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Short communication

UCL 1684: a potent blocker of Ca^{2+}-activated K^+ channels in rat adrenal chromaffin cells in culture

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Abstract

The novel K^+ channel blocker 6,10-diaza-3(1,3)8,(1,4)-dibenzena-1,5(1,4)-diquinolinacyclodecaphane (UCL 1684) has been tested for its ability to inhibit Ca^{2+}-activated K^+ currents in cultured rat chromaffin cells. Low nanomolar concentrations of UCL 1684 produced a rapid and reversible inhibition of the slow, apamin-sensitive, tail current activated by a depolarizing voltage command. This compound also inhibited the muscarine activated outward current with an IC_{50} of 6 nM. These results confirm UCL 1684 to be the most potent non-peptidic blocker of the apamin-sensitive Ca^{2+}-activated K^+ channel so far described.

Keywords: K^+ channel; Ca^{2+}-activated; Apamin; Chromaffin cell; UCL 1684

1. Introduction

Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels are present in many cell types including hepatocytes (Burgess et al., 1981), gastro-intestinal smooth muscle (Gater et al., 1985; Vogalis and Goyal, 1997), chromaffin cells (Neely and Lingle, 1992; Park, 1994) and in both peripheral (Kawai and Watanabe, 1986) and central (Bourque and Brown, 1987) neurones. In many (though not all) tissues, the channel is blocked by nanomolar concentrations of the bee venom toxin apamin.

Molecular cloning techniques have identified three SK_{Ca} channel sequences (Köhler et al., 1996; Chandy et al., 1998), each capable of forming functional homomeric channels. An additional channel described as *hSK4* (Joiner et al., 1997) would appear to belong to the intermediate conductance (IK_{Ca}) subfamily (Ishii et al., 1997b; Logsdon et al., 1997). The potential formation of hetero-multimeric channels (Ishii et al., 1997a) and the possible involvement of an addition β-subunit (see Wadsworth et al., 1997) means that the molecular identity of the endogenous channels still remains to be determined.

These channels have both a physiological and pathophysiological role. The aberrant expression of SK_{Ca} channels in skeletal muscle is thought to be the underlying defect in myotonic muscular dystrophy (Renaud et al., 1986; Behrens et al., 1994), while intracerebroventricular injection of apamin has implicated these channels in the control of sleep (Gandolfo et al., 1996) and of certain kinds of learning (Messier et al., 1991). More recently, a defect in the gene for the *SKCa3* channel protein has been implicated in type 2 spinocerebellar ataxia (Imbert et al., 1996) and some psychiatric disorders (Chandy et al., 1998). Thus, apamin-sensitive SK_{Ca} channels may be suitable targets for the development of novel therapeutic agents. A particularly interesting possibility is raised by the finding that blockade of SK_{Ca} channels in the adrenal medulla increases agonist-evoked release of catecholamines (Montiel et al., 1995). Since intracerebral grafting of adrenal chromaffin cells has been used with some success in animal models (for reviews see Fine, 1990; Freed et al., 1990) and in the clinical treatment of Parkinson's Disease (Barker and Dunnett, 1993; Date et al., 1996), selective blockers of the SK_{Ca} channel expressed in chromaffin cells could prove useful adjuncts to increase the efficacy of such grafts.

In earlier work on SK_{Ca} channel blockers, the anti-fungal, anti-bacterial drug dequalinium was found to be an effective inhibitor of the apamin sensitive channel with an

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IC_{50} of approximately 1 μM (Castle et al., 1993; Dunn, 1994). More recently, another non-peptidic compound 6,10-diaza-3(1,3)8,(1,4)-dibenzena-1,5(1,4)-diquinolinacylodecaphane (UCL 1684), which is structurally related to dequalinium, was found to be a remarkably active blocker of the SK_{Ca} channel present in sympathetic neurones, with a potency comparable to that of apamin (Campos Rosa et al., 1998). However, there are differences in the sensitivity of the three homomeric recombinant SK_{Ca} channels to blocking agents (Köhler et al., 1996; Ishii et al., 1997a). Furthermore, UCL 1530, a cyclophane structurally related to UCL 1684, exhibits some selectivity between SK_{Ca} channels in different tissues (Dunn et al., 1996), as does gallamine (Wadsworth et al., 1994; Dunn et al., 1996). In the present study, UCL 1684 has been tested for its ability to block the SK_{Ca} channel that is found on rat chromaffin cells which can be activated either by Ca^{2+} influx through voltage gated Ca^{2+} channels or by Ca^{2+} release from intracellular stores. The results allow the pharmacology of the SK_{Ca} channel present on chromaffin cells to be compared with that of sympathetic neurones and also confirm the high potency of UCL 1684 as an SK_{Ca} channel blocker.

