challinor-Rogers and McPherson, 1994; Edwards and Weston, 1993). This proposition is based on the findings that sulfonylureas such as glibenclamide, which is a potent and selective K_{ATP} channel blocker in the pancreas, is able to antagonise the actions of the K^+ channel openers in smooth muscle cells (see Challinor-Rogers and McPherson, 1994; Edwards and Weston, 1993). Recently, using rat thoracic aorta, we found that two quaternary compounds, tetraphenylphosphonium and tetraphenylarsonium, were two of the most potent antagonists of levcromakalim-mediated vasorelaxation responses identified so far (McPherson and Piekarska, 1994), having a potency similar to that of glibenclamide (Challinor and McPherson, 1993). In addition, we showed that the blockade of levcromakalim-mediated responses caused by tetraphenylphosphonium can be reversed by the negatively charged quaternary ion, tetraphenylboron (McPherson and Piekarska, 1994).

Some quaternary ions, such as tetraphenylphosphonium, are highly lipophilic which enables them to enter the hydrophobic core of the membrane. As yet, the exact mode of interaction between the lipophilic quaternary ions and K^+ ion channels has not been clarified. Whatever the mechanism of action, the activity of quaternary compounds as levcromakalim antagonists may prove to be useful tools in the study of the K_{ATP} channel. Consequently in this study we wished to characterise further the interaction between quaternary ions and the functional effects mediated by K^+ channel openers. To this end we have examined a range of structurally related quaternary compounds

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and tried to relate their chemical structure to the potency with which they antagonise levcromakalim in vascular smooth muscle. In addition, we tested the ability of two other structurally unrelated quaternary ions, guanethidine and clofilium, to determine whether these agents too were able to antagonise the smooth muscle relaxant action of levcromakalim.

2. Materials and methods

2.1. Isolation and study of porcine coronary artery

The porcine 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, $MgSO_4.7H_2O$ 1.17, KH_2PO_4 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 5.5). The artery was cut into 4 mm long segments and each segment was 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^+ depolarising solution (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 the K^+ depolarising solution was reached, vessels were washed twice and left until the response returned to the initial baseline, before commencing the experiment.

After this period each coronary ring was submaximally constricted with the thromboxane-mimetic U46619 (10–30 nM). Once a plateau response to the constrictor was reached, a cumulative concentration–response curve to levcromakalim; 0.01–30 μM, was then constructed (0.5 log increments). Concentrations of levcromakalim where 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. The antagonist tested was added 20 min prior to submaximally constricting the tissue with U46619. Preliminary experiments indicated that this incubation period was sufficient for equilibrium antagonism to be produced. In some cases levcromakalim did not cause full tissue relaxation, hence sodium nitroprusside (100 μM) was added at the end of each curve to obtain maximal vessel relaxation.

2.2. Analysis of results

The contraction to U46619 (10–30 nM) was taken as 100% response and relaxation produced by different concentrations of levcromakalim expressed as a percentage of this response. The percentages obtained were then represented graphically and the pD_2 ($-\log EC_{50}$) value calculated as the concentration of relaxant required to cause 50% of the maximal relaxant response. To determine whether the antagonists directly affected the tissue's ability to contract, the response to U46619 was expressed as a percentage of the maximal K^+ depolarising solution contraction.

It was observed that at high concentrations, most of the active antagonists shifted the levcromakalim concentration–response curve in a non-parallel fashion such that the slope of the curve was reduced and the maximum response to levcromakalim appeared to be depressed. This suggested that the type of antagonism displayed by the quaternary ions was non-competitive, preventing us from the use of Schild analysis (Jenkinson, 1991) as a means of determining the potency of the active ions. Instead, an 'apparent pK_B' was estimated, based on a single concentration of antagonist.

The following equation was used:

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

The single concentration of the antagonist used to calculate the 'apparent pK_B' was selected on the basis that it produced a 'parallel' shift in the levcromakalim concentration–response curve without overtly affecting the maximal levcromakalim response.

One-Way Repeated Measures Analysis of Variance (ANOVA) was used to test several values which were dependent. Mann-Whitney *t*-test or paired *t*-test was used to compare two values. Either Student-Newman-Keuls' method (for all pairwise multiple comparisons) or Dunnett's method (for comparisons of all groups vs. control) were used as post ANOVA tests. All statistical calculations were performed using SigmaStat (Jandel Scientific, USA). Results in the text are given as means ± S.E.M.

