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Blockade of delayed rectifier K^+ currents in neuroblastoma × glioma hybrid (NG 108-15) cells by clofilium, a class III antidysrhythmic agent

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1 The whole-cell patch-clamp technique was used to examine the effects of the class III antidysrhythmic agent, clofilium, on voltage-activated delayed rectifier K^+ currents (IK_v) in undifferentiated mouse neuroblastoma × rat glioma hybrid (NG 108-15) cells. Ca^{2+}-activated K^+ currents also seen in these cells were abolished by bath application of 4 mM Co^{2+}.

2 Bath application of clofilium (0.3 to 70 μM) caused dose-dependent, irreversible inhibition of IK_v in these cells. Under control conditions, activated currents were sustained during 200 ms depolarizing steps, but in the presence of clofilium, or after its wash-out, currents were reduced in amplitude and showed a time-dependent decay.

3 Clofilium blockade of IK_v was voltage-dependent: the degree of current inhibition increased with increasing depolarizations. The transient nature of IK_v seen in the presence of clofilium was also more apparent at higher test potentials.

4 The effects of clofilium were use-dependent: when cells were left unstimulated during drug application, and then depolarizations were resumed, several pulses were required for clofilium blockade to reach a steady level. Similar results were obtained post-clofilium, when cells were unstimulated during application and then removal of clofilium, suggesting that although the blocking action of the drug was use-dependent, it bound to the closed, delayed rectifier K^+ channel.

5 High concentrations (100 or 300 μM) of sotalol, another class III antidysrhythmic agent, were without discernible effects on IK_v in NG 108-15 cells.

6 The effects of clofilium on a neuronal IK_v described here, and its possible mechanism of action, are compared with previously reported effects of clofilium on the cardiac IK_v.

Keywords: K^+ current; delayed rectifier; sotalol; clofilium; class III antidysrhythmic; neuroblastoma × glioma (NG 108–15) cells

Introduction

The activity of K^+ channels is fundamental to the control of membrane potential in both excitable and non-excitable cells. In the heart, many K^+ channel types exist, and their complicated interactions contribute to shaping the cardiac action potential waveform (see Noble, 1984, for review). Their importance in such events has been exploited in the search for agents which can correct dysrhythmias. Class III antidysrhythmic agents exert their stabilizing effects by prolonging the cardiac action potential and hence the effective refractory period (they are particularly useful in severe dysrhythmia e.g. ventricular tachycardia and fibrillation; Osterrider & Waterfall, 1990). These compounds are structurally diverse, and the mechanisms by which they prolong the cardiac action potential are complex and not fully understood. However, one commonly found feature is that they inhibit the voltage-gated delayed rectifier K^+ current (IK_v) which contributes to the repolarization of cardiac myocytes following each action potential (Cook & Quast, 1990).

IK_v is also present in neuronal tissue, where it contributes to the termination of the neuronal action potential. Although IK_v activates at a much greater rate in neuronal tissue compared with cardiac tissue, the currents from the two tissue types show several other pharmacological and kinetic similarities (Adams *et al.*, 1980; Noble, 1984; Halliwell, 1990). The original aim of this study was to examine the effects of the two class III antidysrhythmic agents clofilium and sotalol, which have both been demonstrated to inhibit cardiac IK_v (Carmeliet, 1985; Arena & Kass, 1988), on a neuronal IK_v, in the undifferentiated neuroblastoma × glioma hybrid cell line NG 108-15. While sotalol was without effect on neuronal IK_v, clofilium was seen to have a potent inhibitory effect. Further experiments indicated that its mechanism of blockade is quite different from that reported on cardiac IK_v. A preliminary account of some of these findings has appeared in abstract form (Reeve & Peers, 1991).

Methods

Mouse neuroblastoma × rat glioma hybrid (NG 108-15) cells (Hamprecht, 1977) were continuously grown in culture flasks containing Dulbecco's modified Eagle's medium (DMEM) with 10% foetal calf serum, 1 × HAT (0.1 mM hypoxanthine, 0.4 μM aminopterin and 160 μM thymidine), penicillin (100 iu ml^{-1}), streptomycin (100 μg ml^{-1}) and L-glutamine (2 mM). They were maintained in a humidified incubator at 37°C (10% CO_2). No chemicals were added to promote or induce cell differentiation. When required for electrophysiological study, cells were removed from their culture flasks with gentle mechanical agitation and plated onto polylysine-coated coverslips in 35 mm culture dishes. They were left to adhere for between 3 and 5 days, in the same culture medium and incubating conditions.