2. Methods

Adult male Sprague–Dawley rats were killed by inhalation of a rising concentration of CO_2, and their adrenal glands were removed into ice cold Ca^{2+} and Mg^{2+} free Hank's Balanced Salt Solution (HBSS). The medullae were dissected out and dissociated using collagenase and trypsin (using a method previously applied to cultured sympathetic neurones by Dunn et al. (1996), where further details can be found). The resultant suspension of chromaffin cells was plated onto collagen (Vitrogen 100, Imperial Laboratories) coated plastic culture dishes (Falcon), and maintained in DMEM supplemented with 10% fetal calf serum in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were used after 3 to 7 days in culture. For electrophysiological recordings, the dishes were mounted on the stage of an inverted microscope (Diaphot, Nikon), and perfused with physiological salt solution of the following composition (mM): NaCl 154, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 5.6, Hepes 10; adjusted to pH 7.4 with NaOH. Cells were viewed at 400× magnification. Whole cell recordings were made using the 'perforated patch' technique (Horn and Marty, 1988; Rae et al., 1991) using a List EPC7 amplifier. Patch electrodes were pulled from 1.5 mm borosilicate capillaries (GC1.5TF, Clark Electromedical) and had a resistance of 2–5 MΩ when filled with internal solution of the following composition (mM) K_3 citrate 56, KCl 25, NaCl 10, $MgCl_2$ 1, Hepes 35, EGTA 0.1, adjusted to pH 7.2 with KOH. For perforated patch recording, the internal solution also contained 240 μg/ml amphotericin B. Cells were routinely voltage clamped at −60 mV. A slow tail current (see Park, 1994) was activated by a 500 ms depolarization to 0 mV, and the amplitude measured 100 ms after termination of the pulse, by which time the currents carried by other K^+ channels were minimal. Voltage commands were generated, and data acquired using the pClamp software package and a Digidata 1200 interface (Axon Instruments) Drugs were applied locally to the cell using a micro-perfusion manifold as described previously (Dunn et al., 1996). All drugs and chemicals were obtained from Sigma except for UCL 1684 (6,10-diaza-3(1,3)8,(1,4)-dibenzena-1,5(1,4)-diquinolinacyclodecaphane) which was synthesised by Dr J. Campos-Rosa (Dept of Chemistry, University College London).

Values are given as the mean ± S.E.M. It was not usually possible to obtain a full dose response curve and determine an IC_{50} for each cell. Instead, the pooled data from a number of cells were fitted with the Hill equation using an iterative least squares fitting routine (Origin, Microcal), which gives a fitted parameter ± an approximate standard error.

3. Results

Ca^{2+}-activated K^+ currents in chromaffin cells were evoked either by a prior depolarization which allowed Ca^{2+} to enter the cells through voltage gated Ca^{2+}-channels, or by the application of muscarine to release Ca^{2+} from intracellular stores.

3.1. Tail currents

The slow tail current (corresponding to the slow afterhyperpolarization which follows the action potential) activated by a prolonged depolarizing voltage command provides a convenient means for studying SK_{Ca} channels in chromaffin cells (see, e.g., Park, 1994). This tail current was found to be inhibited by nanomolar concentrations of the bee venom toxin apamin (Fig. 1), with 10 nM producing 71 ± 4% inhibition ($n = 5$). While this effect was maximal in less than 1 min, recovery was much slower and could be described by a single exponential with a time constant of 600 ± 100 s ($n = 3$). This is close to the value of ≈800 s estimated by Park (1994).

Having confirmed the sensitivity of the tail current to apamin, the novel SK_{Ca} channel blocker UCL 1684 was then tested. At a concentration of 10 nM this compound produced a 68 ± 5% inhibition ($n = 4$) of the tail current while at 30 nM the current was reduced by 82 ± 4% ($n = 5$; Fig. 1). In contrast to the action of apamin, the effect of UCL 1684 reversed rapidly, with full recovery apparent after 4 min washout.

Another drug known to block SK_{Ca} channels is gallamine (Cook and Haylett, 1985; Wadsworth et al., 1994). Furthermore, this compound exhibits some selectivity between channels in sympathetic neurones and those in liver

cells (Dunn et al., 1996). At a concentration of 3 μM, gallamine produced a 45 ± 9% ($n = 4$) reduction in the tail current (Fig. 1B). This effect also reversed rapidly on washout.