2.3. Drugs

Drugs used and their sources were: levcromakalim (SmithKline Beecham, UK); sodium nitroprusside (David Bull, Australia); U46619 (11α,9α-epoxymethano-prostaglandin H_2; Upjohn, USA); tetraphenylphosphonium chloride, tetrabutylammonium iodide, tetrabutylphosphonium chloride, tetrapropylammonium iodide, tetrapentyltin, methyltriphenylphosphonium bromide, ethyltriphenylphosphonium bromide, butyltriphenylphosphonium bromide, propyltriphenylphosphonium bromide, hexyltriphenylphosphonium bromide (Aldrich, USA); tetraphenylarsonium

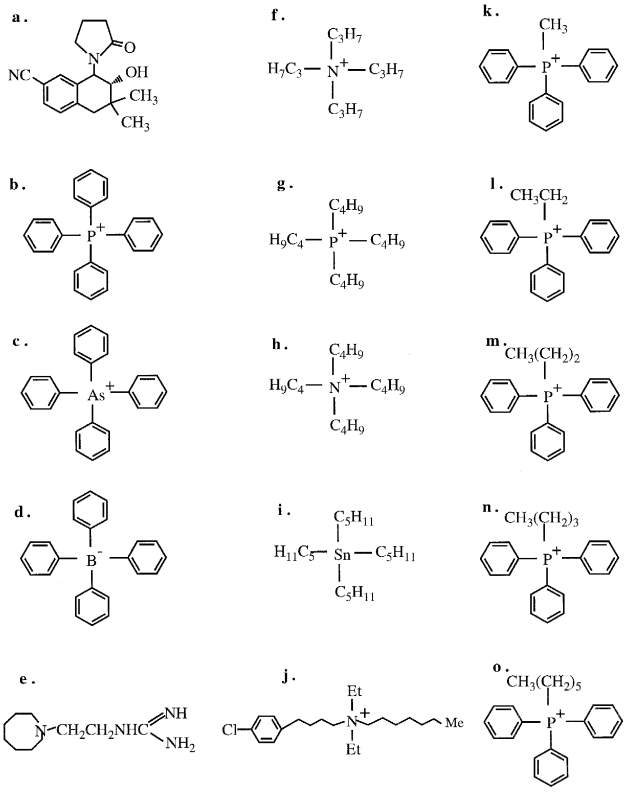

Fig. 1. Chemical structures of the quaternary ions and the K^+ channel opener, levcromakalim. (a) Levcromakalim; (b) tetraphenylphosphonium; (c) tetraphenylarsonium; (d) tetraphenylboron; (e) guanethidine; (f) tetrapropylammonium; (g) tetrabutylammonium; (h) tetrabutylphosphonium; (i) tetrapentyltin; (j) clofilium; (k) methyltriphenylphosphonium; (l) ethyltriphenylphosphonium; (m) propyltriphenylphosphonium; (n) butyltriphenylphosphonium; (o) hexyltriphenylphosphonium.

chloride, tetraphenylboron sodium (Sigma, USA), clofilium tosylate (Research Biochemicals International), guanethidine monosulfate (Ciba-Geigy, Switzerland).

Table 1
Mean 'apparent pK_B' values obtained for the compounds tested for levcromakalim antagonistic activity, in isolated pig right circumflex coronary artery constricted with U46619 (10–30 nM) $n = 3–6$

Compound	Apparent pK_B [a]	Antagonist (μM) [b]
Propyltriphenylphosphonium	7.33 ± 0.06	0.3
Butyltriphenylphosphonium	7.04 ± 0.10	0.3
Ethyltriphenylphosphonium	6.70 ± 0.16	0.3
Hexyltriphenylphosphonium	6.63 ± 0.16	1
Methyltriphenylphosphonium	5.25 ± 0.20	3
Tetraphenylarsonium	6.86 ± 0.07	1
Tetraphenylphosphonium	6.81 ± 0.18	1
Tetrabutylphosphonium	6.06 ± 0.07	3
Tetrabutylammonium	5.12 ± 0.18	30
Clofilium	5.66 ± 0.25	10
Guanethidine	5.61 ± 0.15	10

[a] 'Apparent pK_B' values estimated for each of the active compounds.
[b] Concentration of the quaternary ion used to calculate 'apparent pK_B'.

