British Journal of Pharmacology (2001) 132, 889–898

www.nature.com/bjp

Clotrimazole analogues: effective blockers of the slow afterhyperpolarization in cultured rat hippocampal pyramidal neurones

[1]M.M. Shah, [2]Z. Miscony, [2]M. Javadzadeh-Tabatabaie, [2]C.R. Ganellin & *,[1]D.G. Haylett

[1]Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT and [2]Department of Chemistry, University College London, Gower Street, London, WC1E 6BT

1 The pharmacology of the slow afterhyperpolarization (sAHP) was studied in cultured rat hippocampal pyramidal neurones.

2 Clotrimazole, its *in vivo* metabolite, 2-chlorophenyl-bisphenyl-methanol (CBM) and the novel analogues, UCL 1880 and UCL 2027, inhibited the sI_{AHP} with similar IC_{50}s (1–2 μM).

3 Clotrimazole and CBM also inhibited the high voltage-activated (HVA) Ca^{2+} current in pyramidal neurones with IC_{50}s of 4.7 μM and 2.2 μM respectively. UCL 1880 was a less effective Ca^{2+} channel blocker, reducing the HVA Ca^{2+} current by 50% at 10 μM. At concentrations up to 10 μM, UCL 2027 had no effect on the Ca^{2+} current, indicating that its effects on the sI_{AHP} were independent of Ca^{2+} channel block.

4 Clotrimazole also inhibited both the outward holding current ($IC_{50}=2.8$ μM) present at a potential of -50 mV and the apamin-sensitive medium AHP (mAHP; $IC_{50}\approx 10$ μM). The other clotrimazole analogues tested had smaller effects on these two currents. The present work also shows that 100 nM UCL 1848, an inhibitor of apamin-sensitive conductances, abolishes the mAHP.

5 Currents were recorded from HEK293 cells transfected with hSK1 and rSK2. The SK currents were very sensitive to inhibition by UCL 1848 but were not significantly reduced by the sI_{AHP} inhibitor, UCL 2027 (10 μM). 10 μM UCL 1880 reduced the hSK1 current by 40%.

6 UCL 2027 appears to be the first relatively selective blocker of the sAHP to be described. Furthermore, the ability of UCL 2027 to block the sAHP with minimal effect on SK1 channel activity questions the role of this channel in the sAHP.

British Journal of Pharmacology (2000) **132,** 889–898

Keywords: Slow afterhyperpolarization; hippocampal pyramidal neurones; SK potassium channels; clotrimazole; UCL 1848; UCL 1880; UCL 2027

Abbreviations: BK, IK and SK channels, large, intermediate and small conductance potassium channels respectively; CBM, 2-chlorophenyl-bisphenyl-methanol; HEK293 cells, human embryonic kidney 293 cells; mAHP and sAHP, slow and medium afterhyperpolarizations

Introduction

An afterhyperpolarization (AHP) comprising fast (fAHP), medium (mAHP) and slow (sAHP) components follows a train of action potentials in hippocampal pyramidal neurones (for reviews see Storm, 1990 and Sah, 1996). Large conductance Ca^{2+}-activated K^+ channels (BK channels) underlie the fAHP and contribute to the repolarization of action potentials (Lancaster & Nicoll, 1987; Yoshida *et al*., 1991). Multiple channels including the apamin-sensitive small conductance Ca^{2+}-activated K^+ channels (SK channels) underlie the mAHP (Storm, 1987; Stocker *et al*., 1999). The channels which underlie the sAHP have not yet been determined.

The sAHP is characterized by its slow time-course (see Storm, 1990) and its lack of sensitivity to the bee venom toxin, apamin and the non-specific K^+ channel blockers tetraethylammonium (TEA) and 4-aminopyridine (4-AP) (Lancaster & Adams, 1986; Lancaster & Nicoll, 1987). There are no specific blockers or openers of the sAHP current though it can be modulated by the activation of G-protein coupled receptors (see Sah, 1996).

The sAHP is important for spike frequency adaptation (Storm, 1990; Sah, 1996) and may serve to limit neuronal damage due to excessive rises in Ca^{2+} concentration within the cells (Vergara *et al*., 1998). A combination of behavioural and electrophysiological studies have shown that the sAHP may also have a role in learning and memory (Moyer *et al*., 1992; Disterhoft *et al*., 1996; Giese *et al*., 1998; Weiss *et al*., 2000). However, a direct test of this hypothesis is hampered by a lack of specific blockers of the sAHP.

It has been shown that K^+ channels that are dependent upon Ca^{2+} for activation underlie the sAHP (Lancaster & Adams, 1986). As noise analysis studies have revealed that a small-conductance channel underlies the sAHP (Sah & Isaacson, 1995; Valiante *et al*., 1997), it has been postulated that the sAHP results from the opening of apamin-insensitive SK channels (Bond *et al*., 1999). Recently, three SK channels (SK1, SK2 and SK3) have been cloned (Kohler *et al*., 1996).

*Author for correspondence.

When expressed in *Xenopus* oocytes, SK2 and SK3 formed apamin-sensitive channels whereas those from SK1 were insensitive (Kohler *et al*., 1996; Ishii *et al*., 1997a). These results have led to the suggestion that SK1 channels may underlie the sAHP in hippocampal pyramidal neurones (Vergara *et al*., 1998; Bond *et al*., 1999). However, the recent finding that in mammalian cell lines, transfection of SK1 cDNA results in the formation of predominantly apamin-sensitive channels (Shah & Haylett, 2000a; Strøbæk *et al*., 2000) casts some doubt on this. Also, in contrast to the sAHP, there is no evidence that SK1 channels are subject to modulation by protein kinases (Xia *et al*., 1998).

To extend the pharmacology of the sAHP, the present study has explored the actions of two known types of K^+ channel blockers. Firstly, we have tested the action of UCL 1848, a blocker of apamin-sensitive conductances (Benton *et al*., 1999) and also of SK1 channels (Shah & Haylett, 2000a). Secondly, we have examined the effects of clotrimazole and its major *in vivo* metabolite 2-chlorophenyl-bisphenyl-methanol (CBM) as well as some related compounds. Clotrimazole is a potent blocker of the intermediate conductance Ca^{2+}-activated K^+ channels (IK channels) found in red blood cells and lymphocytes (Alvarez *et al*., 1992; Brugnara *et al*., 1993; Rittenhouse *et al*., 1997b; Logsdon *et al*., 1997; Dunn, 1998; Jensen *et al*., 1999). CBM is also a potent inhibitor of the IK channels in red blood cells (Rittenhouse *et al*., 1997b). The IK channel (commonly referred to as SK4 or IK1) has recently been cloned (Joiner *et al*., 1997; Ishii *et al*., 1997b; Jensen *et al*., 1998) and has about 55% sequence identity to the cloned SK channels.

Using the perforated patch technique, we have recently demonstrated that the sAHP can be recorded from cultured hippocampal pyramidal neurones (Shah and Haylett, 2000b). The characteristics of the current underlying the sAHP (sI_{AHP}) in cultured cells are very similar to those observed when the sI_{AHP} is recorded from slices and the improved drug access to isolated cells makes them well suited to a study of agents modifying the sI_{AHP}. In our experiments, the sI_{AHP} was elicited by a train of action potentials. As Ca^{2+} entry through high voltage-activated (HVA) Ca^{2+} channels is involved in the generation of the sI_{AHP} in hippocampal pyramidal neurones (Shah & Haylett, 2000b), it was necessary to test the effects of clotrimazole and its analogues on Ca^{2+} currents as well as the sI_{AHP}.

Some of this work has been communicated at the American *Society for Neuroscience* meeting (November, 1999).