3.2. Muscarine-activated currents

An alternative method for activating SK_{Ca} channels is to elevate the cytosolic [Ca^{2+}] through the activation of metabotropic muscarinic receptors (Neely and Lingle, 1992). In cells voltage clamped at -60 mV, application of 3 μM muscarine evoked a small transient outward current of 83 ± 10 pA ($n = 11$). Despite the use of the perforated patch recording technique, and an interval of 4 min between agonist applications, there was often a progressive decline in these responses. Application of 30 nM UCL 1684 produced a dramatic and reversible inhibition of the response to muscarine (Fig. 2A). The concentration response curve for this effect, shown in Fig. 2B, yielded an IC_{50} value of 6.6 ± 1.3 nM (fitted value ± approximate S.E. based on data from 11 cells).

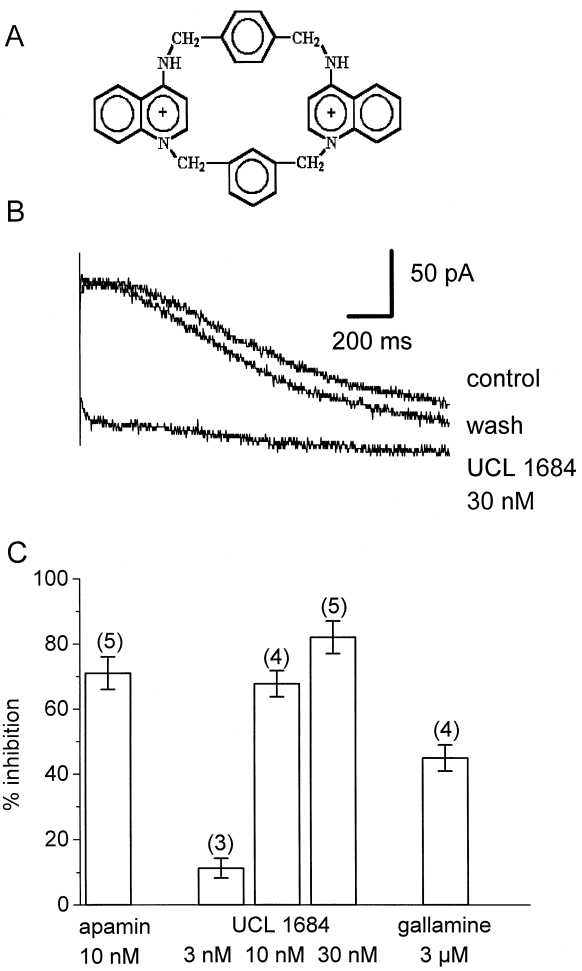

Fig. 1. Inhibition of tail currents by SK_{Ca} channel blockers including UCL 1684 (structure shown in A). (B) Tail currents recorded from a chromaffin cell voltage clamped at −60 mV following a 500 ms depolarization to 0 mV. The three superimposed traces were recorded before, after 2 min in the presence of, and 4 min after washout of 30 nM UCL 1684. (C) Histograms comparing the inhibition of the tail current produce by three concentrations of UCL 1684 with that by apamin (10 nM) and gallamine (3 μM). The columns represent the mean ± S.E.M. inhibition determined from the number of cells given in parenthesis.

