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Block of cardiac delayed-rectifier and inward-rectifier K^+ currents by nisoldipine

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1 The objective of this study was to determine the concentration-dependent effects of nisoldipine, a dihydropyridine Ca^{2+} channel blocker, on K^+ currents in guinea-pig ventricular myocytes.

2 Myocytes in the conventional whole-cell configuration were bathed in normal Tyrode's solution or K^+-free Tyrode's solution for the measurement of the effects of 0.01–100 μM nisoldipine on rapidly activating delayed-rectifier K^+ current (I_{Kr}), slowly activating delayed-rectifier K^+ current (I_{Ks}), inwardly rectifying K^+ current (I_{K1}), and reference L-type Ca^{2+} current ($I_{Ca,L}$).

3 Nisoldipine inhibited I_{Kr} with an IC_{50} of 23 μM, and I_{Ks} with an IC_{50} of 40 μM. The drug also had weak inhibitory effects on inward- and outward-directed I_{K1}; the IC_{50} determined for outward-directed current was 80 μM.

4 Investigation of nisoldipine action on I_{Ks} showed that inhibition occurred in the absence of previous pulsing, and with little change in the time courses of activation and deactivation. However, the drug-induced inhibition was significantly weaker at $\geqslant +30$ mV than at $+10$ mV.

5 We estimate that nisoldipine is about 30 times less selective for delayed-rectifier K^+ channels than for L-type Ca^{2+} channels in fully polarised guinea-pig ventricular myocytes, and several orders less selective in partially depolarised myocytes.

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Keywords: Guinea-pig ventricular myocytes; nisoldipine; L-type Ca^{2+} current; rapidly activating delayed-rectifier K^+ current; slowly activating delayed-rectifier K^+ current; inward-rectifying K^+ current

Abbreviations: DMSO, dimethyl sulphoxide; EGTA, ethylene glycol-bis(b-aminoethyl)-*N,N,N,N*-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; *I–V*, current–voltage; IC_{50}, concentration that produces 50% of maximal inhibition; $I_{Ca,L}$, L-type Ca^{2+} current; I_K, delayed-rectifier K^+ current; I_{Kr}, rapidly activating component of I_K; I_{Ks}, slowly activating component of I_K; I_{Kur}, ultrarapid K^+ current; I_{K1}, inward-rectifying K^+ current; I_{to}, transient outward current

Introduction

Members of the 1,4-dihydropyridine class of compounds have important clinical application in the management of cardiovascular disorders, and the basis of this use is the binding of drug molecules to high-affinity inhibitory sites on L-type Ca^{2+} channels (Kohlhardt & Fleckenstein, 1977; Singh *et al.*, 1983; Sanguinetti & Kass, 1984; Schwartz & Triggle, 1984; McDonald *et al.*, 1994; Roden, 1996; Kamp *et al.*, 1999). Dihydropyridines can also block other ion channels, including voltage-dependent Na^+ channels (Yatani & Brown, 1985) and delayed-rectifier K^+ channels, in a variety of excitable and inexcitable cells (Nishi *et al.*, 1983; Hume, 1985; Terada *et al.*, 1987; Richard *et al.*, 1988; Jacobs & DeCoursey, 1990; Tatsuta *et al.*, 1994).

Delayed-rectifier K^+ channels in cardiac cells are also affected by dihydropyridines. In particular, ca. 10 μM concentrations of nifedipine and/or nisoldipine have been shown to have marked inhibitory effects on the fast-activating K^+ channels that carry transient outward current (I_{to}) (Kass, 1982; Gotoh *et al.*, 1991; Jahnel *et al.*, 1994) and ultrarapid K^+ current (I_{Kur}) (Zhang *et al.*, 1997). However, the susceptibility of these channels to nifedipine/nisoldipine action is not representative of all cardiac delayed-rectifier K^+ channels. For example, we have recently reported that 10 μM nifedipine has no significant effect on the two components of delayed-rectifier K^+ current (I_K) in guinea-pig ventricular myocytes, rapidly activating I_{Kr} and slowly activating I_{Ks}; however, higher concentrations produced a concentration-dependent block with IC_{50} values near 300 μM (Zhabyeyev *et al.*, 2000). Whether nisoldipine (which is a much more potent inhibitor of L-type Ca^{2+} channel blocker than nifedipine) is an equally weak inhibitor of these K^+ currents has not been established.

