Actions and mechanisms of action of novel analogues of sotalol on guinea-pig and rabbit ventricular cells

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1 The actions and mechanisms of action of novel analogues of sotalol which prolong cardiac action potentials were investigated in guinea-pig and rabbit isolated ventricular cells.

2 In guinea-pig and rabbit cells the compounds significantly prolonged action potential duration at 20% and 90% repolarization levels without affecting resting membrane potential. In guinea-pig but not rabbit cells there was an increase in action potential amplitude and in rabbit cells there was no change in the shape or position of the 'notch' in the action potential.

3 Possible mechanisms of action were studied in more detail in the case of compound II (1-(4-methanesulphonamidophenoxy)-3-(N-methyl 3,4 dichlorophenylethylamino)-2-propanol). Prolongation of action potential duration continued to occur in the presence of nisoldipine, and calcium currents recorded under voltage-clamp conditions were not reduced by compound II (1 μ M). Action potential prolongation by compound II was also unaffected in the presence of 10 μ M tetrodotoxin.

4 Compound II (1 μ M) did not influence I_{K1} assessed from the current during ramp changes in membrane potential (20 mV s⁻¹) over the range -90 to -10 mV.

5 Compound II (1 μ M) blocked time-dependent delayed rectifier potassium current (I_K) activated by step depolarizations and recorded as an outward tail following repolarization. When a submaximal concentration (50 nM) was applied there was no change in the apparent reversal potential of I_K .

6 Submaximal concentrations of compound II were without effect on activation of I_K with time at a membrane potential of +40 mV, and no changes were detected in the time constants of the two components of I_K decay over the range of potentials -60 to 0 mV. Compound II (50 nM) appeared to cause a small shift in the activation of I_K with membrane potential (an apparent shift of approximately 10 mV in the depolarizing direction at the mid-point of the curve).

7 Log dose-response curves for action potential prolongation and for blockade of I_K by compound II were similar. The IC₅₀ for compound II was approximately 30 nM.

8 It is concluded that this novel series of compounds prolongs action potential duration, and that in the case of compound II the evidence supports a potent selective effect on the time-dependent potassium current I_K , an effect which can account for this prolongation.

Keywords: Sotalol; Class III antiarrhythmic; potassium channel; cardiac, ventricular cell

Introduction

Class III antiarrhythmic agents have been defined by Vaughan Williams (1970) as any compound which increases the duration of the cardiac action potential. Previously the drugs, amiodarone and sotalol, have been the best examples of this type of antiarrhythmic. However, both compounds are not selective in their actions; sotalol is a nonspecific β -adrenoceptor blocker (Singh *et al.*, 1987) while amiodarone has been shown to block both sodium (Sheldon *et al.*, 1989) and calcium channels (Nokin *et al.*, 1986). In a multicentre trial study (Kehoe *et al.*, 1990) with 236 patients, sotalol proved effective in suppression of sustained ventricular tachyarrhythmias of patients that had been refractory to at least 3 other antiarrhythmics. β -Adrenoceptor blockade may be a disadvantage (Soyka *et al.*, 1990) and it is of interest that (+)-sotalol has little or no β -blocking action but retains the ability to prolong cardiac action potentials (Lynch *et al.*, 1984; Carmeliet, 1985). With recent clinical evidence supporting the effectiveness of class III agents there has been great interest in the search for more selective compounds.

The first success in this search was the synthesis of the compounds sematilide (Lumma *et al.*, 1987) and CK-1649c (Randall *et al.*, 1987) from Berlex laboratories. These compounds were shown not to possess any β -blocking ability and

to be approximately 10 times more potent than sotalol in prolonging the action potential duration (APD) in canine Purkinje fibres. Subsequently, E-4031 (Oinuma *et al.*, 1990) was developed by Eisai Tsukuba Research Laboratories and even more recently UK-68,798 by Pfizer (Cross *et al.*, 1990; Gwilt *et al.*, 1991). This latter compound has been reported to prolong APD in the concentration range 5 nM–1 μ M and is the most potent compound that has been described to date.