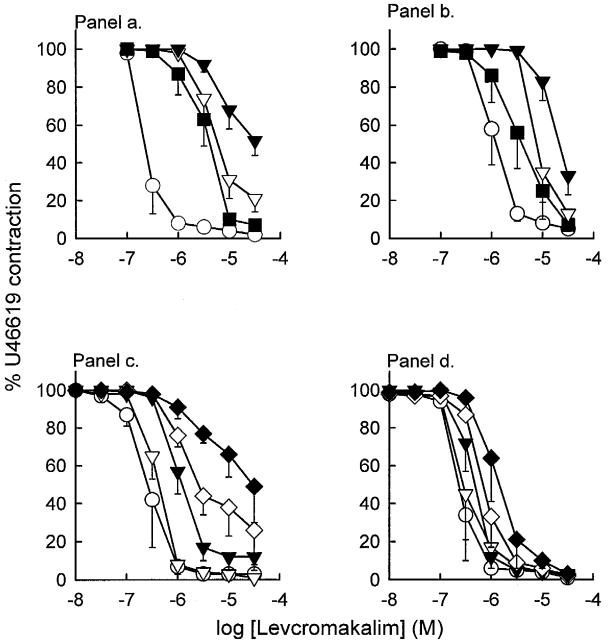

Fig. 2. Mean levcromakalim concentration–response curves obtained in the isolated porcine right circumflex artery in the absence (○) and in the presence of increasing concentrations of the quaternary ions. Panel a, tetraphenylphosphonium chloride; panel b, tetraphenylarsonium; panel c, tetrabutylphosphonium; panel d, tetrabutylammonium. The concentrations used were: 0.3 (■), 1 (▽), 3 (▼), 10 (◇) and 30 (◆) μM. Error bars are ±S.E.M. and if not visible are contained within the symbol ($n = 3–6$).

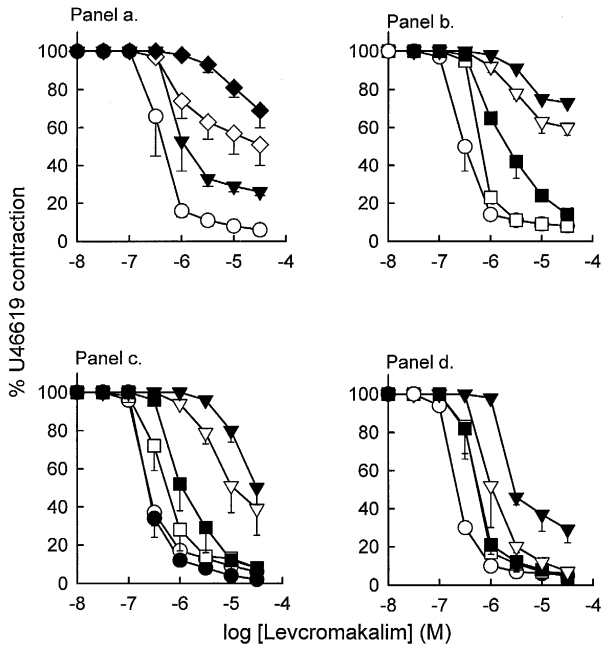

Fig. 3. Mean levcromakalim concentration–response curves obtained in the isolated porcine right circumflex artery in the absence (○) and in the presence of increasing concentrations of the quaternary ions. Panel a, methyltriphenylphosphonium; panel b, propyltriphenylphosphonium; panel c, butyltriphenylphosphonium; panel d, hexyltriphenylphosphonium. The concentrations used were: 0.03 (●), 0.1 (□), 0.3 (■), 1 (▽), 3 (▼), 10 (◇) and 30 (◆) μM. Error bars are ±S.E.M. and if not visible are contained within the symbol ($n = 3–5$).

Levcromakalim was made up in 100% methanol as stock solutions of 10 mM. All dilutions were made in distilled water. The quaternary ions were made up fresh each day in distilled water.