The primary objective of the present study was to determine the concentration-dependent effects of nisoldipine on delayed-rectifier I_{Kr} and I_{Ks} in guinea-pig ventricular myocytes. For comparison with these data, we also measured the effects of the drug on inwardly rectifying K^+ current (I_{K1}) and on $I_{Ca,L}$. Nisoldipine had concentration-dependent inhibitory actions on all the three K^+ currents, and these are discussed and compared with pertinent findings in earlier studies.

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Methods

All the procedures were carried out in accordance with the national and university regulations on the care and treatment of laboratory animals.

Ventricular myocytes

Male guinea-pigs (250–300 g) were killed by cervical dislocation, and single ventricular myocytes were enzymatically isolated as described previously (Ogura *et al*., 1995). The excised hearts were mounted on a Langendorff column, and retrogradely perfused through the aorta with Ca^{2+}-free Tyrode's solution (37°C) containing collagenase (0.08–0.12 mg ml^{-1}; Yakult Pharmaceutical Co., Tokyo, Japan) for 10–15 min. The cells were dispersed and stored at 22°C in a high-K^+, low-Na^+ solution supplemented with 50 mM glutamic acid and 20 mM taurine. A few drops of the cell suspension were placed in a 0.3-ml perfusion chamber mounted on an inverted microscope stage. After the cells had settled to the bottom, the chamber was perfused (2 ml min^{-1}) with Tyrode's solution at 36°C. The Tyrode's solution contained (in mM) NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) 5 (pH 7.4 with NaOH). In some experiments, the Tyrode's solution was replaced by a Na^+-free solution (equimolar substitution with trimethylammonium ion), or by a K^+-, Ca^{2+}-free Tyrode's solution (KCl and $CaCl_2$ omitted) that also contained 0.2 mM Cd^{2+} to suppress Ca^{2+} channel current.

Whole-cell membrane currents were recorded using an EPC-7 amplifier (List Electronic, Darmstadt, Germany). Recording pipettes were fabricated from thick-walled borosilicate glass capillaries (H15/10/137, Jencons Scientific Ltd, Bedfordshire, U.K.) and filled with a solution that contained (in mM) KCl 40, potassium aspartate 106, $MgCl_2$ 1, K_2-ATP 4, ethylene glycol-bis(b-aminoethyl ether)-*N,N,N,N*-tetraacetic acid (EGTA) 5, and HEPES 5 (pH 7.2 with KOH). The pipettes had resistances of 1.5–2.5 MΩ when filled with pipette solution, and liquid junction potentials between external and pipette-filling solution were nulled prior to patch formation. Series resistance ranged between 3 and 7 MΩ, and was compensated by 60–80%. Membrane current signals were filtered at 3 kHz, and digitised with an A/D converter (Digidata 1200A, Axon Instruments) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz prior to analysis.

Drugs

Nisoldipine was kindly provided by Bayer Inc. (Etobicoke, ON, Canada). The drug was dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St Louis, MO, U.S.A.) (0.1 M stock solution) and stored in the dark at −20°C. Appropriate amounts of stock solution were freshly added to bathing solutions, and these were protected from the light during all experiments. The highest final concentration of DMSO was 0.1%, a concentration that has no significant effect on membrane currents in guinea-pig ventricular cells (Ogura *et al*., 1995). Nevertheless, the appropriate concentrations of DMSO were included in the control solutions used in experiments with high concentrations of nisoldipine. E4031 was obtained from Eisai (Tokyo, Japan) and dissolved in the bathing solution.

Statistics

Results are expressed as means ± s.e.m., and Student's *t*-test or one-way ANOVA followed by Dunnett's multiple comparison test was used to determine the significance of drug effects. Differences were considered to be significant when $P<0.05$.