The mechanism by which sotalol prolongs the cardiac action potential has been studied in rabbit Purkinje fibres and been shown to be inhibition of the time-dependent potassium current (I_K) and a small decrease in the inward rectifier current (I_{K1}) (Carmeliet, 1985). The aim of the work presented here was to investigate the mechanism of action of a novel series of potent class III agents which have been synthesized in these laboratories. A report on the synthesis of these compounds has been published elsewhere along with preliminary observations on their effects (Connors *et al.*, 1991). The compounds are devoid of β_1 -blocking activity as assessed from the suppression of an increase in heart rate induced by intravenous injection of isoprenaline in pithed rats (Connors *et al.*, 1991).

Methods

Experiments were performed on single cardiac ventricular myocytes isolated from guinea-pig and rabbit. The animals

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were stunned then killed by cervical dislocation. Hearts were excised and single cells isolated as previously described by Powell *et al.* (1980) and Mitchell *et al.* (1984). After isolation, cells were superfused with an oxygenated solution at 36°C containing (mM): NaCl 118.5, NaHCO₃ 14.5, KCl 4.2, KH₂PO₄ 1.2, MgSO₄ 1.2, glucose 11.1 and CaCl₂ 2.5.

All compounds tested were water-soluble either as their free bases or as the corresponding hydrochloride salt (except Pfizer UK 68,798 which was dissolved in 50% v/v aqueous ethanol). Stock solutions were made up to a concentration of 5 mM in twice glass-distilled water. Further dilutions were carried out as needed. Switch to a drug-containing solution was made by a tap placed close to the inflow of the bath. All effects were reversible on washout of the drugs.

Electrical recordings were made via electrodes containing 0.5 M K₂SO₄ and 300 μ M KCl; in some voltage-clamp experiments 75 mM BAPTA (1,2-bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid) was added to this solution. Action potentials were stimulated by 2 ms current pulse injections at 1 Hz (Axoclamp 2 single-microelectrode system; bridge mode). Each measurement of APD was made after continuous recording in the same cell when the effect had reached a steady state approximately 4 min after exposure to the drug.

A switch clamp method of voltage clamping the cells was used. The rate of switching between current passing and voltage recording modes was adjusted to allow settling between transitions, and was normally in the range 2–8 kHz.

In experiments where calcium currents were investigated, both peak current and current at the end of step depolarizations (applied at a frequency of 0.3 Hz) were measured with respect to zero current.

I_K was activated by depolarizing pulses (0.3 or 0.1 Hz) from a holding potential of –50 mV (except where otherwise indicated). Analysis of the activation of I_K is difficult because of additional currents which may be simultaneously activated by depolarizing pulses. Several additional currents flow during the depolarization including second inward current I_{si} , sodium-calcium exchange current (Beukelmann & Weir, 1989) and calcium activated nonspecific channels (Ehara *et al.*, 1988; Beukelmann & Weir, 1989). Upon repolarization from an activating voltage step an outward tail current is observed which is thought to represent the slow deactivation of I_K and serves as a more reliable index of I_K current. Any residual calcium current would be expected to de-activate much more rapidly than I_K , and currents associated with sodium-calcium exchange or calcium-activated channels are expected to be inward and of much shorter duration (see e.g. Mitchell *et al.*, 1987). Such calcium activated inward tail currents might lead to underestimation of peak I_K . To minimize this possibility, inward tails were suppressed by

minimizing the calcium transient in the cell with either nisolipine applied extracellularly (3 μ M) or intracellular BAPTA (Tsien, 1972) from appropriately filled electrodes. The amplitude of the I_K tails measured under these conditions was used as an index for revealing the degree of inhibition by the compounds. Such outward I_K tail currents were similar to those previously described by Matsuura *et al.* (1987). Under the conditions of our experiments, stable records of I_K were obtained for periods of at least 20 min and all experiments were completed within this period.