On each experimental day, fragments of coverslip with attached cells were transferred to a continually perfused (1 ml min^{-1}) low-volume (approximately 80 μl) recording chamber. The perfusate was composed of (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 10, glucose 10 (pH 7.4, 21–24°C). Whole-cell patch-clamp recordings (Hamill *et al.*, 1981) were made from cells by use of patch electrodes filled with a solution of composition (in mM): KCl 117, EGTA 11, HEPES 11, $CaCl_2$ 1, $MgSO_4$ 2, NaCl 10, ATP 2 (pH 7.2).

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When filled with this solution, electrodes had resistances of between 3 and 10 MΩ. Cells were voltage-clamped at −70 mV, and 200 ms step changes in the membrane potential were applied at a frequency of 0.2 Hz. Capacitive transients were minimized by analogue means. Data were stored on computer, and current amplitude measurements were subsequently obtained using VCAN software (J. Dempster, Strathclyde University) following leak subtraction, which was performed by the appropriate scaling and subtraction of the mean current amplitude evoked by small depolarizing and hyperpolarizing steps. Currents recorded under control conditions were sustained during 200 ms step depolarizations (i.e. showed no time-dependent inactivation), and so their amplitudes were measured by averaging the last 20 to 30 ms of the evoked current. In the presence of clofilium, currents showed a time-dependent decay (see Results), and so were measured at their peak (I_{peak}) and also over the final 20 to 30 ms of the step depolarization, as for controls (I_{end}). Current-voltage (I–V) relationships were constructed and all analysis of current amplitudes was performed after leak subtraction, but as the correction changed values of current amplitude by only a maximum of approximately 5%, and was rarely completely successful in removing capacitive transients, the example current traces shown are unsubtracted. Statistical comparisons were made by the paired, two-tailed Student's t test, unless otherwise indicated.

Results

Isolation of the delayed rectifier K^+ current in NG 108–15 cells

Figure 1a shows current-voltage (I–V) relationships obtained from an undifferentiated NG 108-15 cell. Under control conditions K^+ currents activated at between −30 mV and −10 mV, and increased with increasing test potential. In chemically-differentiated NG 108-15 cells these currents are known to be composed of both Ca^{2+}-dependent K^+ currents (IK_{Ca}) and Ca^{2+}-independent, delayed rectifier K^+ currents (IK_v; Brown & Higashida, 1988). The Ca^{2+}-dependent component relies upon Ca^{2+} influx via voltage-dependent Ca^{2+} channels for activation, and so can be indirectly inhibited with Ca^{2+} channel blockers (Brown & Higashida, 1988). In undifferentiated NG 108-15 cells used here, we found that the Ca^{2+} channel blocker, Co^{2+}, was effective in inhibiting IK_{Ca} (e.g. Figure 1a). For example, at a test potential of +20 mV, 4 mM Co^{2+} reduced K^+ currents by 39.8 ± 3.2% ($n = 6$, $P < 0.005$) and at +60 mV by 31.9 ± 4.3% ($P < 0.02$). In the presence of 4 mM Co^{2+}, a brief delay in the activation of the residual K^+ current was apparent (e.g. Figure 1b), characteristic of IK_v. A concentration of 4 mM Co^{2+} was maximal in inhibiting IK_{Ca}, because application of 0.2 mM Cd^{2+} in the continued presence of 4 mM Co^{2+} did not produce further inhibition ($n = 3$; e.g. Figure 1a). Cd^{2+} alone could not be used to isolate IK_v as it is toxic to cells after several minutes exposure. Further increases in the Co^{2+} concentration only produced a parallel shift of the I–V curve to the right (not shown), indicating the membrane surface charge screening effect of high doses of divalent cations (Hille, 1984). Indeed, currents recorded in the presence of Co^{2+} only began to activate at around −10 mV (see Figure 1a), indicating a possible screening effect of the cation at this dose, similar to that reported in pancreatic β cells (Smith *et al.*, 1990). We also found that the currents recorded in the presence of 4 mM Co^{2+} were inhibited in a concentration-dependent manner by bath application of tetraethylammonium (TEA, not shown), with an IC_{50} of approximately 1.5 mM. This further supports the idea that the Ca^{2+}-independent K^+ currents in these cells are delayed rectifier (IK_v) currents, as this IC_{50} value for TEA is similar to that reported on IK_v in other preparations (Adams *et al.*, 1980; Stanfield, 1983). All subsequent experiments were designed to study the actions of clofilium and sotalol on IK_v alone, and were therefore carried out in the presence of 4 mM Co^{2+}.