Methods

Cell culture methods

Hippocampal pyramidal cell culture Hippocampal cells were cultured as previously described (Shah & Haylett, 2000b). Briefly, a 4-day old Sprague-Dawley rat was decapitated and coronal brain slices were cut using a McIlwain tissue chopper. The CA1 and CA3 regions of the hippocampus were dissected and incubated together for 1 h in Hanks buffered saline solution (HBSS) containing 0.125% trypsin. Individual cells were then released by trituration and resuspended in Neurobasal medium supplemented with 2% B27 serum free supplement, 0.5 mM L-glutamine and 10% foetal calf serum (FCS). The cells were then plated onto 35 mm Nunc tissue-culture treated plastic dishes (Gibco, U.K.) that had been previously coated with 0.05 mg ml^{-1} poly-D-lysine (MW > 300,000). Cells were maintained in culture for 15 days using the same growth medium but without the FCS.

Maintenance and transfection of HEK293 cells HEK293 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 50 u ml^{-1} penicillin, 50 μg ml^{-1} streptomycin and 10% FCS. Cells were transfected with both hSK1 (a generous gift from Dr J.P. Adelman, Vollum Institute, U.S.A.) and CD8 cDNAs using a modified form of the calcium phosphate method described previously (Shah & Haylett, 2000a).

A few experiments were performed with rSK2 transfected stably in HEK293 cells (kindly provided by Dr W. Joiner, Yale University, U.S.A.).

Electrophysiological studies

Recording of the sI_{AHP} Electrophysiological recordings were obtained from cells that had been in culture for at least 8 days. All experiments were done at a temperature of approximately 28°C. The culture dishes were superfused at 5 ml min^{-1} with a bathing solution of composition (mM): NaCl 130, KCl 3, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES free acid 5, glucose 10, $NaHCO_3$ 26, pH maintained at 7.2 by continuously gassing with 95% O_2/5%CO_2. 5 μM DNQX (6,7-dinitroquinoxaline-2,3-dione) was added to the external solution to block the AMPA and kainate receptors. Patch electrodes with resistances of 4–10 MΩ were pulled from thin borosilicate glass (Clark Electromedical Instruments; GC15OTF-15). The tips of the pipettes were coated with Sylgard (Dow Corning, U.S.A.) and fire-polished. The tips of the electrodes were initially dipped into a solution composed of (mM): $KMeSO_4$ 126, KCl 14, HEPES 10, $MgCl_2$ 3, Na_2ATP 2, Na_2GTP 0.3. The pH was adjusted to 7.25 with 1 M KOH. To obtain perforated patches, the patch electrodes were then backfilled with the same solution containing 1.2 mg ml^{-1} amphotericin B.

The sI_{AHP} was recorded with an Axoclamp 2A amplifier (Axon Instruments) using a hybrid current/voltage clamp protocol in which a train of 13 action potentials (see Shah & Haylett, 2000b) was evoked by passing 5 ms current pulses (at a frequency of 76.5 Hz) under discontinuous current clamp conditions following which the cell was voltage-clamped at approximately −50 mV to record the sI_{AHP} (Axoclamp sampling rate 3–5 kHz). The sI_{AHP} was evoked every 10 s. The current signals were filtered using the Axoclamp 2A low pass filter at 0.3 kHz. For action potential recording, the voltage signals were filtered at 3 kHz. Signals were recorded on a chart recorder and an oscilloscope. They were also digitized at 48 kHz (VR-10 digital data recorder; Instrutech Corporation) and recorded continuously on a video recorder. sI_{AHP}s were also stored on a computer using pClamp6 (Axon Instruments) for later analysis. The first 2 s of the sI_{AHP} were acquired at a sampling frequency of 2 kHz and the remainder at a sampling frequency of 0.5 kHz. To measure drug effects on action potentials, these were acquired separately at a sampling frequency of 20 kHz.

Drugs were applied by switching to a superfusion fluid containing the drug using a multiway tap. The inlet tube was positioned such that the flow was directed onto the patched cell.

Recording of Ca^{2+} currents The presence of an extensive dendritic tree made it difficult to voltage-clamp cultured hippocampal pyramidal cells, so Ca^{2+} currents were studied using freshly dissociated cells. The pyramidal cells were isolated and plated as described above. Recordings were made from cells between 3.5 and 8 h after isolation.

The cells were superfused with a solution of the following composition (mM): NaCl 115, KCl 2, $CaCl_2$ 2, $MgCl_2$ 0.5, glucose 11, HEPES 10, pH adjusted to 7.4 with 1 M NaOH. 25 mM TEA, 0.3 μM tetrodotoxin (TTX) and 5 μM DNQX were added to the external solution to block K^+ channels, Na^+ channels and AMPA and kainate receptors respectively. Whole cell patch-clamp recordings were made with a List EPC-7 amplifier using 7–10 MΩ pipettes filled with the following solution (mM): CsCl 135, $CaCl_2$ 0.5, $MgCl_2$ 2, HEPES 10, EGTA 3, Na_2ATP 2, Na_2GTP 0.3, pH adjusted to 7.3 with 1 M NaOH. The Ca^{2+} current was elicited using a procedure similar to that described by Deak *et al*., (1998). The cells were voltage-clamped at −80 mV. Then, using a ramp protocol, the cells were depolarized from −100 mV to +40 mV at a speed of 1400 mV s^{-1} every 10 s. The leak current was determined by using Cd^{2+} (200 μM) to block the high voltage-activated (HVA) calcium currents. As the measured series resistance was less than 5 MΩ and the maximum voltage error calculated to be less than 5 mV, series resistance compensation was not applied. The signals were filtered at a frequency of 1 kHz (8 pole Bessel filter) and digitized at a sampling frequency of 3.33 kHz using pClamp6 software (Axon Instruments).

Experiments using HEK293 cells transfected with hSK1 cDNA Transiently transfected cells were identified by adding CD8 antibody coated microspheres (Dynabeads M-450 CD8, Dynal, U.K.) to the cells 30 min before use (Jurman *et al*., 1994). HEK293 cells were used a minimum of 24 h after transfection. Cells were superfused at 5 ml min^{-1} with the following solution (mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 10, pH adjusted to 7.4 with 1 M NaOH. Whole cell recordings from isolated cells were made with pipettes of resistances of 3–5 MΩ when filled with the following solution (mM): KCl 130, HEDTA 5, HEPES 10, Na_2ATP 2, $MgCl_2$ 3, $CaCl_2$ 0.67, pH adjusted to 7.2 using 1 M KOH. The calcium concentration was calculated to give 1 μM free calcium using the program 'REACT' (G.L. Smith, Dept. of Physiology, University of Glasgow, U.K.). Cells were voltage-clamped at −80 mV using a List EPC-7 amplifier. 100 ms voltage steps were made at 100 ms intervals from −140 mV to +40 mV in 20 mV increments every minute (see Figure 8). Signals were stored on a computer using pClamp6 (sampling frequency of 5 kHz) for further analysis.

Data analysis

Data were analysed using pClamp6 software. The current traces shown in the illustrations are the average of three records. Drug effects on the sI_{AHP} are recorded as the average amplitude of three successive records of the sI_{AHP} in the presence of the drug expressed as a percentage of the average amplitude of six successive records prior to drug application. The effects of drugs on action potential duration were measured at −20 mV. The effects of drugs on the time-course of the I_{AHP} were measured by fitting the following empirical equation to the recorded current:

$$y = y_0 + A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3} \quad (1)$$

where A_1 and τ_1 are the amplitude and decay time constant respectively of the current underlying the mAHP (mI_{AHP}); A_2 and τ_2 are the amplitude and time constants of the growth phase of the sI_{AHP}; and A_3 and τ_3 are the amplitude and time constants of the decay phase of the sI_{AHP}. Note that this requires A_2 to be negative. t is the time from switching to voltage-clamp and y_0 represents the holding current at the voltage-clamp potential. Results are expressed as mean ± s.e.mean. Statistical analysis was carried out using Student's t-test (paired or unpaired as appropriate).

When analysing the effects of drugs on the HVA Ca^{2+} current, the average peak current of the last two records in the presence of the drug (2 min bath application) was expressed as a percentage of the average peak current of two successive records obtained both before application of the drug and after its washout. This was necessary in order to allow for run-down of the Ca^{2+} currents with time. Data was collected only from cells in which the Ca^{2+} current recovered to within 70% of the control after washout.