Voltage and current were recorded on a Racal Store 4 FM tape recorder at 3.75 in/s. Records were displayed on a digital storage oscilloscope (Gould 4020). The data were then either digitized (Data Translation DT 2801A) and analysed with the VCAN software package kindly provided by Dr J. Dempster or sent as an output via an IEEE interface to an Epson H180 plotter.

Results

When the compounds listed in Table 1 were applied to individual guinea-pig ventricular cells the action potential was seen to prolong in a concentration-dependent fashion (see Figure 1). The prolongation reached a steady state in less than two minutes and was reversible. Recovery was slower than onset (see Figure 1b). Observations were compared to those for sotalol since this was used as a lead compound in the development of the series. Sotalol at a concentration of 100 μ M gave a prolongation of the action potential duration at 90% repolarization (APD₉₀) of 25 $\pm$ 2% under the conditions of our experiments. This compares well with a published value for prolongation of APD₉₀ in guinea-pig papillary muscle of 30% at the same concentration (Carmeliet, 1985). UK 68,798 was also tested and found to produce a prolongation of 30 $\pm$ 2% at 250 nM. At 50 nM an 8 $\pm$ 3% increase in APD₉₀ was observed compared with a published value of 28% seen in guinea-pig right papillary muscle (Cross *et al.*, 1990).

The most potent sotalol analogues were 10,000 times more effective than the parent compound (see Table 1). For example, compound I caused a 31% prolongation at a concentration of 10 nM. A detailed study has been made of another potent analogue (compound II). In addition to APD₉₀ measurements were made of action potential duration at 20% repolarization (APD₂₀), resting membrane potential and amplitude of the action potential. No change was observed in the resting membrane potential with any of the compounds that were tested, while significant changes were seen in APD₂₀ and APD₉₀ (see Table 2). An interesting feature of the action potential prolongation was that the maximum achievable

Table 1 Compounds used in this study

Sotalol	Dose	n	% Δ APD ₉₀
50 μ M	4	10 $\pm$ 2
	100 μ M	7	25 $\pm$ 2
Pfizer UK 68,798	5 nM	4	2 $\pm$ 1
50 nM	6	8 $\pm$ 3
	100 nM	4	21 $\pm$ 3
	250 nM	4	30 $\pm$ 2
Compound I	1 nM	4	16 $\pm$ 4
10 nM	4	31 $\pm$ 2
	100 nM	4	32 $\pm$ 2
Compound II	1 nM	6	7 $\pm$ 3
10 nM	6	14 $\pm$ 1
	50 nM	6	25 $\pm$ 3
	100 nM	6	28 $\pm$ 3
	1 μ M	6	30 $\pm$ 2

Figure 1 (a) Effect of varying doses of compound II on guinea-pig isolated ventricular cell action potentials at 1 Hz: (1) control; (2) wash (12 min after final exposure to compound II); (3) 10 nM II; (4) 50 nM II. Each dose was allowed to equilibrate for 4 min. (b) The time course of action of 100 nM compound II applied to a guinea-pig isolated ventricular cell. The trace represents a measure of APD₉₀ determined from action potentials recorded at 1 Hz. (Each vertical spike represents a ramp voltage the height of which is proportional to the duration of the signal above the threshold. In this case the threshold was set at the 90% repolarization level of the action potential (APD₉₀). The trace above is at a time scale such that all the vertical spikes merge). (c) Influence of compound II (100 nM) on an action potential recorded from rabbit isolated ventricular myocyte. Note prolongation without effect on 'notch'.

effect was 30–35%, an effect also seen with a related compound E-4031 (Sanguinetti & Jurkiewicz, 1990). All the active compounds that were tested were never observed to prolong the action potential more than this amount under our experimental conditions.