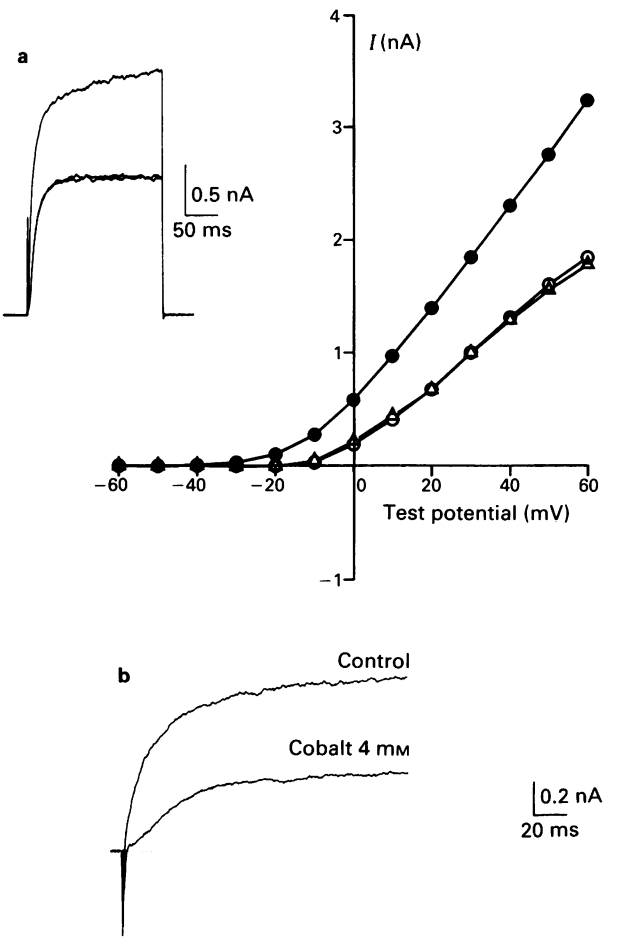

Figure 1 (a) Current-voltage (I–V) relationships all obtained from the same undifferentiated NG 108-15 cell under control conditions (●), in the presence of Co^{2+} 4 mM (○) and in the presence of Co^{2+} 4 mM and Cd^{2+} 0.2 mM (△). Inset shows examples of traces of K^+ currents recorded under these conditions, all at the test potential of +40 mV. (b) Superimposed currents, both obtained at a test potential of +10 mV, showing the effects of Co^{2+} 4 mM. Note the delay in activation of the current in the presence of Co^{2+}, characteristic of the delayed rectifier current.

Effects of clofilium on IK_v in NG 108-15 cells

The effects of bath application of 10 μM clofilium on IK_v recorded in a NG 108-15 cell are shown in Figure 2a. In the presence of the drug, currents were reduced in amplitude as compared with controls and became increasingly transient with increasing depolarizations, showing a time-dependent decay during 200 ms depolarizing test depolarizations. This contrasted with the currents recorded under control conditions, which were sustained through the 200 ms depolarizations (Figure 2a). However, the initial rising phase, or upstroke, of IK_v was unaffected by clofilium (e.g. Figure 2b). The current-voltage relationship from this cell is plotted in Figure 2c and shows that blockade of IK_v is voltage-dependent: the degree of blockade increased with increasing test potential. Also, the progressively more transient appearance of the currents seen at more positive test potential values is indicated by the differences in the current amplitudes measured at their peak and near the end of the test depolarization. This information is averaged for each concentration of clofilium tested (0.3 to 70 μM) in Figure 3, which plots the percentage reduction in current amplitudes as measured at their peak (I_{peak}; Figure 3a) and near the end of the step depolarization