To minimize series resistance errors and the contribution of a significant amount of delayed rectifier current, the amplitude of SK currents was measured at a potential of −40 mV. The amplitude of the SK current in the presence of the drug was expressed as a percentage of the average of the amplitude before application of the drug and that after washout.

Concentration-inhibition curves were fitted with the Hill equation:

$$y = y_{max}[I^n]/(IC_{50}{}^n + [I^n]) \quad (2)$$

where y is the percentage inhibition, [I] is the drug concentration, y_{max} is the maximum percentage inhibition and n is the Hill coefficient. The IC_{50} value is the concentration of drug causing 50% of the maximal attainable inhibition. y_{max} was constrained to 100%, because the currents could be abolished by high concentrations of all agents tested.

Materials

Tissue culture reagents and materials were purchased from Life Technologies, U.K. All chemicals were obtained from Sigma, U.K. except for CBM, UCL 1880 and UCL 2027 which were synthesized by the authors (Z. Miscony, M. Javadzadeh-Tabatabaie and C.R. Ganellin) using procedures that will be described elsewhere. UCL 1848 was made by Dr J. Chen (Dept. of Chemistry, UCL).

Stock solutions of clotrimazole (Figure 1A), 2-chlorophenyl-bisphenyl-methanol (CBM; Figure 1B), 3-{N-[1-(2-chlorophenyl)-1,1-diphenyl]methyl}aminopyridine (UCL 1880; Figure 1C) and 2-triphenylmethylaminothiazole (UCL 2027; Figure 1D) and 1,1′-(pentane-1,5-diyl-)-N,N′-(pentane-1,5-diyl)bis-(4-aminoquinolinium)ditrifluoro-acetate (UCL 1848; Figure 1E) were made up in dimethyl sulphoxide (DMSO) and stored at 4°C. These were then diluted to the appropriate

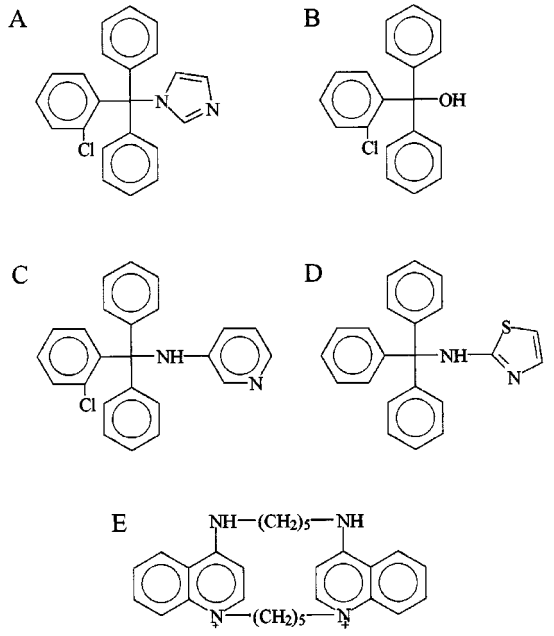

Figure 1 Chemical structures of clotrimazole (A), CBM (B), UCL 1880 (C), UCL 2027 (D) and UCL 1848 (E).

concentrations in the external bathing solution. The maximum concentration of DMSO applied (0.1%) had no effect on the sI_{AHP}.

Results

Stable recordings of the sI_{AHP} lasting for up to 60 min could be obtained from the cultured hippocampal cells, as described previously (Shah & Haylett, 2000b). In this series of experiments, the currents peaked on average 500 ms after the train of action potentials and had a decay time constant (τ_3, Equation 1) of 1.03 ± 0.04 s ($n = 57$).

Effects of clotrimazole and its derivatives on the sI_{AHP}

Clotrimazole was first tested at a concentration of 3 μM which, within 2 min of application, reduced the amplitude of the sI_{AHP} by $69.1 \pm 3.7\%$ ($n = 6$; Figure 2A). This effect was reversible within 5 min of washout. In light of the blocking effect of clotrimazole, the related compounds CBM, UCL 1880 and UCL 2027 were all tested at 3 μM. CBM, UCL 1880 and UCL 2027 inhibited the sI_{AHP} at this concentration by $61.7 \pm 5.8\%$ ($n = 7$; Figure 2B), $71.8 \pm 7.0\%$ ($n = 8$; Figure 2C) and $77.6 \pm 3.9\%$ ($n = 5$; Figure 2D) respectively. With all three compounds, block of the sI_{AHP} was maximal within 2 min and the effect was reversible within 10 min (see Figure 5 for examples). The concentration-inhibition curves for each agent were determined next and these are illustrated in Figure 3. The IC_{50} values for clotrimazole, CBM, UCL 1880 and UCL 2027 came to 1.69 ± 0.04 μM, 2.2 ± 0.5 μM, 1.2 ± 0.1 μM and 1.1 ± 0.2 μM respectively with corresponding Hill coefficients of 1.34 ± 0.04, 1.3 ± 0.3, 1.4 ± 0.1 and 1.5 ± 0.2. The sI_{AHP} could be abolished at the maximal concentrations tested and indeed there was some suggestion that a small inward current was revealed, though this was not investigated further.

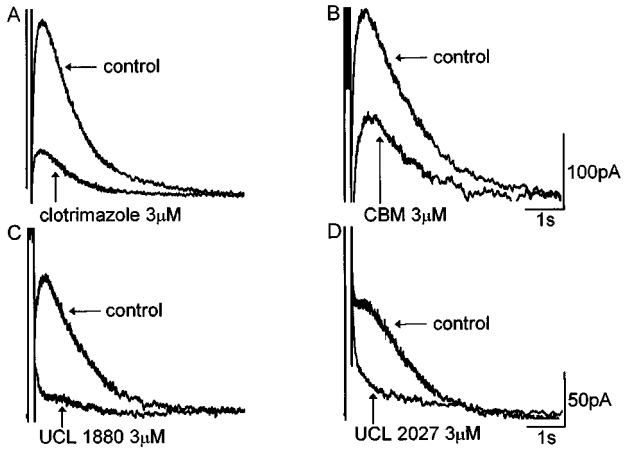

Figure 2 Effects of clotrimazole and analogues on the sI_{AHP} recorded from hippocampal pyramidal neurones. The traces under control conditions and in the presence of clotrimazole (A), CBM (B), UCL 1880 (C) and UCL 2027 (D) have been superimposed in each panel. The calibration bars in (B) and (D) also apply to (A) and (C) respectively.

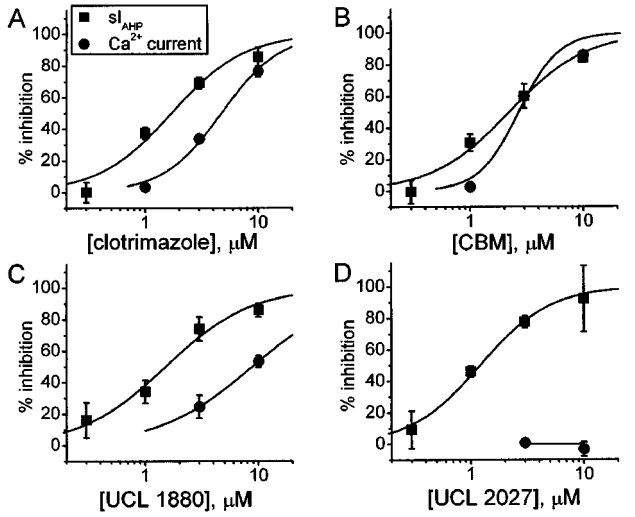

Figure 3 Concentration-inhibition curves for the sI_{AHP} and the HVA Ca^{2+} current. Each point is the mean $\pm$ s.e.mean of 3–7 observations. The lines show least squares fits of the Hill equation with y_{max} constrained to 100%. The IC_{50} values are given in the text. The key shown in panel (A) applies to panels (B), (C) and (D), also.

