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The effects of cetiedil and its congeners on levcromakalim-stimulated ^{86}Rb efflux from smooth and skeletal muscle

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Abstract

The effects of cetiedil on levcromakalim-stimulated ^{86}Rb efflux from rat aorta, rat anococcygeus and frog sartorius muscle have been investigated. In experiments on rat aorta, cetiedil inhibited the tracer efflux stimulated by 10 μM levcromakalim with an IC_{50} of 1.3 ± 0.4 μM. In the rat anococcygeus and frog sartorius 10 μM cetiedil caused 67 ± 11% and 84 ± 4% inhibition of the responses to 10 μM and 50 μM levcromakalim respectively. The effect of two analogues of cetiedil, UCL 1285 (cetiedil methiodide) and UCL 1495 (triphenyl acetic acid-2-*N*[5-ethyl-2-methylpiperidinoethyl] ester) were also tested on rat aorta. UCL 1285 caused inhibition of the response to 10 μM levcromakalim with an IC_{50} of approximately 6 μM. In contrast, UCL 1495 (10 μM) had no significant effect. It is concluded that cetiedil is an effective blocker of the action of levcromakalim in smooth and skeletal muscle but does not distinguish between tissues. The relative activities of cetiedil, UCL 1285 and UCL 1495 are discussed in relation to their activity at other cetiedil-sensitive K^+ channels.

Keywords: Levcromakalim; Cetiedil; Smooth muscle; Skeletal muscle

1. Introduction

Since the characterisation of cromakalim (BRL 34915) and levcromakalim ((−)-cromakalim, BRL 38227) as K^+ channel openers a number of compounds have been found to block their effects (see Edwards and Weston (1993) for a review). These include the sulphonylureas (Quast and Cook, 1988), phentolamine (McPherson and Angus, 1989), guanethidine (Berry et al., 1992a,b), ciclazindol (Noack et al., 1992) and tedisamil (Bray and Quast, 1991). Most of these agents are known to affect other K^+ channels and the majority of the studies have focused on a single tissue, vascular smooth muscle. There is still a need for selective compounds to facilitate the characterisation of K^+ channel opener-activated channels.

Cetiedil (Fig. 1a) was first introduced as a vasodilator agent (Boissier et al., 1978) and has also been shown to have potential as an anti-sickling agent (Cabannes and Maron, 1977; Benjamin et al., 1986). The latter action has been suggested (Berkowitz and Orringer, 1982) to result from the ability of cetiedil to block Ca^{2+}-activated K^+ channels (K_{Ca}) in the erythrocyte membrane. Cetiedil has also been shown to block a number of other K^+ channels including the cell-volume sensitive channels of lymphocytes (Sarkadi et al., 1985) and hepatocytes (Sandford et al., 1992) and the G-protein-coupled K^+ channels found in atrial myocytes and submucous plexus neurones (Jones et al., 1994). In studies in this laboratory of the structure-activity relationship of cetiedil analogues as blockers of erythrocyte K_{Ca} it has been found that the activity increased with lipophilicity (Benton et al., 1994). Thus the highly lipophilic compound UCL 1495 (Fig. 1b) was found to be 20-times more active than cetiedil whereas the quaternary, and therefore less lipophilic, derivative of cetiedil, UCL 1285 (Fig. 1c) was considerably less effective. The aims of the present study were, first, to find whether cetiedil would block the levcromakalim-induced increase in K^+ permeability in smooth and skeletal muscle and, second, to compare its action with that of the related compounds UCL 1285 and UCL 1495, in order to establish whether lipophilicity is as important in determining the blockade of the effect of levcromakalim as it had been shown to be in investigations of agents that block the Ca^{2+}-activated K^+ permeability in erythrocytes. It was

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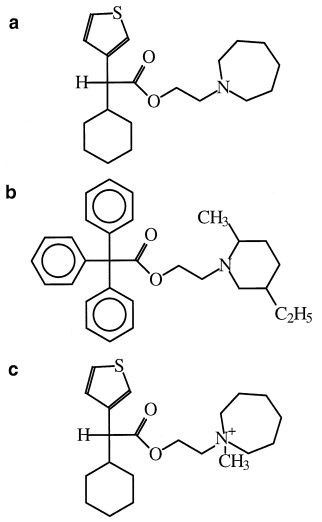

Fig. 1. Structures of (a) cetiedil, (b) UCL 1495 (triphenyl acetic acid-2-*N*[5-ethyl-2-methylpiperidinoethyl] ester) and (c) UCL 1285 (cetiedil methiodide).

also hoped that the work would be of value in characterising the types of K^+ channels involved.