Results

Inhibition of total I_K

Figure 1a shows records of membrane currents obtained from a representative myocyte bathed in normal Tyrode's solution and depolarised for 500 ms from −40 mV to potentials up to +70 mV. The depolarisations elicited inward $I_{Ca,L}$ that reached a maximal amplitude near 0 mV, and activated time-dependent outward K^+ current that increased with positive potential and deactivated when the membrane was repolarised

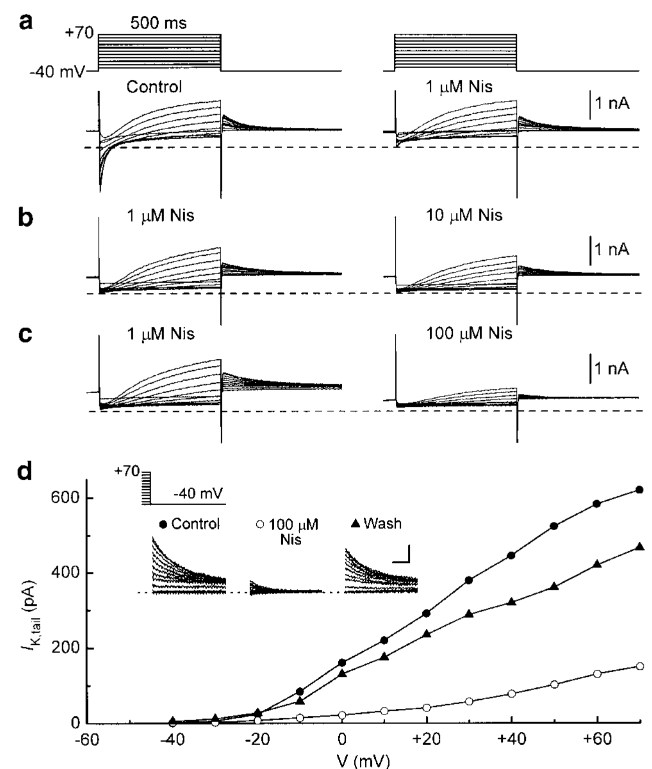

Figure 1 Effects of nisoldipine on membrane currents in guinea-pig ventricular myocytes. The myocytes were bathed in normal Tyrode's solution, held at −80 mV, and depolarised after 200-ms prepulses (−40 mV) to more positive potentials for 500 ms at 0.1 Hz; tail currents were recorded on repolarisations to −40 mV. (a) Selective inhibition of inward $I_{Ca,L}$ by 1 μM nisoldipine (Nis). (b,c) Inhibition of outward K^+ currents at positive potentials, and K^+ tail currents at −40 mV, by 8-min exposures to 10 and 100 μM nisoldipine. The myocytes were pretreated with 1 μM nisoldipine for 10 min. The dashed lines on the records here and in other figures indicate zero-current levels. (d) $I_{K,tail}-V$ relationships determined before, during, and 10 min after removal of 100 μM nisoldipine. The calibration bars on the $I_{K,tail}$ records (top) indicate 100 ms and 200 pA.

to −40 mV ($I_{K,tail}$). Exposure to 1 μM nisoldipine for 10 min resulted in near-complete inhibition of $I_{Ca,L}$, but had little effect on the amplitudes of I_K elicited by depolarisations and repolarisations. However, concentrations of nisoldipine higher than 1 μM had marked concentration-dependent inhibitory effects on I_K. In the examples shown in Figure 1b and c, 10 μM drug depressed the time-dependent current by about 20% and 100 μM depressed it by about 80%. These inhibitions were registered in myocytes pretreated with 1 μM nisoldipine, that is, they were essentially independent of changes in $I_{Ca,L}$.

The pronounced inhibition of I_K by high concentrations of nisoldipine was not a manifestation of toxicity because ca. 10-min washouts generally restored the current to at least 50% of predrug amplitude (e.g., Figure 1d) (also see below). By comparison, washout of 1 μM nisoldipine for 13±2 min only restored $I_{Ca,L}$ to 29±5% of control ($n=4$), and washout of 50 μM nisoldipine for 14±2 min only restored the current to 9±4% of control ($n=5$).

Myocytes were repolarised to −40 mV for measurement of $I_{K,tail}$ elicited after 500-ms depolarisations to test potentials (V) between −30 and +70 mV. Average $I_{K,tail}-V$ relationships recorded before and 7–10 min after additions of 3, 30, and 100 μM nisoldipine are shown in Figure 2a. The low concentration had little effect on the $I_{K,tail}-V$ relationship, whereas the 30 and 100 μM concentrations depressed its amplitude by about 50 and 80%, respectively. For reference in the interpretation of these data, Figure 2a also shows the effects of 3 μM E4031 on the $I_{K,tail}-V$ relationship. The selective I_{Kr} inhibitor (Sanguinetti & Jurkiewicz, 1990) almost completely abolished $I_{K,tail}$ elicited after depolarisations from −40 mV up to test potential 0 mV, but had little effect on the increment in tail amplitude induced by depolarisations to more positive potentials.