The I_{AHP}s were also fitted with Equation 1 to give estimates of the time to peak and the decay time constant (τ_3). These estimates did not include measurements of the sI_{AHP}s in which the current amplitude was reduced to below 20 pA in the presence of the drug (at concentrations of 3 μM and 10 μM) when τ_3 and time to peak could no longer be reliably measured (see Figure 8A,C). For clotrimazole, UCL 1880 and UCL 2027, at concentrations of 3 μM and lower, there were no significant differences in the times to peak and the τ_3 of the sI_{AHP} in the absence and presence of the drug (see Table 1). Although it is not immediately apparent from

the trace presented (Figure 2B), on average both τ_3 and the time to peak were significantly altered (Table 1) in the presence of 3 μM CBM.

Effects of clotrimazole and its derivatives on action potentials

Since action potentials were used to evoke the sI_{AHP} and a change in the action potential width can affect the amplitude of the sI_{AHP} (see Shah & Haylett, 2000b), it was important to establish whether any of the agents modified the action potential. To take into account possible use-dependence of their action, the effects of the compounds on the width of the last action potential in the train were measured. At concentrations up to 3 μM, clotrimazole and UCL 1880 had little effect on the action potential width (Figure 4A and Table 2). In the presence of either clotrimazole or UCL 1880 at a concentration of 10 μM, however, the action potential tended to widen though the increase was not significant (Table 2). The action potentials became broader in the presence of CBM and UCL 2027 at concentrations of 3 μM and 10 μM (Table 2; Figure 4B), though the effects were statistically significant only at 3 μM. The amplitude of the action potentials were little affected in the presence of all drugs tested (see Figure 4C,D for examples).

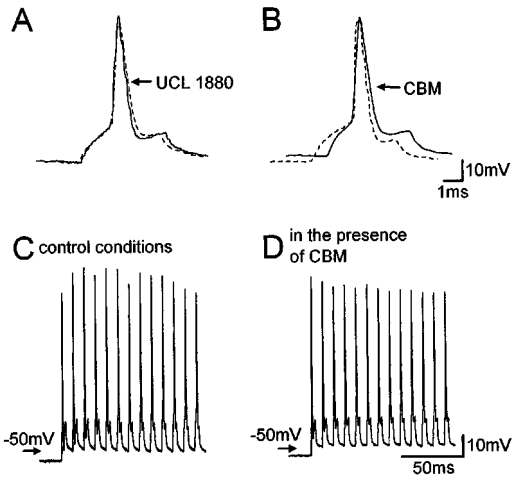

Figure 4 Examples of the effects of the compounds on action potential width and amplitude. Effects of (A) 3 μM UCL 1880 and (B) 3 μM CBM on the last action potential in a train. The dotted lines represent the last action potential in the train before application of the agents. The records are superimposed so as to align the upstroke. The negligible effect of CBM on the amplitude of the action potentials in a train is seen by comparing (C) (in the absence) with (D) (in the presence) of the drug. The action potentials shown in (B) are from the records in (C) and (D). The calibration bars in (B) and (D) also apply to (A) and (C) respectively.

Table 1 Effect of the compounds on the decay time constant (τ_3; (A)) and the time to peak (TP; (B)) of the sI_{AHP}

(A)

Drug	*Control* τ_3 (s)	τ_3 *in the presence of 1* μM *drug* (s)	*Control* τ_3 (s)	τ_3 *in the presence of 3* μM *drug* (s)
Clotrimazole	1.38±0.22	1.09±0.13	0.91±0.08	0.79±0.09
CBM	0.99±0.09	0.81±0.10	1.11±0.15	0.77±0.07*
UCL 1880	0.96±0.09	0.92±0.09	1.00±0.07	1.07±0.15
UCL 2027	0.62±0.10	0.57±0.09	1.24±0.11	0.98±0.14

(B)

Drug	*Control TP* (s)	*TP in the presence of 1* μM *drug* (s)	*Control TP* (s)	*TP in the presence of 3* μM *drug* (s)
Clotrimazole	0.50±0.06	0.50±0.04	0.46±0.07	0.50±0.06
CBM	0.38±0.03	0.39±0.06	0.49±0.06	0.58±0.06*
UCL 1880	0.45±0.03	0.45±0.01	0.48±0.03	0.51±0.05
UCL 2027	0.43±0.02	0.43±0.02	0.47±0.04	0.52±0.05

τ_3 and the time to peak were estimated by fitting the sI_{AHP} traces with Equation 1. Each value is the mean±s.e.mean of 3–7 observations. *Indicates significant effects at the 5% level (paired *t*-test).

Table 2 Effect of the compounds on action potential width (APW)

Drug	*Control APW* (ms)	*APW in the presence of 1* μM *drug* (ms)	*Control APW* (ms)	*APW in the presence of 3* μM *drug* (ms)	*Control APW* (ms)	*APW in the presence of 10* μM *drug* (ms)
Clotrimazole	0.95±0.12	1.07±0.14	1.07±0.17	1.18±0.22	0.66±0.11	1.84±0.52
CBM	1.14±0.20	1.18±0.20	1.00±0.13	1.33±0.16**	1.13±0.24	1.70±0.35
UCL 1880	1.38±0.38	1.43±0.42	1.35±0.17	1.66±0.22	1.25±0.24	2.05±0.40
UCL 2027	0.90±0.15	1.17±0.37	1.36±0.25	2.08±0.38*	1.80±0.17	2.26±0.35

The width of the last action potential in the train was measured at a potential of −20 mV. Each value is the mean±s.e.mean of 3–7 observations. * and ** indicate significant effects at the 5% and 1% level respectively using the paired *t*-test.

Effect of clotrimazole on the outward holding current at −50 mV

The sI_{AHP} was recorded at a holding potential of −50 mV. As the resting membrane potential of these cultured hippocampal pyramidal neurones is usually in the range −55 to −70 mV, there is normally an outward holding current (see Figure 5A,C) Clotrimazole inhibited the outward holding current (Figure 5A), the effect being concentration-dependent. The concentration-inhibition data were fitted with a Hill equation having a Hill coefficient of 1.2±0.1 and an IC_{50} value of 2.8±0.1 μM (Figure 5B). In keeping with this, the resting membrane potential was also significantly decreased in the presence of 3 μM and 10 μM clotrimazole by 5.4±1.1 mV ($n=7$; $P<0.05$) and 8.7±2.4 mV ($n=4$; $P<0.05$) respectively.

CBM, UCL 1880 and UCL 2027 had smaller and more variable effects on the outward holding current (see Figure 5C for an example). The percentage inhibition of the holding current and the decrease in the resting membrane potential were 44.4±8.1% ($n=3$; $P=0.01$) and 4.6±1.9 mV ($n=3$; $P=0.1$) for 10 μM CBM; 29.5±14.3% ($n=4$; $P=0.1$) and 3.7±1.8 mV ($n=4$; $P=0.1$) for 10 μM UCL 1880; and 36.8±19.0% ($n=3$; $P=0.2$) and 5.2±1.7 mV ($n=3$; $P=0.1$) for 10 μM UCL 2027.

Effect of the compounds on Ca^{2+} currents

Clotrimazole has been reported to inhibit the L-type Ca^{2+} channels in cardiac myocytes (Thomas *et al*., 1999) and is also a potent inhibitor of channels formed by the expressed human cardiac L-type Ca^{2+} channel α_{1c} subunits (Fearon *et al*., 2000). Since hippocampal pyramidal neurones express L-type Ca^{2+} channels as well as N-, P-, Q-, R- and T-type channels (Christie *et al*., 1996), it seemed essential to test the effects of the compounds on the Ca^{2+} currents. As cultured hippocampal pyramidal neurones cannot be adequately voltage-clamped, we necessarily studied the Ca^{2+} currents in freshly dissociated neurones. Both low voltage-activated (LVA) and high voltage-activated (HVA) Ca^{2+} currents can be evoked in these cells (see Figure 6). At a holding potential of −50 mV, only the HVA Ca^{2+} currents would be expected to contribute to the generation of the sI_{AHP} (Shah and Haylett, 2000b). Hence, only the effects of the compounds on the HVA Ca^{2+} currents were examined in detail.