2. Methods and materials

2.1. Smooth muscle

Adult male Sprague-Dawley rats (200–300 g) were killed by cervical dislocation and exsanguination. In all experiments on smooth muscle the bathing solution was a modified Krebs' of the following composition (in mM): NaCl 116, KCl 4.6, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11. The solution was gassed with 95% O_2/5% CO_2 to maintain pH at 7.4. The experiments were conducted at 37°C.

The thoracic aorta was removed and cleared of fat and connective tissue and cut into 6 rings. These were then slit longitudinally and denuded of endothelium by gently rubbing the luminal surface with moist cotton wool.

Both anococcygeus muscles were removed and divided transversely yielding four preparations per animal.

2.2. Frog skeletal muscle

Adult *Rana temporaria* were killed by a blow to the head followed by destruction of the brain and spinal cord and the sartorius muscles removed from each leg. Experiments were carried out at ambient temperature (20–25°C) in a modified Ringer solution containing (in mM): NaCl 116, KCl 2.5, $CaCl_2$ 1.8, NaH_2PO_4 0.75 and Na_2HPO_4 1.25 (pH 7.2). One muscle from each pair served as a control while the other was exposed to cetiedil.

2.3. Measurement of ^{86}Rb efflux

Freshly dissected tissues were tied to frames made from stainless steel tubing, through which gas could be bubbled to provide mixing. They were then transferred to a solution containing ^{86}Rb at a radioactive concentration of 0.1 MBq ml^{-1} for 90 min (the concentration of Rb^+ was less than 100 μM). At the end of this period the tissues were placed in 250 ml of non-radioactive solution in order to allow ^{86}Rb to diffuse from the extracellular spaces. After 30 min (when the rate constant for tracer efflux from the cells had stabilised) the tissues were transferred at regular intervals through a series of test tubes containing 5 ml of solution with or without drugs as indicated. At the end of the efflux period the tissue was blotted, removed from the frame and dissolved in 200 μl of concentrated nitric acid to allow the determination of the remaining radioactivity. The amounts of activity in the washout samples and tissue extract were determined by Cêrenkov counting in a scintillation counter (Beckman LS1801). A quench correction for the muscle extract was determined by internal standardisation ('spiking'). The rate constant for ^{86}Rb efflux was expressed as the fractional loss of tracer per minute.

In experiments on smooth muscle the size of the response to levcromakalim is expressed as the difference between the peak in the presence of levcromakalim and the mean of the rate constants for the two collection periods immediately before exposure to the drug. In experiments on frog sartorius, where efflux reached a broad peak during the 2nd, 3rd and 4th agonist periods, it is calculated from the means of the two periods prior to exposure to levcromakalim and of the 2nd, 3rd and 4th periods during drug application.

2.4. Data analysis

With the exception of values obtained by non-linear curve fitting, results are presented as means ± S.E.M. The significance of differences between the effect of levcromakalim in the presence and absence of test compound was determined by Student's *t*-test. Differences were considered significant when $P < 0.05$.The Hill equation,

$$y = y_{max}\frac{[A]^{n_H}}{[A]^{n_H} + IC_{50}^{n_H}}$$

where y is the percentage inhibition, y_{max} is the maximum inhibition, n_H is the Hill coefficient and [A] is the concentration of test compound, was fitted to the concentration-response curve for cetiedil. Fitting was performed by a weighted least squares method as implemented in the program CVFIT by Prof. D. Colquhoun, Department of Pharmacology, UCL. This program provides an estimate of the parameters ± an approximate standard deviation (see Colquhoun et al. (1974) for details).