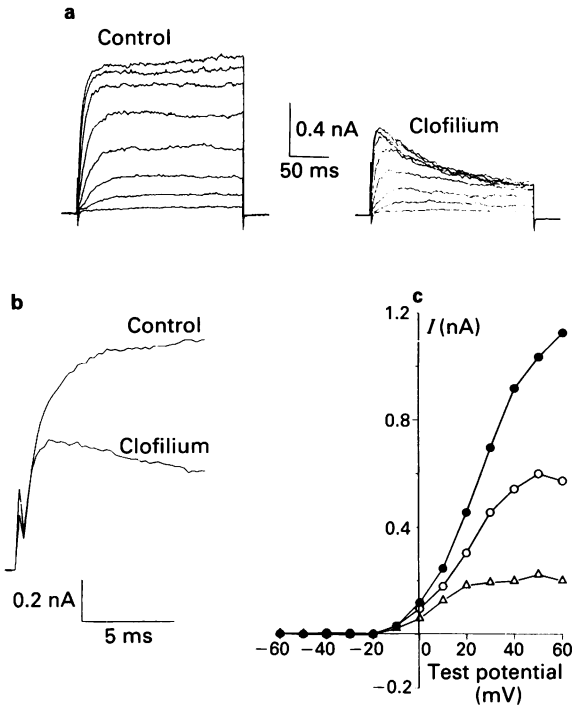

Figure 2 (a) Superimposed families of currents obtained from the same NG 108-15 cell under control conditions and in the presence of clofilium 10 μM. Currents were evoked by step changes in the membrane potential from −70 mV to between −10 mV and +60 mV, in 10 mV increments. (b) Superimposed currents shown on a fast timebase, indicating the lack of effect of clofilium on the initial part of the upstroke or activation of IK_v. Currents were obtained from the same cell as in (a), using a test potential of +60 mV. (c) *I–V* relationship obtained from the same cell as in (a) and (b), under control conditions (●), and in the presence of 10 μM clofilium, when currents were measured at their peak (○) and at the end of the test depolarization (△).

(I_{end}; Figure 3b) versus test potential. At all concentrations examined, clofilium reduced both peak and end current amplitude in a manner that increased with increasing test potential. At higher concentrations (10 μM or greater) the voltage-dependence of inhibition of I_{end} (Figure 3b) was steeper than that for I_{peak} (Figure 3a), indicating the increasingly transient nature of the currents with test potential. Due to the voltage-dependence of clofilium blockade of IK_v in NG 108-15 cells (Figure 3), and its effects on changing the time-course of activated currents, a dose-response curve has not been constructed. However, if the values of percentage current inhibition as measured at the end of depolarizations to +40 mV are considered, an IC_{50} value of approximately 4 μM is obtained. The effects of TEA, measured identically, yielded an IC_{50} value of approximately 1.5 mM.

Mechanism of action of clofilium on IK_v in NG 108-15 cells

Time series experiments (in which the cell is repeatedly depolarized from −70 mV to +40 mV, pulse duration 200 ms, 0.2 Hz) were performed to investigate the mechanism by which clofilium (at a concentration of 30 μM) causes blockade of IK_v in NG 108-15 cells. For comparison, we also investigated the effects of 10 mM TEA, which gave a similar, final degree of current inhibition. The rate of reduction in current amplitude caused by clofilium was markedly slower than that for TEA; in 6 cells tested, the reductions in current amplitude caused by 10 mM TEA were 50% complete after 10.9 ± 0.4 s (mean ± s.e.mean) following change of the bath solution for solution containing the drug. However, the effects of 30 μM clofilium were only half-maximal after 33.6 ± 2.1 s exposure (n = 5 cells), a significantly slower effect ($P < 0.0001$, unpaired