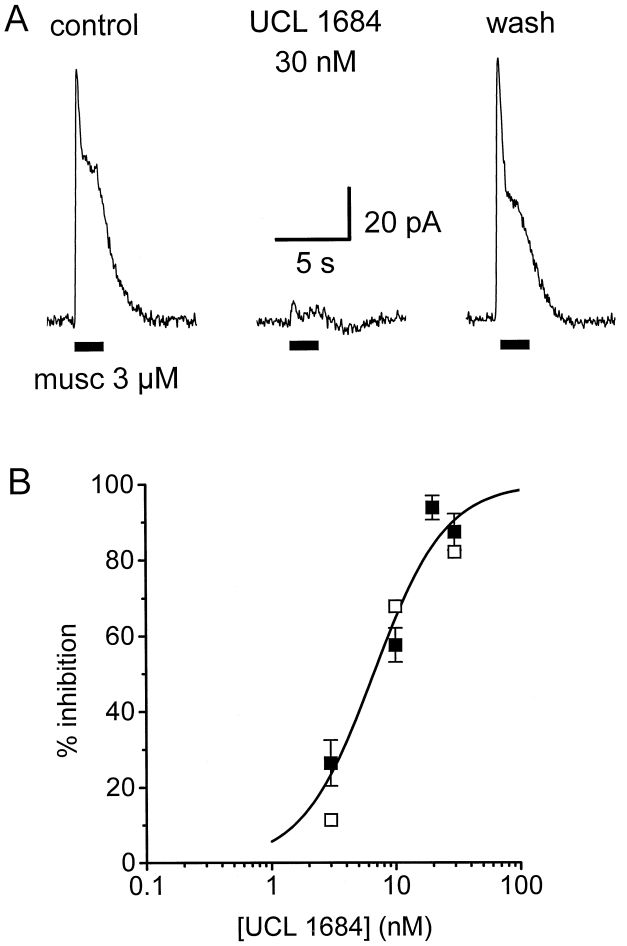

Fig. 2. Inhibition of muscarine activated outward currents. (A) Membrane currents evoked by 3 μM muscarine in a chromaffin cell voltage clamped at −60 mV before, after 4 min in the presence of 30 nM UCL 1684, and 4 min after washout of UCL 1684. (B) Log–concentration response curve for inhibition of muscarine activated currents by UCL 1684 (■). The points represent the mean ± S.E.M. from 3 to 5 cells. The solid line shows the least squares fit of the Hill equation to the data, with an IC_{50} of 6.6 ± 1.3 nM (fitted value ± S.E.). For comparison, data for inhibition of the tail current shown in Fig. 1 have been superimposed (□).

4. Discussion

The novel bisquinolinium cyclophane UCL1684 is a potent blocker (IC_{50} 3 nM) of the afterhyperpolarization in sympathetic neurones (Campos Rosa et al., 1998). In this study, UCL 1684 was tested for its ability to block two SK_{Ca}-mediated responses in rat chromaffin cells, namely the slow tail current activated by Ca^{2+} entry during a 500 ms depolarization, and the muscarine evoked outward current resulting from the release of Ca^{2+} from intra-

cellular stores. The IC_{50} for inhibition of the muscarine-activated current was 6.6 ± 1.3 nM, which is of the same order as that (3 nM) for inhibition of the afterhyperpolarization in sympathetic neurones. Furthermore, UCL 1684 inhibited the slow tail current in rat chromaffin cells at similar concentrations (see Fig. 2B). Although the actions of UCL 1684 could perhaps be explained by supposing that it is able to block both voltage gated Ca^{2+} channels and muscarinic receptors, and moreover at equal concentrations, a single site of action at the SK_{Ca} channel seems much more likely. This view is strongly supported by the observation that nanomolar concentrations of UCL 1684 inhibit ^{131}I-apamin binding to guinea-pig hepatocytes (D.C.H. Benton and D.G. Haylett, personal communication). The specificity of UCL 1684 for SK_{Ca} channels has not yet been systematically investigated. However, at a concentration of 100 nM this compound had no obvious effect on the action potential in sympathetic neurones apart from abolishing the slow afterhyperpolarization (P.M. Dunn, unpublished observation). Furthermore, at this concentration it did not affect the carbachol evoked contracture of the frog rectus abdominis muscle (A. Wong, personal communication), and at 2 μM had no effect on nerve evoked contraction of the rat vas deferens (D.H. Jenkinson, personal communication).

In the present study, gallamine was found to be a moderately effective blocker of the slow tail current in chromaffin cells, and considerably more potent than it is on either sympathetic neurones (IC_{50} 68 μM; Dunn et al., 1996) or on guinea-pig hepatocytes ($IC_{50} \approx 12$ μM; Cook and Haylett, 1985). This may indicate that the SK_{Ca} channel present in chromaffin cells differs somewhat from those in sympathetic neurones and liver cells, a view supported by the different single channel conductances reported for these three cell types (Capiod and Ogden, 1989; Park, 1994; Selyanko and Brown, 1996). Although the molecular identity of the SK_{Ca} channel in chromaffin cells remains to be determined, the transcript for *SKCa2*, but not *SKCa1* is present in the adrenal gland, and although *SKCa3* is widely distributed in the periphery its absence or presence in the adrenal gland has yet to be described. Whether UCL 1684 is able to discriminate between the recombinant *SKCa* channels remains to be tested.

In conclusion, this study has demonstrated that UCL 1684 is a potent inhibitor of the SK_{Ca} channel in chromaffin cells, adding to the evidence that it is the most active non peptide blocker of this channel so far described. It can be expected to increase the release of catecholamines from these cells both in situ and possibly in intracerebral grafts.

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