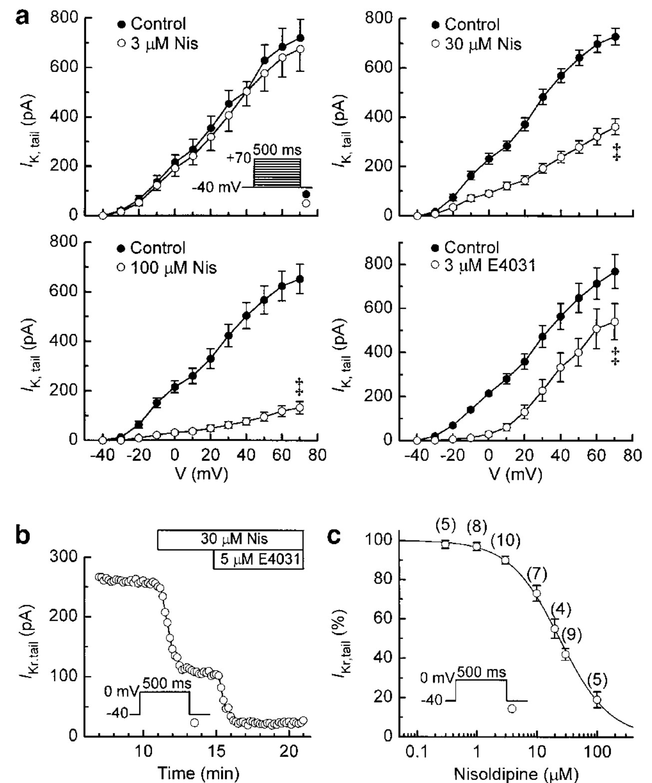

Figure 2 Inhibition of I_{Kr} by nisoldipine. Myocytes were bathed in normal Tyrode's solution and depolarised from prepulse −40 mV to more positive potentials for 500 ms at 0.1 Hz for the measurement of $I_{K,tail}$ on repolarisations to −40 mV. (a) Average $I_{K,tail}-V$ relationships determined from myocytes treated with single concentrations of nisoldipine (3 μM ($n=4$), 30 μM ($n=5$), 100 μM ($n=6$)). Also shown (lower right) are the effects of the I_{Kr}-selective inhibitor E4031 (3 μM) ($n=7$); the results of the E4031 series (near complete inhibition of $I_{K,tail}$ at ⩽0 mV) validate the method ($I_{K,tail}$ amplitude after pulses to 0 mV) used for measurement of I_{Kr} in (b, c) below. ‡$P<0.001$ (paired t-test at +70 mV). (b) Time course of inhibition of $I_{Kr,tail}$ (0 mV) by 30 μM nisoldipine. (c) Dependence of I_{Kr} inhibition on nisoldipine concentration. Myocytes were treated for 7–10 min with one or two concentrations of the drug. The Hill equation fitting the data has an IC_{50} of 23±2 μM and a coefficient of 1.05. Numbers of observations are shown in parentheses.

Inhibition of I_{Kr}

The result with E4031 in Figure 2a indicated that the amplitudes of tail currents after 500-ms depolarisations to 0 mV could be used to evaluate the inhibition of I_{Kr} by nisoldipine. In practice, the amplitude of $I_{Kr,tail}$ was measured following 500-ms depolarisations to 0 mV at 0.1 Hz. The data in Figure 2b indicate that the inhibition by nisoldipine reached a steady state within 5 min. As illustrated by the significant recovery of current at 0 mV following 10-min washout of 100 μM drug in Figure 1d, the inhibition of I_{Kr} by high concentrations of nisoldipine was slowly reversible. For example, in four myocytes treated with 30 μM nisoldipine for 5–8 min, I_{Kr} recovered to 89±6% of predrug amplitude after a 10–15 min washout period.

The dependence of $I_{Kr,tail}$ inhibition on the concentration of nisoldipine is shown in Figure 2c. The lowest concentration that produced a significant inhibition was 3 μM (reduction to 90±2% of predrug control ($n=10$), $P<0.05$, paired t-test). The overall data are well described by the Hill equation with an IC_{50} of 23±2 μM and a coefficient of 1.05.