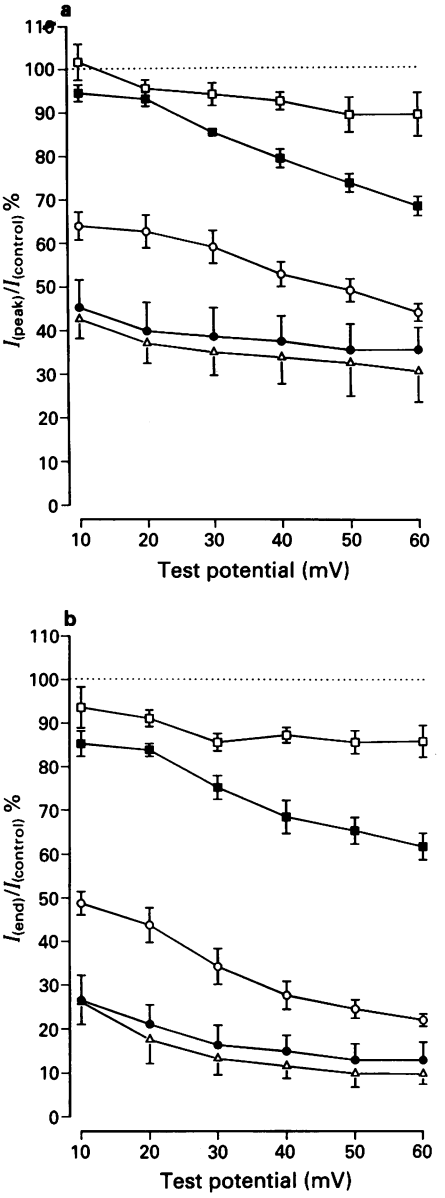

Figure 3 Plots of percentage reductions in IK_v caused by clofilium versus test potential. Currents were measured at their peak (I_{peak}; 3a) and at the end of the step depolarizations (I_{end}; 3b) in the presence of different doses of clofilium: 0.3 μM (□; n = 3 cells), 3 μM (■, n = 4), 10 μM (○, n = 5), 30 μM (●, n = 4) and 70 μM (△, n = 6). Points plotted are mean values, with vertical bars showing s.e.mean.

t test). This observation suggested that some factor(s) other than bath exchange time (which limits the rate of blockade by TEA) slowed the blockade of IK_v by clofilium.

One possible factor was that the action of clofilium was use-dependent i.e. blockade depended on repeated activation of IK_v in these cells. Typical examples of experiments designed to investigate this possibility are shown in Figure 4. As for experiments described above, time-series studies were used, where cells were repeatedly depolarized from −70 mV to +40 mV. Figure 4a illustrates the time course of blockade caused by 30 μM clofilium, when the cell was not depolarized during exchange of the bath solution for the solution containing the drug (a period of 45 s). It can be seen that, on resuming depolarizations, clofilium did not cause an immediate inhibition of the current. Indeed, several depolarizations were required for clofilium to reach a steady level of inhibition of IK_v in this cell. Similar results were obtained in a total of 6 cells in which this protocol was used. This effect was in marked contrast to blockade caused by 10 mM TEA. Figure 4b shows an experiment which followed the same protocol as