3. Results

3.1. Antagonism of levcromakalim-induced vasorelaxation in vascular smooth muscle

All compounds tested were structurally similar and related to tetraphenylphosphonium with the exception of guanethidine and clofilium. The structures of these agents are shown in Fig. 1. In the first series of experiments, we tested three tetraphenyl compounds containing varying central ions: tetraphenylphosphonium, tetraphenylarsonium and tetraphenylboron. Also, compounds with different number of carbons in the side chains as well as differing central ions, were tested: tetrapentyltin, tetrabutylphosphonium, tetrabutylammonium and tetrapropylammonium. The majority of these compounds antagonised levcromakalim-induced vasorelaxation (Table 1, Fig. 2). On the basis of the apparent pK_B value (Table 1), the two tetraphenyl compounds (tetraphenylphosphonium and tetraphenylarsonium) were found to be significantly more potent, producing a shift in the levcromakalim concentration–response curve at concentrations as low as 0.3 μM (Fig. 2). The tetrabutyl compounds (tetrabutylphosphonium and tetrabutylammonium) shifted the levcromakalim curve at 3 μM and 30 μM, respectively. Tetrapropylammonium (30 μM) and tetrapentyltin (30 μM) and tetraphenylboron (10 μM) were found to be inactive ($n = 2$–4; data not shown).

Out of the quaternary ions tested, the tetraphenyl compounds (tetraphenylarsonium and tetraphenylphosphonium) appeared to have the optimal chemical structure for levcromakalim antagonism (Fig. 4a). In the next series of experiments, a number of tetraphenylphosphonium analogues was investigated to determine whether subtle changes in structure can significantly alter the potency. All analogues tested had a positively charged phosphonium ion at the centre, while one of the four surrounding phenyl rings has been substituted with a variety of different length carbon chains (see Fig. 1k–o) The compounds tested were: methyltriphenylphosphonium, ethyltriphenylphosphonium, propyltriphenylphosphonium, butyltriphenylphosphonium and hexyltriphenylphosphonium. On the basis of the apparent pK_B value (Table 1, Fig. 3), the analogues with two, three, four and six carbon groups (see Fig. 4) were found

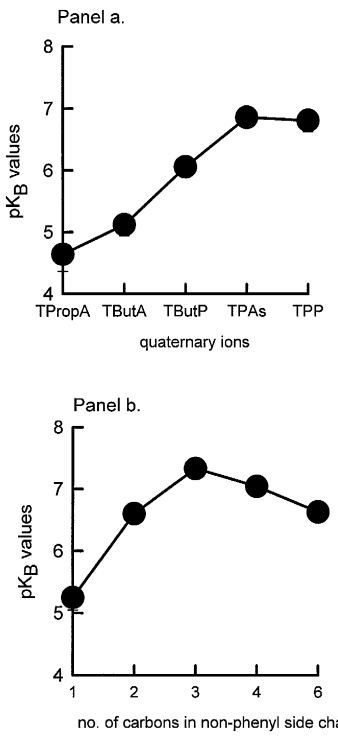

Fig. 4. The structure–activity relationship. Mean of 'apparent pK_B' values for compounds tested for levcromakalim antagonistic activity vs. (panel a) examples of quaternary ions containing different length side chains and varying central ions: tetrapropylammonium (TPropA), tetrabutylammonium (TButA), tetrabutylphosphonium (TButP), tetraphenylarsonium (TPAs), tetraphenylphosphonium (TPP); (panel b) increasing number of carbons in the non-phenyl residue of the tetraphenylphosphonium analogues, in isolated pig right circumflex coronary artery. Error bars are ±S.E.M. and if not visible are contained within the symbol ($n = 3$–5).