Inhibition of I_{Ks}

Dependence on drug concentration Inhibition of I_{Ks} by nisoldipine was evaluated in myocytes that were superfused with a solution (K^+-, Ca^{2+}-free plus 0.2 mM Cd^{2+}) that enhances I_{Ks} and suppresses I_{Kr} and Ca^{2+}-dependent currents (Sanguinetti & Jurkiewicz, 1990; Jones *et al*., 1998). Under these conditions, both the time-dependent currents elicited by 500-ms depolarisations and the subsequent tail currents at −30 mV were insensitive to 5 μM E4031 (Figure 3a). However, I_{Ks} isolated in this manner was rapidly inhibited by high concentrations of nisoldipine, and the inhibition was only slowly and partially reversible upon removal of the drug (Figure 3b). In four myocytes treated with 100 μM nisoldipine for 5 min, a 10-min washout period restored I_{Ks} to 73±5% of predrug control amplitude.

The dependence of I_{Ks} inhibition on nisoldipine concentration was evaluated from measurements of the amplitudes of tail currents after 2-s depolarisations to +70 mV before and during steady-state drug action (Figure 3c). The data from these determinations are well described by the Hill equation with an IC_{50} of 40±1 μM and a coefficient of 1.06 (Figure 3c, open circles).

The dependence of I_{Ks} inhibition on ⩾10 μM nisoldipine was also assessed from changes in the amplitude of time-dependent outward currents elicited by 500-ms depolarisations

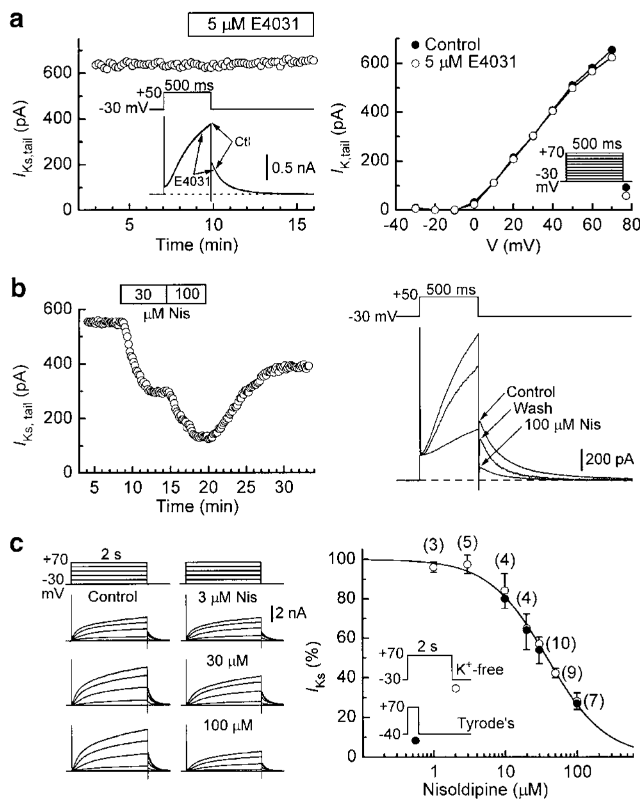

Figure 3 Inhibition of I_{Ks} by nisoldipine. Myocytes were bathed in K^+-, Ca^{2+}-free Cd^{2+} solution to suppress I_{Kr} and Ca^{2+}-dependent currents, and I_{Ks} was activated by 500-ms or 2-s depolarisations from −30 mV to more positive potentials at 0.1 Hz. (a) Results from two myocytes demonstrating the lack of effect of I_{Kr} inhibitor E4031 on the I_K activated under the foregoing experimental conditions. (b) Time course of inhibition of $I_{Ks,tail}$ by 30 and 100 μM nisoldipine. (c) Dependence of inhibition on nisoldipine concentration. Left: examples of records obtained from myocytes depolarised with 2-s pulses. Right: data (open symbols) obtained from myocytes exposed to one or two concentrations of the drug for 6–10 min each. Inhibition was measured from changes in the amplitudes of tail currents after 2-s depolarisations to +70 mV, and the Hill equation fitting the data has an IC_{50} of 40±1 μM and a coefficient of 1.07. Numbers of observations are shown in parentheses. For comparison, the plot also shows data (filled symbols) obtained when the inhibition of I_{Ks} was evaluated as the drug-induced change in the amplitude of time-dependent current elicited by 500-ms pulses to +70 mV in myocytes bathed in normal Tyrode's solution (n = 4–10).

to +70 mV in myocytes superfused with normal Tyrode's solution and pretreated with 1 μM nisoldipine (see Figure 1 for representative traces). The filled circles on the plot in Figure 3c indicate that the nisoldipine–I_{Ks} relationship determined in this manner was similar to that determined from measurements of tail I_{Ks} under K^+-free conditions.