Clotrimazole reduced the HVA Ca^{2+} current in a concentration-dependent manner (see Figures 3A, 6A,B). The fitted Hill equation had a Hill coefficient of 1.7±0.2 and an IC_{50} of 4.7±0.4 μM (Figure 3A). At concentrations of 3 μM and 10 μM, a significant effect on the LVA Ca^{2+} current was also observed (see Figure 6A,B). CBM also inhibited the HVA Ca^{2+} current in a concentration dependent manner (Figure 3B). The concentration-inhibition curve yielded an IC_{50} value of 2.4±1.0 μM with a Hill coefficient of 2.7±0.4. UCL 1880 was a less potent blocker of the Ca^{2+} current. The block of the HVA Ca^{2+} current by UCL 1880 was dose-dependent (Figure 3C) with an IC_{50} value of approximately 10 μM. The low solubility of the compound in the external solution did not allow concentrations greater than 10 μM to be tested.

UCL 2027 did not block the HVA Ca^{2+} current at concentrations of either 3 μM or 10 μM (Figures 3D and 6C). Thus, in terms of Ca^{2+} channel and sI_{AHP} block, it is the most selective of the four compounds tested.

Effects of UCL 1848, clotrimazole and its analogues on the mI_{AHP}

Slice recordings from hippocampal pyramidal neurones have shown that a mAHP precedes the apamin-insensitive sAHP (Storm, 1987; see Storm 1990 for a review). The mAHP current (mI_{AHP}) is at least partially sensitive to the bee-venom toxin apamin (see Stocker *et al*., 1999). In a proportion of our cultured cells, a largely apamin-sensitive mI_{AHP} can also be detected (Shah & Haylett, 2000b). Application of 100 nM UCL 1848 abolished this mI_{AHP} ($n=4$) but had no significant effect on the sI_{AHP} (per cent inhibition = −3.6±5.0%, $n=6$; Figure 7A). The selective effect of UCL 1848 on the mI_{AHP} is clearly seen when a

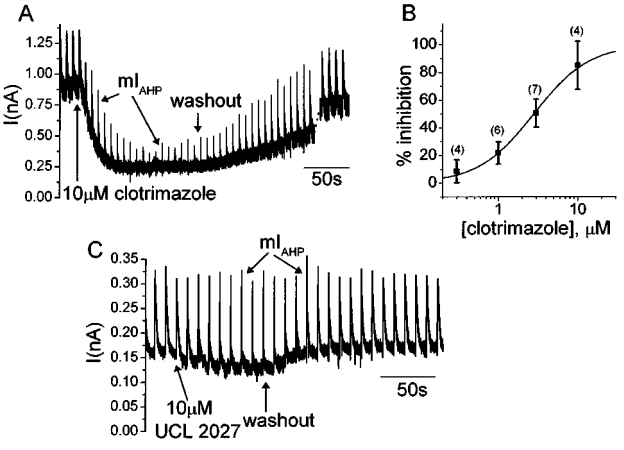

Figure 5 Effects on the outward holding current (at a potential of −50 mV) of (A) 10 μM clotrimazole and (C) 10 μM UCL 2027. In both traces the action potentials have been removed to make it easier to see the effects of the compounds on the outward holding current, the sI_{AHP} and the mI_{AHP}. The axis break shown in (A) represents 150 s. (B) The concentration-inhibition curve for clotrimazole on the outward holding current. The curve was obtained by fitting the points to the Hill equation with y_{max} constrained to 100%. The number of observations for each point are shown in brackets.

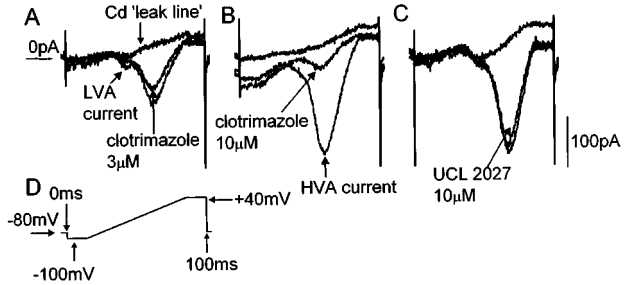

Figure 6 Effects of (A) 3 μM clotrimazole, (B) 10 μM clotrimazole and (C) 10 μM UCL 2027 on the Ca^{2+} current recorded from freshly dissociated hippocampal neurones. The voltage protocol shown in (D) was applied to evoke both the low voltage-activated (LVA) and the high voltage-activated (HVA) Ca^{2+} current. In each case, 200 μM Cd^{2+} was also applied to determine the magnitude of the HVA Ca^{2+} current. The calibration bar shown in (C) also applies to (A) and (B).

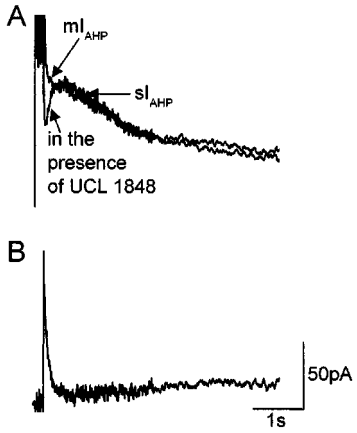

Figure 7 Effects of 100 nM UCL 1848 on the mI_{AHP} and the sI_{AHP}. (A) Traces of the I_{AHP} before and after application of UCL 1848 superimposed. (B) The current sensitive to UCL 1848 (current in the absence of the drug subtracted from the current in its presence).

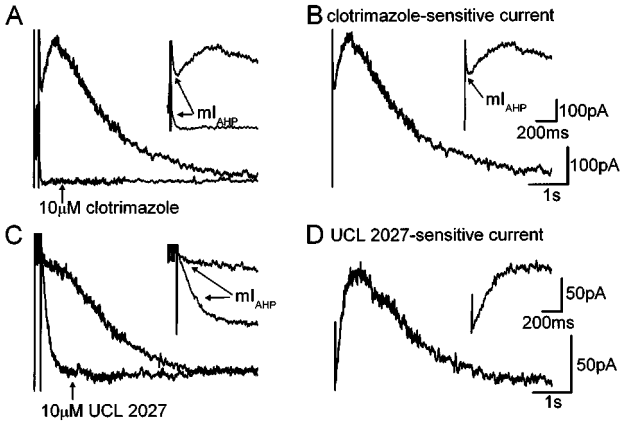

Figure 8 Effects of (A) 10 μM clotrimazole and (C) 10 μM UCL 2027 on the mI_{AHP} and sI_{AHP}. The traces in the presence and absence of the compounds have been superimposed. Clotrimazole and UCL 2027 sensitive currents were obtained by subtraction and are shown in (B) and (D). The insets in (A)–(D) are on an expanded time base. The calibration bars in (B) and (D) apply also to (A) and (C) respectively.

difference plot is constructed (Figure 7B). Since UCL 1848 is a selective inhibitor of apamin-sensitive conductances (Benton *et al*., 1999), these effects are consistent with the previously reported lack of sensitivity to apamin of the sI_{AHP} (Lancaster & Nicoll, 1987; Shah & Haylett, 2000b).

The effects on the mI_{AHP} of clotrimazole, UCL 1880 and UCL 2027 were also studied in cells exhibiting this current. 3 μM clotrimazole had no detectable effect on the mI_{AHP} ($2.1 \pm 4.9\%$ inhibition, $n=3$) whereas at 10 μM, the mI_{AHP} was clearly reduced (Figure 8A). This effect of clotrimazole can be most easily seen when the current underlying the AHP (I_{AHP}) in the presence of clotrimazole is subtracted from the current in its absence (see Figure 8B). The proportion of mI_{AHP} that was reduced by 10 μM clotrimazole was estimated to be $56.8 \pm 3.9\%$ ($n=4$) by fitting to Equation 1. In contrast, UCL 1880 and UCL 2027 at a concentration of 10 μM had little effect on the mI_{AHP} ($-5.1 \pm 1.9\%$ inhibition ($n=3$) and $-11.6 \pm 12\%$ inhibition ($n=3$; Figure 8C) respectively). In support of this data, when the I_{AHP} in the presence of UCL 1880 or UCL 2027 is subtracted from the I_{AHP} present under control conditions, no currents resembling the mI_{AHP} are observed (Figure 8D). These results suggest that at concentrations greater than 3 μM, clotrimazole may inhibit the apamin-sensitive mAHP present in these cells. UCL 1880 and UCL 2027, on the other hand, appear to be selective for the sI_{AHP}.