It is recognized that in guinea-pig ventricular cells there is no significant transient outward current under physiological conditions (e.g. Fedida *et al.*, 1989). However, in rabbit ventricular cells transient outward current is present and gives rise to a 'notched' appearance in the early repolarization phase (Giles & Imaizumi, 1988). Addition of 100 nM compound II to rabbit isolated ventricular cells did not change the appearance of this notch (see Figure 1c). Similar observations were made in six cells. In these cells, compound II did not cause any significant effect on action potential amplitude (115 ± 3 mV before and 116 ± 3 mV during compound II), while APD₂₀ and APD₉₀ were significantly increased ($P < 0.001$, from 54 ± 6 to 67 ± 7 ms, and from

179 ± 14 to 247 ± 18 ms).

In the presence of nisoldipine (to block 'L-type' calcium channels) action potentials were shortened as a consequence of abolition of inward calcium current during the plateau. Under these conditions, exposure to compound II again caused a dose-dependent increase in the duration. A dose-response curve in the presence of nisoldipine along with an example record is shown in Figure 2a and b (compare also Figure 9 and see later). Compound II (100 nM) in the presence of nisoldipine increased action potential amplitude

Figure 2 The effects of compound II on nisoldipine-treated guinea-pig ventricular cells: (a) shows a typical record of an action potential at 1 Hz recorded in the presence of 2 μM nisoldipine before and after the effects of 100 nM compound II, (b) shows a dose-response curve for the effects of compound II on nisoldipine-treated action potentials. Data at each dose from 3–5 cells. Vertical bars represent s.e.mean. In the absence of nisoldipine (c) shows both the peak inward current and the current at the end of the depolarizing pulse plotted as a function of membrane voltage before and after the addition of compound II 1 μM. $n = 6$ cells; s.e. shown ($*P < 0.05$). Panel (d) A typical current record before and after the addition of II. Holding potential -40 mV with a 300 ms depolarization to 0 mV.

from 110 ± 2 mV to 120 ± 2 mV, increased APD_{20} from 17 ± 3 to 39 ± 3 ms, and increased APD_{90} from 93 ± 2 to 143 ± 5 ms ($P < 0.001$ for all three measurements, $n = 5$).

The possibility that compound II might influence calcium currents was further tested under voltage-clamp conditions by applying step depolarizations from a holding potential of -40 mV. No change in the magnitude of the peak inward current over the range -30 mV to $+20$ mV could be detected which was consistent with the hypothesis that there was no significant effect on L-type calcium channels (see Figure 2c and d). At more positive membrane potentials a small reduction in outward current at the end of the pulse (significant at $+30$ to $+50$ mV, see Figure 2c) is consistent with a reduction in I_K which would be expected to contribute more at these depolarized potentials, a possibility which will be considered again later.

The ability to prolong the APD was preserved in the presence of tetrodotoxin (TTX). In Figure 3 it can be seen that $10 \mu\text{M}$ TTX significantly shortened APD_{90} as has been previously observed (Mitchell *et al.*, 1984; Kiyosue & Atria, 1989). When compound II was added in the continued presence of TTX, significant changes were seen in action potential amplitude, APD_{20} , and APD_{90} (see legend to Figure 3). There was no significant difference in prolongation of the action potential by compound II in the presence of TTX: compound II at a concentration of $1 \mu\text{M}$ increased APD by $30 \pm 4.8\%$ in the absence and by $28 \pm 2.9\%$ in the presence of $10 \mu\text{M}$ TTX.

To test for a potential interaction of these compounds with I_{K1} the voltage across the cell membrane was ramped over the range -90 to $+20$ mV at a rate of 20 mV per second (a rate at which most time-dependent currents including those carried by sodium and calcium are at a steady state). It was seen that concentrations of compound II ($1 \mu\text{M}$) which caused maximal prolongation of action potentials produced little change in the I - V relation of the cell over the range -90 mV to -10 mV. At more depolarized membrane potentials where I_{K1} shows marked inward rectification and therefore contributes little to the I - V relation of the cell, the current in the presence of compound II was seen to be less outward (or more inward, see Figure 4). These observations are consistent with those of the experiments described above (Figure 2c) where the current at the end of depolarizations used to evoke I_{si} was less outward for membrane potentials between $+30$ and $+50$ mV while any effects over the range