Time- and voltage-dependent features

Inhibition of fast-activating types of delayed-rectifier K$^+$ current by dihydropyridines generally increases with time during a depolarising pulse to a positive potential (Gotoh *et al.*, 1991; Jahnel *et al.*, 1994; Zhang *et al.*, 1997). In the present study, we examined the effect of 100 μM nisoldipine on the time course of development of I_{Ks} during long (5 s) depolarisations to +50 mV. With the exception of the first 100–200 ms, the current records were satisfactorily fitted with single exponential functions (Figure 4a, left). Although the time constants of these functions were longer in the presence of the drug (587±37 ms) than in its absence (predrug, 424±31 ms; after washout, 518±23 ms) (n = 4 myocytes), the differences were not significant at the 0.05 level (paired t-tests). When records obtained before and after addition of nisoldipine were scaled and superimposed, it was evident that drug treatment did not modify the ratio of the amplitudes of activating I_{Ks} and $I_{Ks,tail}$ (Figure 4a, right). The same figure indicates that the time course of $I_{Ks,tail}$ was also unchanged.

To determine whether block of I_{Ks} by nisoldipine has classical use-dependent features, myocytes were stimulated at 0.1 Hz (500-ms pulses from −30 to +50 mV), rested at −80 mV, exposed to 100 μM drug, and restimulated 4 min later. The records and time plot in Figure 4b indicate that near-maximal inhibition was reached on the first postrest depolarisation. In four experiments of this type, tail I_{Ks} on the first postrest pulse was 32±6% of predrug control, and tail I_{Ks} on the 30th postrest pulse was a similar 33±4%.

The dependence of block on I_{Ks}-activating voltage was determined by measuring the amplitudes of tail currents elicited after 2-s depolarisations to different test potentials. Data from a representative myocyte illustrate that, in contrast to low concentrations of the drug, 30 μM nisoldipine decreased the amplitude of tail currents after depolarisations to all test voltages between +10 and +90 mV (Figure 5a). When tail I_{Ks}–V data from six experiments with 30 μM nisoldipine were normalised, it was evident that tails after small depolarisations

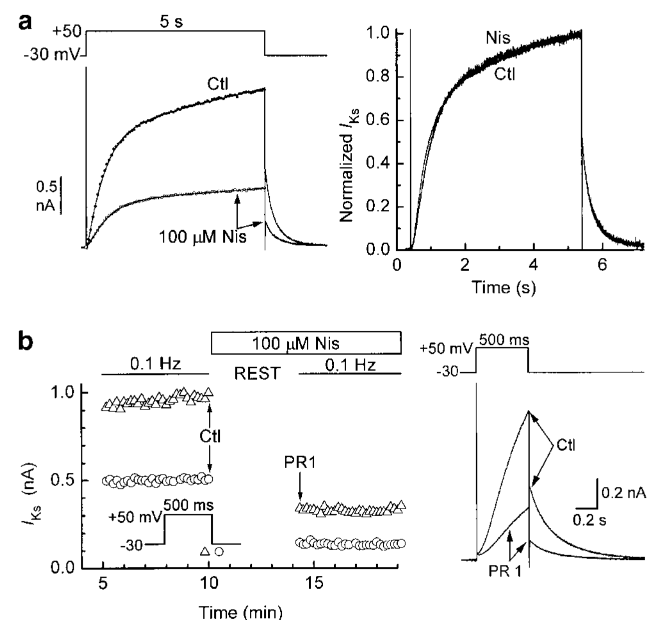

Figure 4 Characteristics of nisoldipine block of I_{Ks} in myocytes bathed in K^+-, Ca^{2+}-free Cd^{2+} solution. (a) Lack of significant effect of 100 μM nisoldipine on the time courses of I_{Ks} activation and deactivation. Left: original records whose activation time courses have been fitted with single exponentials (small dots), having time constants of 488 ms (control) and 576 ms (nisoldipine). Right: superimposition after scaling of the nisoldipine record to the control record. (b) Effect of rest on the development of block. Left: measurements of I_{Ks} amplitudes. Right: records obtained on the last pulse before rest (Ctl) and the first postrest pulse (PR 1).