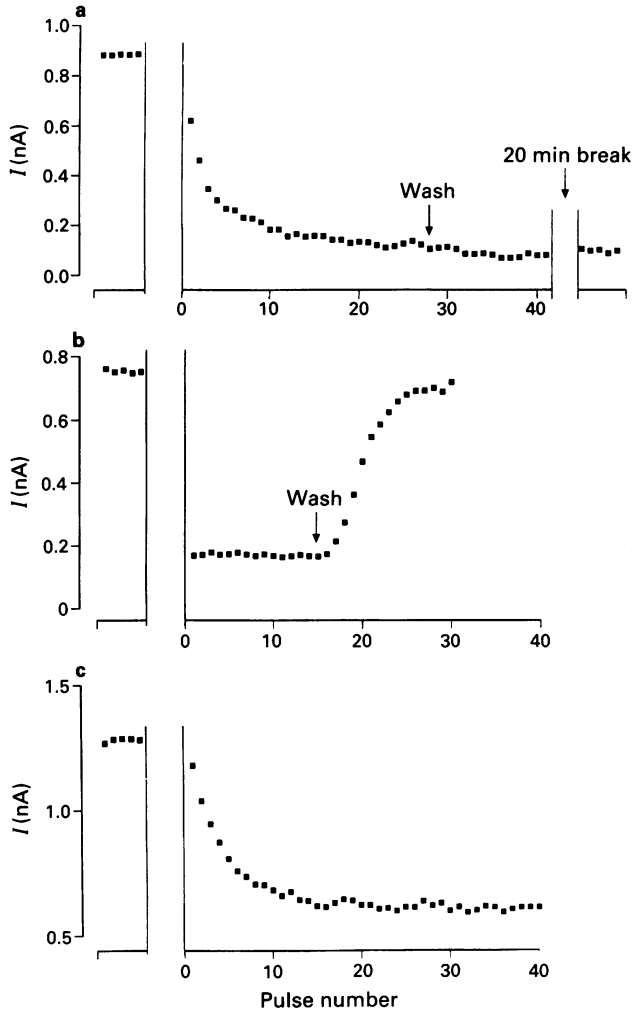

Figure 4 Time-series plots obtained from three different NG 108-15 cells by repeatedly stepping the membrane potential from −70 mV to +40 mV. (a) First break indicates a period of 45 s during which the cell was held at −70 mV without applying step depolarizations, and 30 μM clofilium was applied to the bath perfusate. Note the irreversibility of the drug, as indicated by the lack of recovery of current amplitudes following a 20 min break after the drug was washed out (depolarizations were continued during this period). (b) Break indicates 45 s period during which the cell was not depolarized (as in (a)), but 10 mM tetraethylammonium (TEA) was applied to the perfusate. Note the immediate reduction of current amplitude following resumption of depolarizations, and the full recovery of current amplitude following washout of TEA. (c) Break indicates 45 s period of bath application of 30 μM clofilium followed by 3 min period of washout of the drug, during which the cell was not depolarized.

in Figure 4a, but using TEA rather than clofilium as the test drug. On resuming cell depolarizations, the effects of TEA were immediate: the first current evoked in its presence was strongly inhibited as compared with controls, and subsequent depolarizations showed no further inhibition of IK_v. These effects of TEA were consistently observed in 5 cells. Furthermore the block induced by TEA but not clofilium, was reversible on wash-out of the drug.

Figure 4c shows an experiment (representative of 5 cells examined) in which a similar protocol to that in Figure 4a was used, except that during the period when the cell was not depolarized, clofilium (30 μM) was applied for 45 s and then washed off for 3 min. On resuming cell depolarization, it can be seen that currents were progressively reduced in amplitude and, as in Figure 4a, several pulses were required for clofilium to reach its steady level of inhibition of IK_v. As this progressive reduction in amplitude occurred in the absence of unbound clofilium in the perfusate, it appears that clofilium can bind to the closed channel, does not readily unbind, and repeated channel activation is required for blockade to become maximal i.e. although blockade is use-dependent, drug binding to the channel (or a closely associated site) is not.

Lack of effect of sotalol IK_v *in NG 108-15 cells*

The effects of another class III antidysrhythmic agent, sotalol, which has also been shown to block cardiac IK_v (Carmeliet, 1985) was tested on the neuronal IK_v in NG 108-15 cells. Bath application of sotalol at doses of 100 μM ($n = 13$ cells) or 300 μM ($n = 6$; data not shown) were without any discernible effect on IK_v throughout the range of activating test potentials studied, indicating that sotalol exhibits at least a degree of tissue-selectivity, as such concentrations cause profound electrophysiological changes in cardiac myocytes (Carmeliet, 1985).

Discussion

IK_v contributes to the repolarization of both cardiac and neuronal tissue following action potential generation. Although IK_v in the two tissue types show a number of differences (particularly in their rates of activation), they have a number of common features; most notably they have a similar pharmacological profile (e.g. Cook, 1988). The most common and important feature of the actions of class III antidysrhythmic agents is their ability to inhibit the cardiac IK_v, and the studies presented here were prompted by the lack of information concerning the tissue-selectivity of these compounds. The two examples chosen in this study, clofilium and sotalol, were shown to be profoundly different in their actions on neuronal IK_v, as recorded in NG 108-15 cells.