Figure 3 Preservation of the action of compound II in the presence of $10 \mu\text{M}$ tetrodotoxin (TTX). A typical record is shown on exposure of an action potential stimulated at 1 Hz to first TTX then TTX and compound II together. Changes seen in APD_{20} were as follows: 85 ± 17 ms in control to 80 ± 13 ms on exposure to $10 \mu\text{M}$ TTX alone and 99 ± 16 ms ($P < 0.05$) with $10 \mu\text{M}$ TTX plus compound II. Values for APD_{90} were 187 ± 14 ms, 169 ± 13 ms ($P < 0.001$) and 217 ± 13 ms ($P < 0.001$) respectively. Data from 7 cells.

Figure 4 The effects of $1 \mu\text{M}$ compound II on the I - V relationship in guinea-pig isolated ventricular cells: (a) shows the current-voltage relationship plotted over a range of voltages before and after the addition of compound II. Data are taken from ± 40 mV excursions around holding potentials of -20 mV and -60 mV. Results are from 7 cells. Data are normalized to current at -60 mV ($*P < 0.05$). (b) shows a typical current record in which voltage was varied at 20 mV per s with an excursion of ± 40 mV about a holding potential of -60 mV. The effects before and after compound II are shown.

-30 to $+20$ mV were small.

When the possibility of an effect on I_K was tested more directly (measuring I_K as the outward tail on repolarization following a step depolarization to positive potentials from a holding potential of -50 mV, see Methods) compound II ($1 \mu\text{M}$) was consistently found to suppress I_K in all cells studied. Examples recorded from three different cells are shown in Figure 5. It can be seen that the influence of compound II on current developing during the step depolarization was variable in contrast to the consistent marked reduction of the I_K tail currents. Contribution of currents other than I_K to the current developing during the step depolarization (and thus its variable sensitivity to compound II) is considered in more detail in the Discussion.

The reversal potential of this outward tail current was found to be approximately -75 mV, close to the expected value of E_K (Figure 6). A submaximal concentration of compound II (50 nM), which reduced the amplitude by approximately 60% at -40 mV, caused little or no change in the apparent reversal potential.

In the experiment shown in Figure 7, a range of depolarizations of 350 ms duration from -30 mV to $+40$ mV in 10 mV steps were applied to investigate activation of I_K with membrane potential. The activation of I_K has been described as showing a sigmoid relationship to voltage (Shibasaki, 1987) and the results obtained here followed this pattern. A

Figure 5 The effects of compound II on the current trace in three different cells (from the guinea-pig) in the presence of $2 \mu\text{M}$ nisolipine. Control traces (*) are superimposed on traces in the presence of the compound. Depolarizations are to $+50$ mV for 250 ms from a holding potential of -40 mV. In each case there is a suppression of the decaying outward tail current; however, the effect of compound II on the current during the depolarization is variable. Zero current levels are shown for each cell.

Figure 6 Plot of I_K tail current against membrane potential on repolarization following an activating step depolarization to $+40$ mV for 1 s (currents normalized to that at -40 mV in the absence of compound II). I_K tail currents in the presence of 50 nM compound II (▲); currents in the absence of drug (●). Apparent reversal potential was approximately -75 mV. Data from 5 guinea-pig cells, BAPTA present throughout. Vertical bars show s.e.

submaximal dose of compound II reduced the amplitude of the outward I_K tails, showing little dependence on the membrane potential during the activating step. Activation for 350 ms was investigated because although this was not expected to allow full activation of I_K (Matsuura *et al.*, 1987) the duration of depolarization approximates that of the ventricular action potential. Activation of I_K with membrane potential was also investigated using 1 s depolarizations (see Figure 7b) to allow the current to approximate more closely steady state conditions. The effect of compound II on I_K was seen to be similar when the length of depolarization was increased. Depolarizations greater than 1 s were not used in order to avoid possible harmful effects of such long depolarizations (Terrar & White, 1989). When the voltage activation curves for 1 s depolarizations in the presence and absence of 50 nM compound II were compared (following normalization of the current amplitudes to the maximum current elicited) there appeared to be a slight shift in activation of about 10 mV in the depolarizing direction (see Figure 7c). The observations thus appear to indicate that any voltage-dependence of the action of compound II on I_K is small.