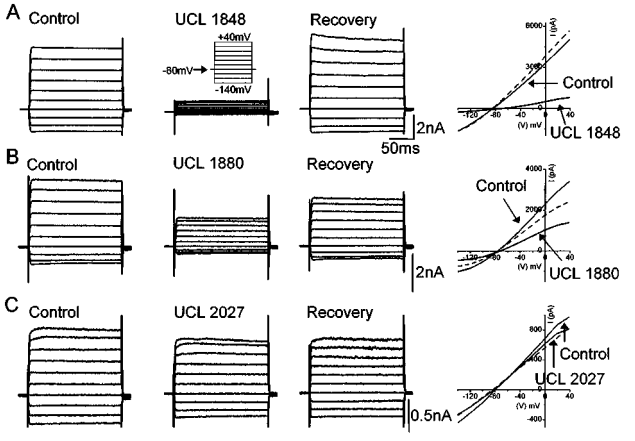

Figure 9 Effects of (A) 10 nM UCL 1848, (B) 10 μM UCL 1880 and (C) 10 μM UCL 2027 on current produced by application of the step protocol shown in (A) to HEK293 cells transfected with SK1 cDNA. The recordings were made under whole cell conditions using patch pipettes containing 1 μM free Ca^{2+}. In each case, the current-voltage curves are also plotted (the dashed lines indicate the maximum recovery after washout). The time calibration shown in (A) also applies to (B) and (C).

Effect of UCL 1880 and UCL 2027 on SK channels

It has been suggested that SK1 channels may underlie the sI_{AHP} (Bond *et al*., 1999). As the rat clone, rSK1, does not form functional channels (Shah, unpublished observations; Hirschberg *et al*., 1999), the human clone, hSK1, was expressed in HEK293 cells. As UCL 1880 and UCL 2027 inhibit the sI_{AHP}, these compounds were tested on the hSK1 current. HEK293 cells transfected with hSK1 cDNA displayed large standing currents when whole cell recordings were made using patch pipettes containing 1 μM free Ca^{2+} and when a step protocol from -120 mV to $+40$ mV was applied (see Figure 9). The hSK1 current was inhibited by 10 nM UCL 1848 (see Figure 9A) as described previously (Shah & Haylett, 2000a). Application of 10 μM UCL 1880 reduced the hSK1 current by $44.2 \pm 3.0\%$ ($n=3$; Figure 9B). However, with UCL 2027, applied at 10 μM, there was little inhibition ($1.6 \pm 5.6\%$, $n=5$; Figure 9C).

UCL 2027 was also tested on HEK293 cells stably expressing rSK2 channels, which have been suggested to underlie the apamin-sensitive part of the mI_{AHP} (Stocker *et al*., 1999). HEK293 cells expressing rSK2 displayed similar currents to those expressing hSK1 (Figure 9) when patched onto with 1 μM free Ca^{2+} and a step protocol from -120 mV to $+40$ mV applied. 10 μM UCL 2027 had only a small effect on the SK2 current (per cent inhibition $= 13.3 \pm 6.5\%$, $n=5$; $P=0.1$). The mI_{AHP} in these

hippocampal neurones is abolished by UCL 1848 and not surprisingly, 1 nM UCL 1848 also caused a large inhibition of the rSK2 current by 73.5±6.2% ($n=3$).

Discussion

In seeking to identify compounds that may block the sI_{AHP}, we selected for investigation clotrimazole and some simple analogues. Clotrimazole proved to be an effective blocker, inhibiting the sI_{AHP} with an IC_{50} of 1.7 μM. The structurally related compounds CBM (Figure 1B), UCL 1880 (Figure 1C) and UCL 2027 (Figure 1D) were also effective with similar IC_{50}s. The imidazole ring in the clotrimazole molecule is not essential for the suppression of the sI_{AHP} as CBM, an *in vivo* metabolite of clotrimazole lacking this ring, was also an effective inhibitor. The activities of UCL 1880 and UCL 2027 provide further evidence that the imidazole ring is not crucial for activity. These results also indicate that the cytochrome P450 inhibitory action of clotrimazole is not involved in the reduction of the sI_{AHP} as this effect requires the presence of the imidazole ring (Rittenhouse *et al*., 1997b). UCL 2027's activity indicates additionally that a chlorine atom in the triphenyl group is not essential.

The value of any pharmacological agent is enhanced if it demonstrates selectivity for a particular target. Clotrimazole is particularly broad in its actions, not only inhibiting IK channels (see introduction) but also BK channels (Rittenhouse *et al*., 1997a; Wu *et al*., 1999), voltage-gated K^+ channels (Hatton & Peers, 1996; Kim & Greger, 1999; Wu *et al*., 1999) and Ca^{2+} channels (Thomas *et al*., 1999; Fearon *et al*., 2000). In this study clotrimazole, not altogether surprisingly, was found to dose-dependently reduce the outward holding current present at a potential of −50 mV (Figure 5A,B), suggesting that it inhibits one or more of the conductances involved in maintaining the resting membrane potential of these neurones. This effect was only weakly shared by the other compounds studied.

It is necessary to consider whether the inhibition of the sI_{AHP} is attributable to block of the underlying K^+ current or to a reduction in the rise in $[Ca^{2+}]_i$ as a consequence of Ca^{2+} channel inhibition. Clotrimazole inhibited the sI_{AHP} with an IC_{50} of 1.7 μM whereas the IC_{50} for block of the HVA Ca^{2+} current was greater, at 4.7 μM. This suggests that Ca^{2+} channel inhibition is unlikely to be the sole mechanism for inhibition of the sI_{AHP} and since direct channel block by clotrimazole has been demonstrated for IK and BK channels, inhibition of K^+ channels seems likely. Compared with clotrimazole, CBM had similar potency as a blocker of the HVA Ca^{2+} channels. The HVA Ca^{2+} current in hippocampal pyramidal neurones has multiple components, with L-, N-, P-, Q- and R-type Ca^{2+} channels contributing to the current (Christie *et al*., 1996). The near complete block of the HVA Ca^{2+} current with clotrimazole and CBM thus shows that these agents block all the channels involved. The large Hill coefficients suggest that the affinities of these agents for the channels are not widely dispersed.

UCL 1880 and in particular UCL 2027 were less potent blockers of the HVA Ca^{2+} current. With UCL 2027 it was possible to obtain a complete block of the sI_{AHP} in the absence of any effect on the Ca^{2+} current, suggesting its effects are independent of a reduction of Ca^{2+} entry.

Since Ca^{2+} entry through HVA Ca^{2+} channels will depend on the time course of the action potential, and in particular its duration, the effects of the agents on this parameter were also determined. At the maximal concentration tested, all the compounds broadened the action potentials. This effect was also observed at the lower concentration of 3 μM for both CBM and UCL 2027. An increase in action potential width is not surprising for clotrimazole which has been shown to block BK channels (Rittenhouse *et al*., 1997a; Wu *et al*., 1999) and voltage-gated K^+ channels (Hatton & Peers, 1996; Kim & Greger, 1999; Wu *et al*., 1999;). A complicating factor is that any Ca^{2+} channel block by the compounds would lead to a reduction in Ca^{2+} entry and reduced activation of BK channels. Nevertheless action potential broadening, by whatever mechanism, would tend to increase Ca^{2+} entry and so potentially enhance the sI_{AHP}, as is indeed seen with charybdotoxin (Shah & Haylett, 2000b). The reduction in the sI_{AHP} produced by, for example, UCL 2027, even though the action potentials were somewhat broader, shows that it is a potent blocker of the current *per se*.