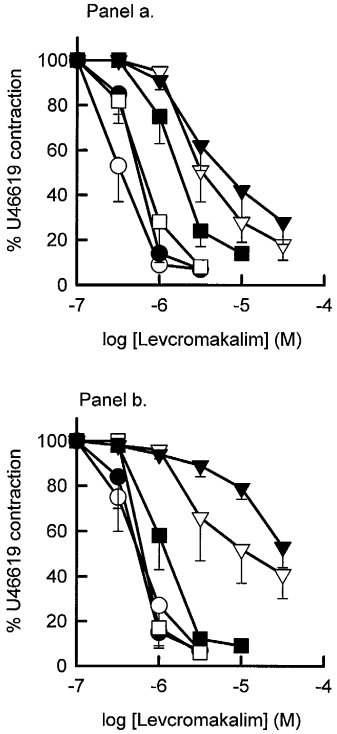

Fig. 5. Mean levcromakalim concentration–response curves obtained in the isolated porcine right circumflex artery in the absence (○) and in the presence of increasing concentrations of (panel a) guanethidine and (panel b) clofilium. The concentrations used were: 1 (●), 3 (□), 10 (■), 30 (▽) and 100 (▼) μM. Error bars are ±S.E.M. and if not visible are contained within the symbol ($n = 5$–7).

to be as potent as tetraphenylphosphonium. Methyltriphenylphosphonium was significantly less potent (Fig. 4b) ($P < 0.05$; ANOVA with Student-Newman-Keuls' test). Hexyltriphenylphosphonium also tended to display lower potency (Fig. 4b), although the difference was not statistically significant.

We also tested two structurally different quaternary compounds: clofilium and guanethidine. They were both found to produce a shift in the levcromakalim concentration–response curve at 10 μM (Fig. 5). They were found to be equi-potent with pK_B values of approximately 5.6 (Table 1).

3.2. Effect of the negatively charged tetraphenylboron on levcromakalim antagonism induced by selected quaternary ions

In the pig right circumflex coronary artery, using U46619 (10–30 nM) to constrict the tissue, we found (Fig. 6) that tetraphenylboron (10 μM) fully reversed the antagonism of levcromakalim vasorelaxant responses caused by the tetraphenylphosphonium and tetraphenylarsonium (3 μM): tetraphenylphosphonium (3 μM) (control levcromakalim $pD_2 = 6.62 \pm 0.09$; plus antagonist (3 μM) $pD_2 = 5.03 \pm 0.14$; antagonist (3 μM) plus tetraphenylboron (10 μM) $pD_2 = 6.35 \pm 0.09$; $n = 6$; not significantly different from control pD_2 $P > 0.05$; ANOVA with Dunnett's test) and tetraphenylarsonium (3 μM) (control levcromakalim $pD_2 = 5.97 \pm 0.11$; plus antagonist (3 μM) $pD_2 = 4.68 \pm 0.11$; antagonist (3 μM) plus tetraphenylboron (10 μM) $pD_2 = 5.82 \pm 0.19$; $n = 6$; not significantly different from control pD_2 $P > 0.05$; ANOVA with Dunnett's test). Similarly tetraphenylboron (100 μM) produced full reversal of tetrabutylammonium (30 μM) induced antagonism (control levcromakalim $pD_2 = 6.83 \pm 0.10$; plus antagonist (30 μM) $pD_2 = 6.38 \pm 0.12$; antagonist (30 μM) plus tetraphenylboron (100 μM) $pD_2 = 7.00 \pm 0.05$; $n =$ 3–4; not significantly different from control pD_2 $P > 0.05$; ANOVA with Dunnett's test) (Fig. 6d).

When the concentration of tetraphenylphosphonium was increased to 10 μM the ability of tetraphenylboron to reverse the antagonism was diminished (Fig. 6b) such that tetraphenylboron (10 μM) only caused partial reversal of tetraphenylphosphonium (10 μM)-induced antagonism (control levcromakalim $pD_2 = 6.52 \pm 0.20$, plus antagonist (10 μM) $pD_2 = 4.25 \pm 0.15$, antagonist (10 μM) plus tetraphenylboron (10 μM) $pD_2 = 5.67 \pm 0.03$; $n = 5–6$; significantly different from control pD_2 $P < 0.05$; ANOVA with Dunnett's test).