Since activation of I_K depends on time in addition to membrane potential, the effects of compound II upon the time-dependent activation of I_K were also investigated (see Figure 8). Cells were depolarized to $+40$ mV for various durations, and the outward I_K tails on repolarization to the holding potential of -50 mV were recorded. The envelope of deactivating outward tails measured in this way revealed the time-dependence of activation of I_K . Compound II at 50 nM reduced I_K with little or no effect on the kinetics of activation while at $1 \mu\text{M}$ I_K was virtually abolished at short times up to 200 ms, with approximately 15% of the current remaining at 1000 ms (records not shown). It appears, therefore, that compound II affects the magnitude and not the kinetics of time-dependent activation of I_K .

The possible effect of compound II on the deactivation of I_K was investigated by fitting exponential components to the current deactivation. This decay conformed to biexponential kinetics and could not be fitted well to monoexponential decay as has been previously described in experiments on single cells (e.g. Bennett *et al.*, 1985). In the voltage range -60 mV to 0 mV, time constants for both the fast and slow components were seen to increase. For voltages positive to this there was a significant steady state activation of I_K such that the deactivating tail current was quite small making analysis difficult. In the absence of I_K inhibitors the time constants were of the order that have been previously reported (Matsuura *et al.*, 1987) and upon addition of compound II no significant change was seen in either the fast or slow component (see Figure 8b and c).

For compound II a dose-response relationship for action potential prolongation and current inhibition is seen in Figure 9. The IC_{50} derived from the relationship of current inhibition and dose was approximately 30 nM and compares with a value of $10 \mu\text{M}$ for sotalol (Carmeliet, 1985) representing a substantial increase in potency. It can also be seen in Figure 9 that the log dose-response curve for inhibition of I_K

Table 2 Characteristics of the effects of sotalol $100 \mu\text{M}$ and compound II 100 nM on the action potentials recorded from guinea-pig isolated ventricular myocytes

	Control ($n = 7$)	Sotalol ($n = 7$)	Control ($n = 6$)	Compound II ($n = 6$)
RMP	-75 ± 0.8 mV	-75 ± 0.9 mV	-75 ± 0.60 mV	-75 ± 0.50 mV
AMP	118 ± 1.0 mV	121 ± 0.4 mV*	122 ± 1.6 mV	126 ± 0.60 mV*
APD ₂₀	101 ± 13 ms	137 ± 17 ms**	109 ± 10 ms	145 ± 9.8 ms**
APD ₉₀	223 ± 16 ms	282 ± 18 ms**	201 ± 12 ms	260 ± 18 ms**

RMP, resting membrane potential.

AMP, amplitude.

APD₂₀, action potential duration at 20% repolarization.

APD₉₀, action potential duration at 90% repolarization.

* $P < 0.01$, ** $P < 0.001$.

Figure 7 This figure demonstrates the current-voltage relationship of I_K in the presence and absence of compound II: (a) shows the current-voltage relationship in the presence and absence of 50 nM II. Currents were elicited within a 350 ms depolarization. $n = 5$ cells, nisoldipine $2 \mu\text{M}$ present. (b) shows again the effects of 50 nM compound II on the current-voltage relationship of I_K but currents in this panel were elicited after depolarization for 1 s. $n = 7$ cells, intracellular BAPTA present. (c) shows the results in (b) normalized to $I_{K\text{max}}$ before and after compound II respectively. Data from 7 cells. s.e. shown. The cells were obtained from guinea-pig.

closely follows that of action potential prolongation providing further evidence that the mechanism of action of the compounds is via selective inhibition of I_K .