Since many of the cells studied also exhibited a mI_{AHP}, it was possible to examine the effects of the compounds on this current as well. In these cells the current is nearly all apamin-sensitive (Shah & Haylett, 2000b) and in keeping with this, UCL 1848 also inhibited a large component of the current. *In situ* hybridization studies have shown that the mRNA for both SK1 and SK2 is detectable in hippocampal pyramidal cells (Stocker *et al*., 1999) and since the IC_{50} for inhibition of the mI_{AHP} by apamin is estimated to be 0.5 nM, a value more in keeping with SK2 than SK1, it is proposed that the underlying channel is a homotetramer of SK2 (though a heteromultimer with SK1 is not excluded; Stocker *et al*., 1999). UCL 1848 blocks not only homomeric SK1 channels (Shah & Haylett, 2000a) but also expressed SK2 channels (the present study). These findings are consistent with the involvement of either homomeric SK1 or SK2 channels or a heteromer of the two in the mI_{AHP}.

Clotrimazole also inhibited the mI_{AHP} (Figure 5 and 8) but in view of its action on the HVA Ca^{2+} current it is not possible to attribute this with any certainty to either SK or Ca^{2+} channel block. At 10 μM, UCL 1880 had little effect on the mI_{AHP}. At this concentration it blocks a large fraction of the HVA Ca^{2+} current so it would appear that SK channel activation is not linearly related to Ca^{2+} entry though HVA channels. It is interesting in this context, to note that it has been suggested that SK channels are specifically activated by Ca^{2+} entry through L-type (HVA) channels with which they co-localize (Marrion & Tavalin, 1998). As yet, it is not known which HVA Ca^{2+} channels are important for the generation of the mI_{AHP} in hippocampal pyramidal neurones. It is possible that UCL 1880 may preferentially inhibit one type of HVA Ca^{2+} channel and thereby allow sufficient Ca^{2+} entry through others for the generation of the mI_{AHP}. The lack of effect on the mI_{AHP} also indicates that UCL 1880 does not block the SK channels underlying this current. This would seem to exclude a role for SK1 channels which were blocked by UCL 1880.

UCL 2027 had only small effects on either the mI_{AHP} or the homomeric SK1 and SK2 channels, while selectively blocking the sI_{AHP}. The lack of effect of UCL 2027 on both the mIAHP and SK channels does of course allow for the

possibility that either channel could contribute to the mIAHP (Stocker *et al*., 1999). If SK subunits are involved in the sI_{AHP}, then clearly the assembly of the subunits into the functional channel must be quite different to their normal incorporation into apamin-sensitive channels, since the channel pharmacology, kinetics and modulation by agonists are so strikingly distinct.

It should, however, be emphasized that the present study used the human SK1 channels whereas the pyramidal cells were from rat. This raises the unlikely possibility that a species difference confounds the interpretation of the results and a that a study with the rat clone might provide evidence for the role of SK1 channels in either the mI_{AHP} or the sI_{AHP} of rat hippocampal pyramidal neurones.

Although the present work has identified UCL 2027 as the first selective blocker of the sI_{AHP}, much remains to be done. In particular, the directness or otherwise of its action has still to be established. This is likely to be difficult for several reasons. Though attempts have been made to identify the native channels that underlie the sAHP in hippocampal neurons (see e.g., Bekkers, 2000), unambiguous single channel recordings have yet to be reported. Also, the molecular composition of the channel is unknown and indeed the present findings (see also Shah & Haylett, 2000a, Strøbæk *et al*., 2000) add to the evidence against the only previous suggestion on the point, namely, that SK1 channels make a major contribution. Finally, the sAHP is subject to modulation by a number of kinases and phosphatases (Pedarzani *et al*., 1998; Krause & Pedarzani, 2000) and it is possible that UCL 2027 could block by activation of an inhibitory mechanism of this kind. Although the structural similarity of UCL 2027 to clotrimazole, a proven K^+ channel blocker, makes it tempting to suppose that the former compound also acts directly on the channels, we have no direct evidence on this crucial point which remains for further study.

In conclusion, the present study has extended the pharmacology of the sI_{AHP} and identified the first selective blocker of the current, UCL 2027. This compound, and more active and selective congeners, could prove useful as tools for establishing the physiological role of the sI_{AHP} as well as in the identification of its molecular correlate.

We are grateful to Professor D.H. Jenkinson and Dr G.W.J. Moss for their helpful discussions. This work was funded by the Wellcome Trust. M. Shah is an MRC scholar.

References

ALVAREZ, J., MONTERO, M. & GARCIA-SANCHO, J. (1992). High-affinity inhibition of Ca^{2+}-dependent K^+ channels by cytochrome-P-450 inhibitors. *J. Biol. Chem.,* **267,** 11789–11793.

BEKKERS, J.M. (2000). Distribution of slow AHP channels on hippocampal CA1 pyramidal neurons. *J. Neurophysiol.,* **83,** 1756–1759.

BENTON, D.C.H., DUNN, P.M., CHEN, J.Q., GALANAKIS, D., GANELLIN, C.R., MALIK-HALL, M., SHAH, M., HAYLETT, D.G. & JENKINSON, D.H. (1999). UCL 1848: A novel bis-quinolinium cyclophane which blocks apamin-sensitive K^+ channels with nanomolar affinity. *Brit. J. Pharmacol.,* **128,** 39P.

BOND, C.T., MAYLIE, J. & ADELMAN, J.P. (1999). Small-conductance calcium-activated potassium channels. *Ann. N.Y. Acad. Sci.,* **868,** 370–378.

BRUGNARA, C., DEFRANCESCHI, L. & ALPER, S.L. (1993). Inhibition of Ca^{2+}-dependent K^+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J. Clin. Invest.,* **92,** 520–526.

CHRISTIE, B.R., MAGEE, J.C. & JOHNSTON, D. (1996). Dendritic calcium channels and hippocampal long-term depression. *Hippocampus,* **6,** 17–23.

DEAK, F., NAGY, G., VARNAI, P., MADARASZ, E. & SPAT, A. (1998). Calcium current activated by potassium ions in voltage-clamped rat hippocampal pyramidal neurones. *J. Physiol.,* **508,** 735–745.

DISTERHOFT, J.F., THOMPSON, L.T., MOYER, J.R. & MOGUL, D.J. (1996). Calcium-dependent afterhyperpolarization and learning in young and aging hippocampus. *Life Sci.,* **59,** 413–420.

DUNN, P.M. (1998). The action of blocking agents applied to the inner face of Ca^{2+}-activated K^+ channels from human erythrocytes. *J. Mem. Biol.,* **165,** 133–143.

FEARON, I.M., BALL, S.G. & PEERS, C. (2000). Clotrimazole inhibits the recombinant human cardiac L-type Ca^{2+} channel alpha(1C) subunit. *Brit. J. Pharmacol.,* **129,** 547–554.

GIESE, K.P., STORM, J.F., REUTER, D., FEDOROV, N.B., SHAO, L.R., LEICHER, T., PONGS, O. & SILVA, A.J. (1998). Reduced K^+ channel inactivation, spike broadening, and after-hyperpolarization in Kv beta 1.1-deficient mice with impaired learning. *Learning Memory,* **5,** 257–273.

HATTON, C.J. & PEERS, C. (1996). Effects of cytochrome P-450 inhibitors on ionic currents in isolated rat type I carotid body cells. *Am. J. Physiol.,* **40,** C85–C92.

HIRSCHBERG, B., MAYLIE, J., ADELMAN, J.P. & MARRION, N.V. (1999). Gating properties of single SK channels in hippocampal CA1 pyramidal neurons. *Biophys. J.,* **77,** 1905–1913.

ISHII, T.M., MAYLIE, J. & ADELMAN, J.P. (1997a). Determinants of apamin and d-tubocurarine block in SK potassium channels. *J. Biol. Chem.,* **272,** 23195–23200.

ISHII, T.M., SILVIA, C., HIRSCHBERG, B., BOND, C.T., ADELMAN, J.P. & MAYLIE, J. (1997b). A human intermediate conductance calcium-activated potassium channel. *Proc. Nat. Acad. Sci. U.S.A.,* **94,** 11651–11656.