Tetraphenylboron (30 μM) completely reversed clofil-

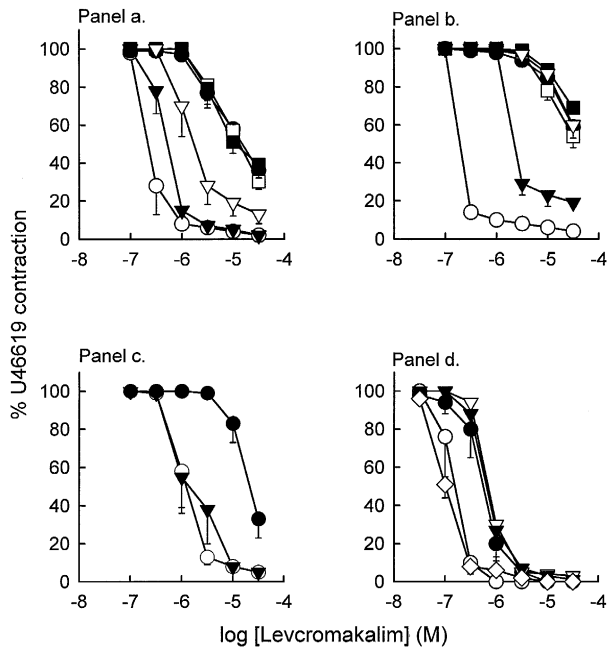

Fig. 6. Interaction between quaternary ions and tetraphenylboron. Mean levcromakalim concentration–response curves obtained in the absence (○) and in the presence of quaternary ions alone: (panel a) tetraphenylphosphonium (3 μM) (●), (panel b) tetraphenylphosphonium (10 μM) (●), (panel c) tetraphenylarsonium (3 μM) (●), (panel d) tetrabutylammonium (30 μM) (●); and in combination with increasing concentrations of tetraphenylboron 0.3 (□), 1 (■), 3 (▽), 10 (▼) or 100 (◇) μM, in the isolated porcine right circumflex artery. Error bars are ±S.E.M. and if not visible are contained within the symbol.

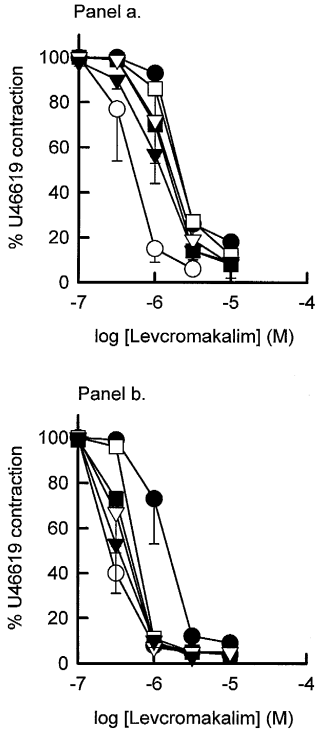

Fig. 7. Interaction between quaternary ions and tetraphenylboron. Mean levcromakalim concentration–response curves obtained in the absence (○) and in the presence of quaternary ions alone and in combination with tetraphenylboron, in the isolated porcine right circumflex artery. Panel a, guanethidine (10 μM); panel b, clofilium (10 μM) alone (●) and combined with 1 (□), 3 (■), 10 (▽) and 30 (▼) μM tetraphenylboron. Error bars are ±S.E.M. and if not visible are contained within the symbol ($n = 4$).

ium (10 μM) induced antagonism (Fig. 7b) (control levcromakalim $pD_2 = 6.57 \pm 0.05$; plus clofilium (10 μM) $pD_2 = 5.86 \pm 0.12$; clofilium (10 μM) plus tetraphenylboron (30 μM) $pD_2 = 6.48 \pm 0.14$; $n = 4$; not significantly different from control pD_2 $P > 0.05$; ANOVA with Dunnett's test). However tetraphenylboron (30 μM) only partially reversed guanethidine (10 μM) antagonism (control levcromakalim $pD_2 = 6.35 \pm 0.14$; plus guanethidine (10 μM) $pD_2 = 5.62 \pm 0.12$; guanethidine (10 μM) plus tetraphenylboron (30 μM) $pD_2 = 5.96 \pm 0.11$; $n = 4$; significantly different from control pD_2 $P < 0.05$; ANOVA with Dunnett's test) (Fig. 7a).

In another set of experiments, tetraphenylphosphonium (3 μM) was combined with increasing concentrations of the neutral tetrapentyltin (up to 100 μM). However, no effect on the antagonism of tetraphenylphosphonium was observed with tetrapentyltin (data not shown).