Sotalol, which has been shown to prolong the cardiac action potential in human and guinea-pig cardiac tissue (e.g. Campbell, 1987), has been demonstrated, by use of conventional voltage-clamp recordings, to cause dose-dependent, reversible inhibitions of guinea-pig cardiac IK_v which were half-maximal at a concentration of 10 μM (Carmeliet, 1985). In contrast to these observations, we found no effect of sotalol at concentrations up to 300 μM on neuronal IK_v in NG 108-15 cells, a finding which is consistent with the idea that sotalol exhibits tissue selectivity in its actions on IK_v.

In marked contrast to the lack of observed effect of sotalol, we found clofilium to be a potent inhibitor of IK_v in NG 108-15 cells (Figures 2–4). However, the blocking effects of clofilium on IK_v in NG 108-15 cells are quite different from its blocking effects reported in guinea-pig isolated ventricular myocytes (Arena & Kass, 1988). In the cardiac tissue, clofilium blockade of IK_v is irreversible, voltage-independent and does not appear to change the kinetics of the activated current. Furthermore, the drug is ineffective at a concentration of 10 μM and causes only 50% inhibition at a concentration of 50 μM (Arena & Kass, 1988). Blockade by clofilium of IK_v in NG 108-15 cells reported here was also irreversible (e.g. Figure 4a), but was voltage-dependent and greatly altered the kinetics of the activated current (e.g. Figure 2a; although was without effect on current activation; Figure 2b). Clofilium was also seen to be a more potent inhibitor of the neuronal than the cardiac IK_v; a concentration of 10 μM, which is without effect in cardiac tissue (see above) caused strong inhibition in NG 108-15 cells (Figures 2 and 3).

Such differences in the effects of clofilium in neuronal tissue, as compared with cardiac tissue (see above), strongly suggest a different mechanism of action of the drug in the two tissue types. For this reason, we performed experiments designed to investigate the mechanism of clofilium blockade of IK_v in NG 108-15 cells, using protocols similar to those of Lee & Tsien (1983), who investigated the mechanism of action of organic Ca^{2+} channel antagonists on cardiac Ca^{2+} currents. Such studies revealed a number of features of clofilium blockade of IK_v in NG 108-15 cells. Firstly, the effects of clofilium were shown to be use-dependent (Figure 4a), repeated depolar-

izations were required for the blockade by clofilium to reach a steady level. This effect was in marked contrast to the effects of TEA, where the first depolarization following its application evoked a much smaller current, which did not diminish further during subsequent depolarizations (Figure 4b). Secondly, it appears that clofilium blocks the open delayed rectifier K^+ channel in NG 108-15 cells; the upstroke, or activation of currents was initially unchanged in the presence of the drug (Figure 2b), but the peak was reduced and the current showed marked time-dependent decay during test depolarizations (Figure 2), suggesting that the drug was only effective when channels were activated, or opened. However, a third feature of the action of clofilium in these cells was that its irreversible binding to the delayed rectifier channel (or a site intimately related to the functioning of the channel) was not dependent on channel activation; a similar pattern of use-dependent inhibition of IK_v was found after application and then washout of unbound clofilium (compare Figures 4a and 4c).

Although the effects of clofilium described here are different from the action of the drug on cardiac IK_v, they are similar in some respects to the recently reported actions of clofilium on batrachotoxin-activated skeletal muscle Na^+ channels incorporated into planar lipid bilayers (Nettleton *et al.*, 1991). These Na^+ channels are blocked by clofilium in a concentration – and strongly voltage-dependent manner, and clofilium appears to act as an open channel blocker in a similar manner to local anaesthetics (Nettleton *et al.*, 1991).