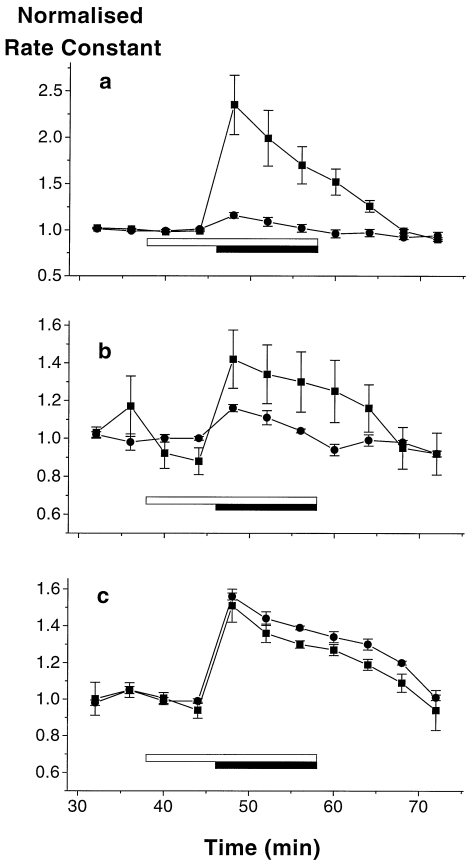

Fig. 2. Effect of 10 μM levcromakalim on ^{86}Rb efflux from rat aorta in the absence (■) and presence (●) of cetiedil (a), UCL 1285 (b) and UCL 1495 (c). All three compounds were present at a concentration of 10 μM. Whereas cetiedil and UCL 1285 caused a marked reduction of the response to levcromakalim ($P < 0.05$), UCL 1495 had no significant effect ($P > 0.05$). Levcromakalim was present during the period indicated by the solid bars while the hollow bars indicate the presence of blocker. Each point is the mean of three observations. The vertical bars indicate S.E.M. Note that in order to take into account variations in baseline efflux between preparations the rate constant for tracer efflux has been normalised by dividing each point from a given experiment by the average of the four periods prior to the application of levcromakalim.

2.5. Materials

^{86}RbCl was obtained from New England Nuclear. Levcromakalim and cetiedil (citrate salt) were generously donated by Smith Kline Beecham and Innothera respectively. UCL 1285 (cetiedil methiodide) and UCL 1495 (oxalate salt) were synthesised in the Department of Chemistry, UCL. Full details of the syntheses will be published subsequently. Stock solutions of Cetiedil, UCL 1495 and UCL 1285 were prepared in de-ionised water at a concentration of 10^{-3} M and further diluted in physiological saline. Levcromakalim was dissolved in DMSO at a concentration of 10^{-2} M; the final concentration of DMSO in the bathing solution was 0.1% (v/v). All other compounds used were of 'Analar' grade and obtained from Merck.

3. Results

3.1. Rat aorta

Exposure of rat aorta to 10 μM levcromakalim increased the rate constant for ^{86}Rb efflux by $81 \pm 10\%$ ($n = 23$). Previous work has shown that this response can be blocked by glibenclamide (Bray and Quast, 1991). The magnitude of the response to levcromakalim varied considerably as was the case with anococcygeus and sartorius. The increase in efflux was inhibited by cetiedil in a concentration-dependent manner. Fig. 2a illustrates the effect of 10 μM cetiedil and the concentration dependence of the action is shown in Fig. 3. The Hill equation was fitted to the data by the method of least squares yielding an estimate for the IC_{50} of 1.3 ± 0.4 μM ($n_H = 0.9 \pm 0.1$ with y_{max} constrained to 100%). UCL 1285 also inhibited the response to levcromakalim but was rather less active with an IC_{50} of approximately 6 μM (Fig. 3). In contrast, UCL 1495 at 10 μM had no significant effect (Fig. 2c).

3.2. Anococcygeus

The annococcygeus seemed rather less sensitive to levcromakalim, 10 μM causing a mean increase of $42 \pm 6\%$. 10 μM cetiedil caused an inhibition of $67 \pm 11\%$ ($n = 4$). The data obtained are shown in Fig. 4a.

3.3. Frog sartorius

Previous work has shown that cromakalim causes a glibenclamide-sensitive increase in ^{86}Rb efflux from frog

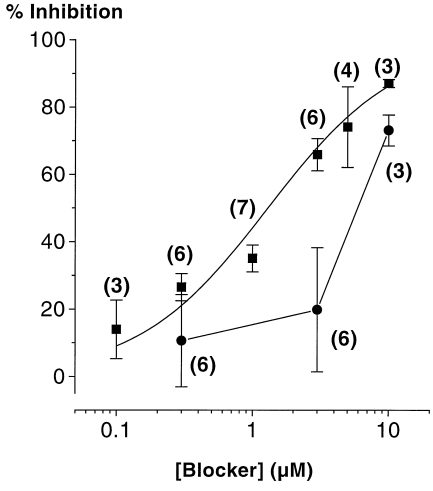

Fig. 3. Concentration-response curves for the inhibition of levcromakalim-stimulated ^{86}Rb efflux from rat aorta by cetiedil (■) and UCL 1285 (●). The curve through the values for cetiedil has been drawn using the Hill equation with the maximum constrained to 100%. The IC_{50} was estimated to be 1.3 ± 0.4 μM with a Hill slope of 0.9 ± 0.1. The IC_{50} for UCL 1285 was approximately 6 μM. Each point is the mean of the number of observations shown in parentheses. Vertical bars indicate S.E.M.