4. Discussion

4.1. Structure–activity relationship of quaternary ions

This study shows that an extensive range of quaternary ions such as propyltriphenylphosphonium and tetraphenylphosphonium are potent antagonists of the relaxation responses mediated by levcromakalim in pig coronary artery. These studies also extend previous work (McPherson and Piekarska, 1994) which examined effects of some quaternary ions in rat thoracic aorta. There is a strong correlation between the structure of the quaternary ions and their potency as levcromakalim antagonists. The overall shape, length of the side chains and the charge carried by the ion all play an important role in determining the magnitude of the levcromakalim concentration–response curve rightward shift. Using the 'apparent' pK_B values, we found that propyltriphenylphosphonium was the most potent quaternary antagonists of levcromakalim action identified so far.

The size of the quaternary ion plays an important role in determining its potency. This idea was supported by the finding that not all tetraphenylphosphonium analogues, which had one phenyl residue substituted with a carbon side chain of varying length (increasing from one to six carbons) were as potent as tetraphenylphosphonium. The less bulky methyltriphenylphosphonium was found to be significantly lower in potency, suggesting that perhaps the short methyl chain reduces the potency of the compound. Hexyltriphenylphosphonium, which carries the six carbons long side chain, also appeared to be less potent but in general the marked structural changes in the molecule resulting from the ethyl to the hexyl substitution had little effect on the potency of the compounds. Further support for the idea that size plays an important role in determining potency is provided by the finding that molecules which carry less compact four carbon butyl side chains (tetrabutylphosphonium and tetrabutylammonium), or tetrapropylammonium, which has only three carbon propyl groups, displayed lower potency (tetrabutylphosphonium and tetrabutylammonium) or are inactive (tetrapropylammonium). It appears that the phenyl side chains are important in determining potency, since most triphenyl (with the exception of methyltriphenylphosphonium) and tetraphenyl (except tetraphenylboron) were found to be highly potent antagonists of levcromakalim. One explanation for this phenomenon could be related to the lipophilic nature of these compounds. It is known that ions such as tetraphenylphosphonium can readily delocalise their positive charge onto the phenyl groups, making the whole quaternary molecule lipophilic, which in turn may allow the ion to effectively interact with the K^+ channel (see McPherson and Piekarska, 1994).

Another finding was that tetrapentyltin had no effect on the levcromakalim-mediated vasorelaxation. Tetrapentyltin does not possess a charge and this is likely to be an important factor in determining its potency as a levcromakalim antagonist. The anionic agent, tetraphenylboron (which has the opposite charge than that of tetraphenylphosphonium) was also found to be inactive, however it did reverse the levcromakalim antagonist action of other quaternary compounds (see below). Tetrapentyltin did not display the similar ability to reverse the antagonism of compounds such as tetraphenylphosphonium. Thus charge appears to be the main determinant of potency, especially since other tetrapentyl compounds (e.g. tetrapentylammonium) have previously been shown to antagonise levcromakalim-mediated vasorelaxation (McPherson and Piekarska, 1994).

A number of structurally unrelated compounds are able to antagonise the vasorelaxant responses to levcromakalim (see Challinor-Rogers and McPherson, 1994). One such group is the imidazolidines represented by alinidine (Challinor and McPherson, 1993). Alinidine, although not a true quaternary ion, is highly positively charged (McPherson and Piekarska, 1994). On the basis of the results obtained with the quaternary ions we suspect that all quaternary, or quaternary like ions such as alinidine and guanethidine, will display some activity as levcromakalim antagonist. This was supported by the results from the present study. Thus, the quaternary anti-arrhythmic, clofilium, which has previously been shown to directly inhibit K_{ATP} channels (Sakuta et al., 1993), in our studies antagonised the vasorelaxant responses to levcromakalim in the pig coronary artery. Similarly, guanethidine, a positively charged quaternary like compound, which has previously been shown to antagonise the bronchial smooth muscle relaxant actions of levcromakalim (Berry et al., 1992), was also active in our studies using pig coronary artery. Obviously this idea has to be tested further before any firm generalisations can be made on the importance of positive charge on levcromakalim antagonistic actions.