In summary, data presented here demonstrate that clofilium can block neuronal delayed rectifier K^+ channels, but its effects are markedly different from those previously described on cardiac IK_v (Arena & Kass, 1988), suggesting a different mechanism of action. The actions of clofilium are voltage-dependent, changes occur in the kinetics of the activated IK_v in NG 108-15 cells, and the drug is a more potent inhibitor of neuronal IK_v than cardiac IK_v. However, sotalol, another class III antidysrhythmic agent, was without effect on IK_v in NG 108-15 cells. Such findings suggest these compounds show differential tissue selectivity and it would be of interest to examine the actions of other examples of this diverse class of compounds in tissues other than the heart.

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References

ADAMS, D.J., SMITH, S.J. & THOMPSON, S.H. (1980). Ionic currents in molluscan soma. *Annu. Rev. Neurosci.*, **3**, 141–167.

ARENA, J.P. & KASS, R.S. (1988). Block of heart potassium channels by clofilium and its tertiary analogs: relationship between drug structure and type of channel blocked. *Mol. Pharmacol.*, **34**, 60–66.

BROWN, D.A. & HIGASHIDA, H. (1988). Voltage- and calcium-activated potassium currents in mouse neuroblastoma × rat glioma hybrid cells. *J. Physiol.*, **397**, 149–165.

CAMPBELL, T.J. (1987). Cellular electrophysiological effects of D- and DL-sotalol in guinea-pig sinoatrial node, atrium and ventricle and in human atrium: differential tissue selectivity. *Br. J. Pharmacol.*, **90**, 593–599.

CARMELIET, E. (1985). Electrophysiologic and voltage clamp analysis of the effects of sotalol on isolated cardiac muscle and purkinje fibers. *J. Pharmacol. Exp. Ther.*, **232**, 817–825.

COOK, N.S. (1988). The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol. Sci.*, **9**, 21–28.

COOK, N.S. & QUAST, U. (1990). Potassium channel pharmacology. In *Potassium Channels, Structure, Classification, Function and Therapeutic Potential.* ed. Cook, N.S. pp. 181–255. Chichester: Ellis Horwood Ltd.

HALLIWELL, J.V. (1990). K^+ Channels in the central nervous system. In *Potassium Channels, Structure, Classification, Function and Therapeutic Potential.* ed. Cook, N.S. pp. 348–381. Chichester: Ellis Horwood Ltd.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflüg. Arch.*, **391**, 85–100.

HAMPRECHT, B. (1977). Structural, electrophysiological, biochemical and pharmacological properties of neuroblastoma-glioma cell hybrids in cell culture. *Int. Rev. Cytol.*, **49**, 99–170.

HILLE, B. (1984). Modifiers of gating. In *Ionic Channels of Excitable Membranes.* pp. 303–328. Sunderland, MA: Sinauer Associates Inc.

LEE, K.S. & TSIEN, R.W. (1983). Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature*, **302**, 790–794.

NETTLETON, J., CASTLE, N.A. & WANG, G.W. (1991). Block of single batrachotoxin-activated Na^+ channels by clofilium. *Mol. Pharmacol.*, **39**, 352–358.

NOBLE, D. (1984). The surprising heart: a review of recent progress in cardiac electrophysiology. *J. Physiol.*, **353**, 1–50.

OSTERRIDER, W. & WATERFALL, J.F. (1990). Therapeutic potential of K^+ channel modulation in heart. In *Potassium Channels, Structure, Classification, Function and Therapeutic Potential.* ed. Cook, N.S. pp. 337–347. Chichester: Ellis Horwood Ltd.

REEVE, H.L. & PEERS, C. (1991). Actions of clofilium on the delayed rectifier K^+ current recorded in undifferentiated neuroblastoma × glioma hybrid (NG 108–15) cell. *J. Physiol.*, (abstract, in press).

SMITH, P.A., BOKVIST, K., ARKHAMMAR, P., BERGGREN, P.-O. & RORSMAN, P. (1990). Delayed rectifying and calcium-activated K^+ channels and their significance for action potential repolarization in mouse pancreatic β-cells. *J. Gen. Physiol.*, **95**, 1041–1059.

STANFIELD, P.R. (1983). Tetraethylammonium ions and the potassium permeability of excitable membranes. *Rev. Physiol. Biochem. Pharmacol.*, **97**, 1–67.

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