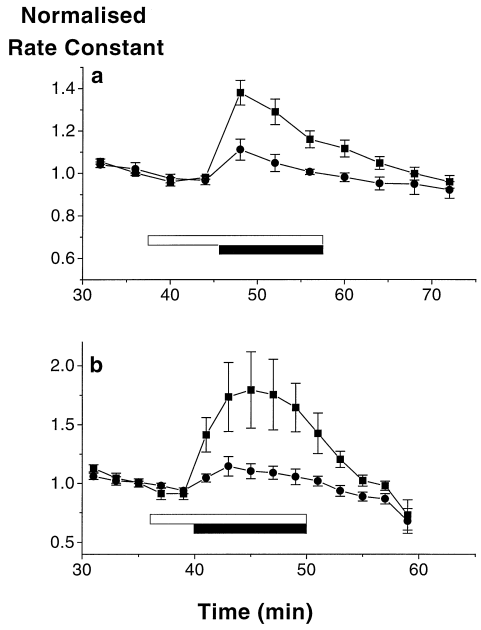

Fig. 4. (a) The effect of 10 μM levcromakalim on ^{86}Rb efflux from rat anococcygeus in the absence (■) and presence (●) of 10 μM cetiedil. At this concentration cetiedil caused 67 ± 11% inhibition ($P < 0.05$). (b) The effect of 50 μM levcromakalim on ^{86}Rb efflux from sartorius in the absence (■) and presence (●) of cetiedil also at a concentration of 10 μM. In this case cetiedil caused 84 ± 4% inhibition ($P < 0.05$). The solid bars indicate the presence of levcromakalim and the hollow bars indicate the presence of cetiedil. Each point is the mean of three observations and the S.E.M. is indicated by the vertical bars. The rate constants for tracer efflux have been normalised as described in the caption to Fig. 2.

sartorius (Benton and Haylett, 1992). However, this tissue is less sensitive to cromakalim than smooth muscle and so a higher concentration was used. At a concentration of 50 μM, levcromakalim increased ^{86}Rb efflux by 92 ± 28% ($n = 3$). This is in keeping with the effect of 100 μM cromakalim previously reported (Benton and Haylett, 1992) and is consistent with the suggestion that the activity of cromakalim resides chiefly in the (−) enantiomer, levcromakalim (Hof et al., 1988). 10 μM cetiedil caused 84 ± 4% inhibition of the response to levcromakalim (Fig. 4b).

4. Discussion

The experiments with rat aorta clearly show that cetiedil is an effective inhibitor of the actions of levcromakalim on ^{86}Rb efflux, with an IC_{50} of 1.3 μM. The quaternised form of cetiedil (UCL 1285) was less active ($IC_{50} \approx 6$ μM) whereas the much more lipophilic derivative UCL 1495 produced little if any block.In previous studies on erythrocyte K_{Ca} it was found that the rank order of potency of the three compounds tested was UCL 1495 > cetiedil > UCL 1285 (Benton et al., 1994). It is clear therefore that lipophilicity does not play the same role in determining activity as in the erythrocyte and that the structural requirements for block of the levcromakalim-activated channel are distinct. It may therefore be possible to develop even more selective inhibitors.

Little is known as yet of the site(s) of action of agents which inhibit K^+ channel opener-sensitive K^+ channels. The present finding that UCL 1285, which contains a quaternary nitrogen and is therefore unlikely to enter the cell, is active suggests that it at least may block the channel from the outside. Since levcromakalim is a lipid-soluble molecule which it is thought may act on the internal surface of the membrane (see e.g. Edwards and Weston (1993) for a discussion of this), it is likely that UCL 1285 and, by inference, possibly also cetiedil do not interfere with the binding of levcromakalim to its receptor. This would set these compounds apart from the sulphonylureas which appear to act at a closely linked site (Bray and Quast, 1992).

Although only a single concentration (10 μM) of cetiedil was tested on the two other tissues examined the similar degrees of inhibition observed suggest that cetiedil does not discriminate strongly between them. This contrasts with the action of phentolamine which did not antagonise the actions of levcromakalim on frog sartorius although it is able to do so in vascular smooth muscle (McPherson and Angus, 1989; Benton and Haylett, 1992). To summarise, cetiedil is an effective inhibitor of the actions of levcromakalim in smooth and skeletal muscle. The structural requirements for this action differ from those for blockade of other K^+ channels.

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