4.2. Effect of tetraphenylboron on levcromakalim antagonism induced by quaternary ions

We found that tetraphenylboron, although inactive as a levcromakalim antagonist, reversed the antagonism induced by tetraphenylphosphonium and tetraphenylarsonium, as well as tetrabutylammonium. It was shown that the ability of tetraphenylboron to reverse the quaternary ion-induced levcromakalim antagonism is directly related to the concentration ratio of tetraphenylboron and the cation used. For example tetraphenylboron (10 μM) produced full reversal of both tetraphenylphosphonium and tetraphenylarsonium ions (both at 3 μM) (Fig. 6a,c), but tetraphenylphosphonium antagonism was only partially reversed when its concentration was increased to 10 μM (Fig. 6b). Similarly the antagonism induced by tetrabutylammonium (30 μM) was not affected by low tetraphenylboron concentrations (up to 10 μM) but full reversal of action was produced when tetraphenylboron dose was increased to 100 μM (Fig. 6d). This indicates that in order to produce full reversal of levcromakalim antagonism, tetraphenylboron should be added in the ratio of approximately 3:1 (tetraphenylboron/cation), but not 1:1 or lower.

The action of clofilium (10 μM) was also fully reversed by tetraphenylboron (30 μM). In this respect and in general, clofilium has the characteristics identical to that displayed by tetraphenylphosphonium, tetraphenylarsonium and tetrabutylammonium. However tetraphenylboron could only partially reverse the antagonistic actions of guanethidine (10 μM). In this respect gaunethidine is similar to the imidazolidine alinidine, the action of which was also only partially reversed by tetraphenylboron (McPherson and Piekarska, 1994). It would appear that while structurally unrelated true quaternary compounds (tetraphenylphosphonium, tetraphenylarsonium and clofilium) have similar characteristics this is not the case for quaternary like compounds (guanethidine and alinidine), the actions of which are resistant to the effects of tetraphenylboron. Although the mechanism of action of tetraphenylboron is still unknown, it is possible the compounds are displaying chemical antagonism, a possibility we have previously suggested (McPherson and Piekarska, 1994). Chemical antagonism occurs when two compounds react chemically to form a product that can not interact with the receptor (Kenakin, 1987). Therefore it is possible that the negatively charged tetraphenylboron forms a non-active complex with cations such as tetraphenylphosphonium, tetraphenylarsonium, tetrabutylammonium or clofilium. The formation of a tetraphenylboron-cation complex would reduce the amount of free antagonist concentration present in the bath and subsequently would diminish the effect of the antagonist. Another possibility is that tetraphenylboron interacts with positively charged quaternary ions within the lipid bilayer itself. Although tetraphenylboron cannot occupy the negatively charged cavity of the ion channel itself, it is known that it has the ability to enhance the equilibration of cations between the inside and outside of the cell (Saito et al., 1992). It is possible that the presence within the lipid bilayer of tetraphenylboron may disrupt agents, such as tetraphenylphosphonium, binding to sites within the K_{ATP} channel itself (see McPherson and Piekarska, 1994). In the case of guanethidine (and presumably alinidine) the incomplete reversal in the presence of tetraphenylboron might suggest that a lower affinity of the tetraphenylboron-cation complex or that both compounds have another site/mechanism of action not shared by the true quaternary compounds.

In conclusion, we examined the structure–activity relationship of triphenyl, tetraphenyl and other quaternary ions by investigating their ability to antagonise the levcromakalim-mediated vasorelaxation responses in pig coronary artery. We found the triphenylphosphonium compounds to be potent antagonists of levcromakalim action, their potency being relative to the number of carbons on the non-phenyl side chain. Propyltriphenylphosphonium was identified as the most potent quaternary ion examined so far (apparent $pK_B = 7.33 \pm 0.06$). Methyltriphenylphosphonium was found to be significantly lower in potency (apparent $pK_B = 5.25 \pm 0.20$). It was confirmed that the anionic tetraphenylboron has the ability to reverse the antagonism displayed by tetraphenylphosphonium. The new finding was that tetraphenylboron can also fully reverse antagonism induced by tetrabutylammonium and partially reverse antagonism of guanethidine. We also showed that clofilium, a chemically unrelated quaternary compound, is able to antagonise the vascular responses to levcromakalim and that these responses are also sensitive to tetraphenylboron. The structure–activity relationship displayed by the quaternary ions is likely to be directly related to their interaction with the active site of the K^+ channel. Therefore these compounds are valuable tools in the study of K_{ATP} channels and their role in the regulation of physiological systems.

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