Discussion

It appears from the observations reported here that under the conditions of our experiments the novel sotalol analogues we have synthesized are among the most potent, perhaps the most potent, agents in prolonging the cardiac action potential described to date. The evidence presented here supports the suggestion that the major mechanism for action potential prolongation is blockade of time-dependent potassium current. This evidence will be briefly discussed below followed by consideration of possible uses of such compounds in electrically dissecting important aspects of cardiac function and as agents of potential therapeutic significance.

Figure 8 This figure shows the effect of compound II 50 nM on the kinetics of deactivation and activation of I_K . Current was elicited upon returning to a family of voltages from a depolarization to $+40$ mV for 1 s: (a) shows the lack of effect on the first time constant ($\bullet$, control) and (b) shows the lack of effect on the second time constant ($\bullet$, control). Deactivating tails were fitted to the biexponential equation: $I(t) = A_1 \exp(-t/t_1) + A_2 \exp(-t/t_2)$ where $I(t)$ is the value of I_K at time t ; A_1 and A_2 the maximum current value of the two components and t_1 and t_2 the values of the first and second time constants. (c) The time activation of I_K at a constant voltage. Currents were elicited with a depolarization to $+40$ mV from a holding potential of -50 mV in the presence of intracellular BAPTA. The effect of compound II 50 nM ($\blacktriangle$) is shown. Currents were normalized to that at 500 ms in control. Data from 6 guinea-pig cells; s.e. shown.

Prolongation of action potential duration might have arisen from elevation of depolarizing plateau currents but it appears that enhancement by the compounds either of fast sodium or of calcium currents is unimportant since prolongation of action potentials persisted in the presence of tetrodotoxin or nisoldipine. More direct evidence that compound II did not influence calcium current was provided by the voltage-clamp experiments.

The observation that compound II did not alter resting membrane potential is not by itself convincing in excluding an action on I_{K1} , since changes in the conductance for this

Figure 9 The dose-response relationship for both action potential duration and inhibition of I_K . The prolongation of action potential duration is that at APD_{90} (▲) and is compared with the inhibition of I_K elicited after a 350 ms depolarization to +40 mV from a holding potential of -50 mV (●). Each point represents data from 4–6 cells. s.e. shown. Nisoldipine 2 μ M present for measurement of the current.

pathway close to the potassium equilibrium potential would be expected to have much less effect than during the repolarization phase of the action potential. However, the ramp voltage-clamp experiments provided additional support for a lack of effect on this pathway.

Compound II caused a consistent suppression of I_K (measured as maximum amplitude of decaying outward tails following an activating depolarization) in all cells studied. No change was detected in the apparent reversal potential of the time-dependent component (I_K) by submaximal concentrations of the drug. It was also shown that compound II did not cause any obvious change either in the activation of I_K with time, or in the two time constants of the decaying I_K tails. Elucidation of precise molecular mechanism requires further experiments but these observations are consistent with an effect to reduce opening of I_K channels without obvious influence on the opening and closing properties of unblocked channels. However, there appeared to be a small change in voltage activation, though the possibility should be considered that this observation might result from differential effects on subtypes of I_K channels (see later) at different membrane potentials. The suggestion that most or all of the action potential prolongation by compound II results from blockade of I_K is supported by the close similarity of log (dose)-response curves for the two effects.