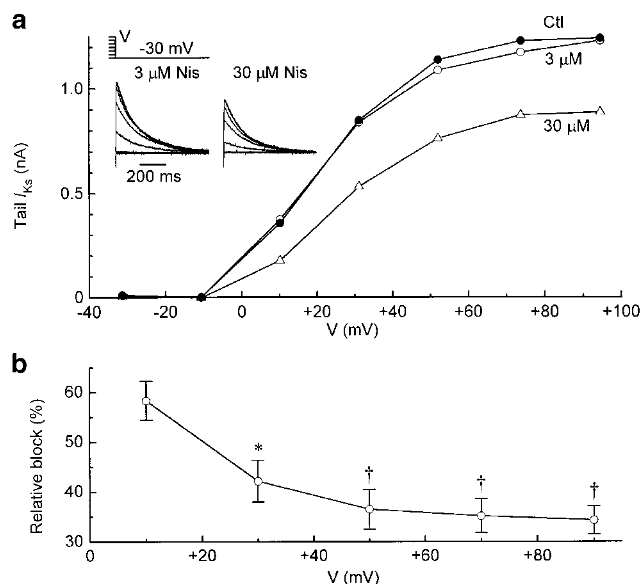

Figure 5 Dependence of nisoldipine block of I_{Ks} on the voltage of the activating pulse. Myocytes were bathed in K^+-, Ca^{2+}-free Cd^{2+} solution and depolarised for 2 s to potentials V for evaluation of $I_{Ks,tail}$ amplitude on repolarisation. (a) Records and $I_{Ks,tail}$ analysis from a myocyte exposed to 3 μM and then 30 μM nisoldipine. (b) Dependence of the degree of block on voltage. The data are from six myocytes that were exposed to 30 μM nisoldipine. *$P<0.05$, ‡$P<0.01$ (*versus* +10 mV data).

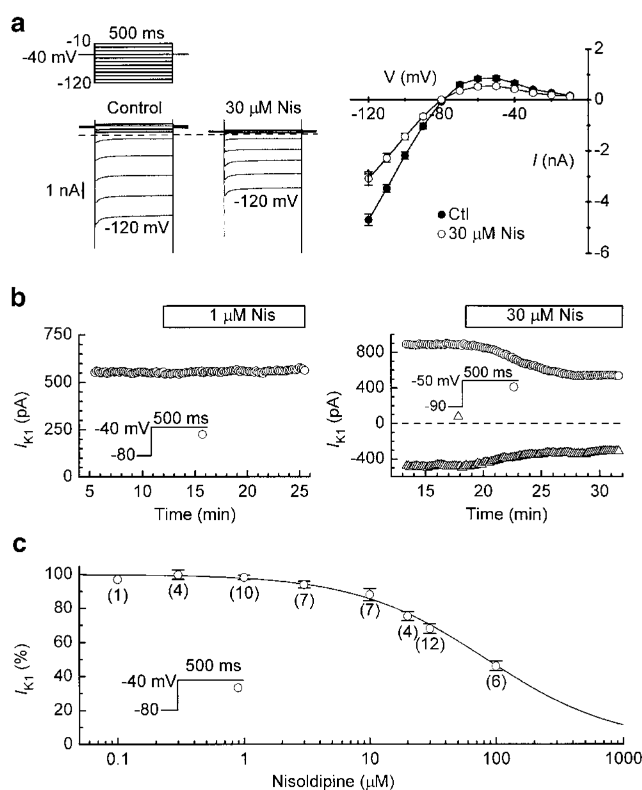

Figure 6 Inhibition of I_{K1} by nisoldipine. Myocytes were bathed in Tyrode's solution that contained 0.2 mM Cd^{2+}, and held at −80 or −90 mV. (a) Effects of 30 μM nisoldipine on I_{K1} measured as the end-of-pulse current amplitude on 500-ms pulses from prepulse −40 mV to potentials between −120 and −10 mV. Left: records from a representative experiment; right: summary of data from four experiments. †$P<0.01$ (multiple comparison test at −120 mV). (b) Time courses of changes in two representative experiments. Left: lack of effect of 1 μM nisoldipine on outward-directed I_{K1} at −40 mV; right: inhibition of inward (−90 mV) and outward (−50 mV) I_{K1} by 30 μM nisoldipine. (c) Dependence of inhibition of outward I_{K1} on the concentration of nisoldipine. The Hill equation fitting the data has an IC_{50} of 80 ± 3 μM and a coefficient of 0.83. Noncumulative drug exposures lasted for 8–10 min. Numbers of myocytes are shown in parentheses.