JENSEN, B.S., ODUM, N., JORGENSEN, N.K., CHRISTOPHERSEN, P. & OLESEN, S.P. (1999). Inhibition of T cell proliferation by selective block of Ca^{2+}-activated K^+ channels. *Proc. Nat. Acad. Sci. U.S.A.,* **96,** 10917–10921.

JENSEN, B.S., STRØBAEK, D., CHRISTOPHERSEN, P., JORGENSEN, T.D., HANSEN, C., SILAHTAROGLU, A., OLESEN, S.P. & AHRING, P.K. (1998). Characterization of the cloned human intermediate-conductance Ca^{2+}-activated K^+ channel. *Am. J. Physiol. Cell Physiol.,* **44,** C848–C856.

JOINER, W.J., WANG, L.Y., TANG, M.D. & KACZMAREK, L.K. (1997). hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc. Nat. Acad. Sci. U.S.A.,* **94,** 11013–11018.

JURMAN, E., BOLAND, L.M., LIU, Y. & YELLEN, G. (1994). Visual identification of individual transfected cells for electrophysiology using antibody-coated beads. *Biotechniques,* **17,** 876–881.

KIM, S.J. & GREGER, R. (1999). Voltage-dependent, slowly activating K^+ current (I-Ks) and its augmentation by carbachol in rat pancreatic acini. *Pflüg. Arch. Eur. J. Physiol.,* **438,** 604–611.

KOHLER, M., HIRSCHBERG, B., BOND, C.T., KINZIE, J.M., MARRION, N.V., MAYLIE, J. & ADELMAN, J.P. (1996). Small-conductance, calcium-activated potassium channels from mammalian brain. *Science,* **273,** 1709–1714.

KRAUSE, M. & PEDARZANI, P. (2000). A protein phosphatase is involved in the cholinergic suppression of the Ca^{2+}-activated K^+ current sI(AHP) in hippocampal pyramidal neurons. *Neuropharmacology,* **39,** 1274–1283.

LANCASTER, B. & ADAMS, P.R. (1986). Calcium-dependent current generating the afterhyperpolarization of hippocampal-neurons. *J. Neurophysiol.,* **55,** 1268–1282.

LANCASTER, B. & NICOLL, R.A. (1987). Properties of two calcium-activated hyperpolarisations in rat hippocampal-neurons. *J. Physiol.,* **389,** 187 – 203.

LOGSDON, N.J., KANG, J.S., TOGO, J.A., CHRISTIAN, E.P. & AIYAR, J. (1997). A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. *J. Biol. Chem.,* **272,** 32723 – 32726.

MARRION, N.V. & TAVALIN, S.J. (1998). Selective activation of Ca^{2+}-activated K^+ channels by co-localized Ca^{2+} channels in hippocampal neurons. *Nature,* **395,** 900 – 905.

MOYER, J.R., THOMPSON, L.T., BLACK, J.P. & DISTERHOFT, J.F. (1992). Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age-dependent and concentration-dependent manner. *J. Neurophysiol.,* **68,** 2100 – 2109.

PEDARZANI, P., KRAUSE, M., HAUG, T., STORM, J.F. & STUHMER, W. (1998). Modulation of the Ca^{2+}-activated K^+ current sI(AHP) by a phosphatase-kinase balance under basal conditions in rat CA1 pyramidal neurons. *J. Neurophysiol.,* **79,** 3252 – 3256.

RITTENHOUSE, A.R., PARKER, C., BRUGNARA, C., MORGAN, K.G. & ALPER, S.L. (1997a). Inhibition of maxi-K currents in ferret portal vein smooth muscle cells by the antifungal clotrimazole. *Am. J. Physiol.,* **273,** C45 – C56.

RITTENHOUSE, A.R., VANDORPE, D.H., BRUGNARA, C. & ALPER, S.L. (1997b). The antifungal imidazole clotrimazole and its major in vivo metabolite are potent blockers of the calcium-activated potassium channel in murine erythroleukemia cells. *J. Mem. Biol.,* **157,** 177 – 191.

SAH, P. (1996). Ca^{2+}-activated K^+ currents in neurones: Types, physiological roles and modulation. *T. I. N. S.,* **19,** 150 – 154.

SAH, P. & ISAACSON, J.S. (1995). Channels underlying the slow afterhyperpolarization in hippocampal pyramidal neurons: neurotransmitters modulate the open probability. *Neuron.,* **15,** 435 – 441.

SHAH, M. & HAYLETT, D.G. (2000a). The pharmacology of hSK1 Ca^{2+}-activated K^+ channels expressed in mammalian cell lines. *Brit. J. Pharmacol.,* **129,** 627 – 630.

SHAH, M. & HAYLETT, D.G. (2000b). Ca^{2+} channels involved in the generation of the slow afterhyperpolarization in cultured rat hippocampal pyramidal neurons. *J. Neurophysiol.,* **83,** 2554 – 2561

STOCKER, M., KRAUSE, M. & PEDARZANI, P. (1999). An apamin-sensitive Ca^{2+}-activated K^+ current in hippocampal pyramidal neurons. *Proc. Nat. Acad. Sci. U.S.A.,* **96,** 4662 – 4667.

STORM, J.F. (1987). Action-potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. *J. Physiol.,* **385,** 733 – 759.

STORM, J.F. (1990). Potassium currents in hippocampal pyramidal cells. *Prog. Brain Res.,* **83,** 161 – 187.

STRØBAEK, D., JORGENSEN, T.D., CHRISTOPHERSEN, P., AHRING, P.K. & OLESEN, S.P. (2000). Pharmacological characterization of small-conductance Ca^{2+}-activated K^+ channels stably expressed in HEK 293 cells. *Brit. J. Pharmacol.,* **129,** 991 – 999.

THOMAS, G.P., KARMAZYN, M., ZYGMUNT, A.C., ANTZELEVITCH, C. & NARAYANAN, N. (1999). The antifungal antibiotic clotrimazole potently inhibits L-type calcium current in guinea-pig ventricular myocytes. *Brit. J. Pharmacol.,* **126,** 1531 – 1533.

VALIANTE, T.A., ABDUL-GHANI, M.A., CARLEN, P.L. & PENNEFATHER, P. (1997). Analysis of current fluctuations during after-hyperpolarization current in dentate granule neurones of the rat hippocampus. *J. Physiol.,* **499,** 121 – 134.

VERGARA, C., LATORRE, R., MARRION, N.V. & ADELMAN, J.P. (1998). Calcium-activated potassium channels. *Curr. Opin. Neurobiol.,* **8,** 321 – 329.

WEISS, C., PRESTON, A.R., OH, M.M., SCHWARZ, R.D., WELTY, D. & DISTERHOFT, J.F. (2000). The M1 muscarinic agonist CI-1017 facilitates trace eyeblink conditioning in aging rabbits and increases the excitability of CA1 pyramidal neurons. *J. Neurosci.,* **20,** 783 – 790.

WU, S.N., LI, H.F., JAN, C.R. & SHEN, A.Y. (1999). Inhibition of Ca^{2+}-activated K^+ current by clotrimazole in rat anterior pituitary GH(3) cells. *Neuropharmacology,* **38,** 979 – 989.

XIA, X.M., FAKLER, B., RIVARD, A., WAYMAN, G., JOHNSON-PAIS, T., KEEN, J.E., ISHII, T., HIRSCHBERG, B., BOND, C.T., LUTSENKO, S., MAYLIE, J. & ADELMAN, J.P. (1998). Mechanism of calcium gating in small-conductance calcium-activated potassium channels. *Nature,* **395,** 503 – 507.

YOSHIDA, A., ODA, M. & IKEMOTO, Y. (1991). Kinetics of the Ca^{2+}-activated K^+ channel in rat hippocampal neurons. *Jap. J. Physiol.,* **41,** 297 – 315.

(Received September 5, 2000
Revised November 20, 2000
Accepted December 8, 2000)

.