No attempt was made to measure I_K during an activating depolarization since it is known that several components of current are superimposed under these conditions. Even when calcium current is suppressed with nisoldipine, current may flow through Na:Ca exchange and through non-selective cation channels activated by a rise in cytosolic calcium (see e.g. Beukelmann & Wier, 1989; Terrar & White, 1989). When cytosolic calcium is buffered with BAPTA, Na:Ca exchange could support outward current (Ca entry and Na extrusion) at positive potentials and it remains possible that calcium-activated channels could still contribute if buffering of calcium in the 'fuzzy space' beneath the sarcolemma were incomplete (Lederer *et al.*, 1990). Outward current continued to develop during step depolarizations under conditions where the outward tail of I_K was completely suppressed. This observation can be accounted for by the lack of effect of compound II on the above additional currents which con-

tribute to the current during depolarization (see Figure 3). Such compound II-insensitive currents are presumably responsible for continued increase in outward current at the end of these step depolarizations. Indeed, it seems possible that compound II may be useful in future experiments to investigate further these additional current components where I_K is selectively abolished by low concentrations of compound II.

Compound II may also be useful in detecting possible contamination by I_K of other current components. For example it is apparent from Figure 2d that for depolarizations to 0 mV, any contribution of compound II-sensitive I_K is negligible compared to peak calcium current under the conditions of our experiments (consistent with previous work, e.g. Matsuda & Noma, 1984; Mitchell *et al.*, 1987).

It has recently been suggested that there may be two components of the delayed rectifier potassium current (Sanguinetti & Jurkiewicz, 1990) and that sotalol tended to block only one of these components. The complex nature of I_K is supported by other work which demonstrates that I_K does not have a simple activation process (Connors *et al.*, 1990) and that those currents induced by longer depolarizations tend to be selectively increased by forskolin (Connors & Terrar, 1990). In the work of Sanguinetti & Jurkiewicz (1990) evidence for subtypes of I_K was based on currents flowing during activation of I_K compared to those on deactivation of I_K . This approach may not be appropriate if there were contaminating currents during activation (such as Na:Ca exchange) as discussed above. However, this work has raised an important issue from which two possible conclusions may be drawn: either there exist subtypes of I_K channels or a single I_K channel exhibits complex kinetics. The resolution of this question would be important in future investigations and may require single channel evidence which is not yet available.

The observation that the amplitude of the action potential was significantly increased by compound II was interesting. It was argued above that the effects of these compounds were not mediated through an action on sodium or 'L-type' calcium channels or by any large change in the background potassium current. Steady state activation of I_K may occur (Boyett & Jewell, 1980; Delmar *et al.*, 1989) under the conditions of our experiments since the interval between action potentials at 1 Hz may not present sufficient time for deactivation. If there were a steady state level of outward current through I_K , inhibition of this pathway would be expected to increase the amplitude of the action potentials. In the case of the rabbit action potentials, little change in the amplitude was seen presumably because rabbit ventricular cells possess a substantial I_{to} (Giles & Imaizumi, 1988) giving them the 'notched' appearance in the early plateau. Since the current through this pathway is much greater than that of I_K (Giles & Imaizumi, 1988) one would not expect to see the effect of inhibiting I_K on the amplitude of the rabbit ventricular action potential. Following this line of argument, in guinea-pig cells where I_{to} is not prominent (Fedida *et al.*, 1989), nisoldipine decreased the amplitude of the action potential presumably because the contribution of the calcium current to peak amplitude was removed.

The observation that the prolongation of action potential duration was greater in the presence than in the absence of nisoldipine may be accounted for in terms of the relative importance of I_K under these two conditions. In the absence of the calcium channel blocker removal of I_K would tend to approach a natural limit in prolonging action potential duration as there will remain repolarizing influences such as decay of the calcium current and activation of I_{K1} . Thus, a prolongation limit of 30–35% may be reached when repolarization is governed entirely by these I_K -independent mechanisms. In the presence of nisoldipine action potentials in the absence of other drugs are much shorter as a consequence of the absence of the calcium current. Under these different initial conditions this natural limit upon removing I_K need no

longer be the same allowing greater increases in action potential duration by these compounds.

In conclusion compound II, the prototype of a novel series of compounds, has been shown to increase action potential duration through a selective inhibition of the time-dependent

potassium current. Future experiments will be required to assess whether compound II and related compounds may have potential therapeutic significance as Class III antiarrhythmic drugs.

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