were inhibited to a greater degree than tails after large depolarisations. As depicted in Figure 5b, the relative block declined from 0.58 ± 0.04 at +10 mV to 0.34 ± 0.03 at +90 mV, with most of the voltage dependence arising between +10 and +50 mV. Analysis of the data (one-way ANOVA followed by Dunnett's multiple comparison test) indicates that block at +10 mV was significantly larger than block at +30, +50, +70, and +90 mV ($P<0.05$–0.01).

Inhibition of I_{K1}

When myocytes were bathed in Tyrode's solution that contained 0.2 mM Cd^{2+} (to block $I_{Ca,L}$), 500-ms pulses from −40 mV to potentials between −120 and −10 mV elicited currents that were large and inward up to −80 mV, and smaller, outward, and quasi-time-independent at more positive potentials (Figure 6a and b); these features, and the negative slope in the I–V relationship at <-50 mV, are typical for I_{K1} over this voltage region (Jones *et al*., 1999). High concentrations of nisoldipine lowered the end-of-pulse amplitudes of both inward and outward I_{K1}; in four myocytes, 30 μM nisoldipine inhibited the inward current at −120 mV by 34 ± 4%, and the outward current at −40 mV by 35 ± 5% (both $P<0.01$, multiple comparison test). Inhibition caused by $\leqslant 30$ μM nisoldipine was only partially reversed by washout of the drug for ca. 10 min (not shown).

The time course and concentration dependence of nisoldipine-induced inhibitions of outward-directed I_{K1} were evaluated by measuring the outward current amplitude at −40 mV (Jones *et al*., 1999). The time plots in Figure 6b indicate that outward I_{K1} at −40 mV was generally stable over tens of minutes, and unaffected by 1 μM nisoldipine, but quickly inhibited by 30 μM nisoldipine. The steady-state effects of the drug are well described by the Hill equation with an IC_{50} of 80 ± 3 μM and a coefficient of 0.83 (Figure 6c).

Inhibition of $I_{Ca,L}$

For comparison with the foregoing data on the inhibition of K^+ currents by nisoldipine, we determined concentration–response relations for inhibition of $I_{Ca,L}$ by the drug. To obtain data that might be applicable to inhibition during an action potential in fully polarised ventricular myocytes, a group of myocytes was bathed in Na^+-free Tyrode's solution to eliminate Na^+ current, held at −90 mV, and depolarised to 0 mV for 200 ms at 0.1 Hz. The myocytes were pretreated with 3 μM E4031 to suppress I_{Kr}, and exposed to 0.4 mM Cd^{2+} after 5–7 min nisoldipine to establish the background current level. A second group of myocytes was bathed in normal Tyrode's solution, and subjected to a voltage protocol similar to that used in many of the experiments on K^+ currents. These

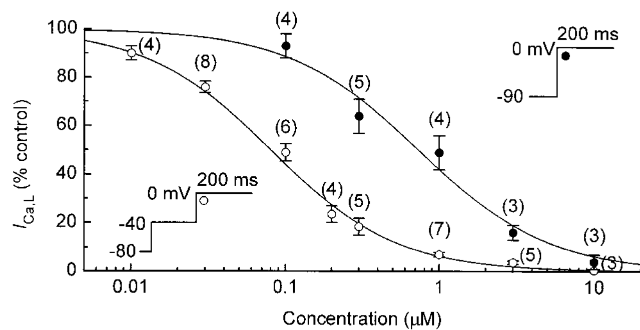

Figure 7 Inhibition of $I_{Ca,L}$ by nisoldipine. Myocytes were either bathed in Na^+-free Tyrode's held at −90 mV, and depolarised to 0 mV for 200 ms (0.1 Hz), or bathed in normal Tyrode's solution, held at −80 mV and depolarised by a prepulse to −40 to 0 mV for 200 ms (0.1 Hz). At 5 min postpatch, the myocytes were pretreated with 3 μM E4031 for 3 min and then exposed to single concentrations of nisoldipine for 5–7 min prior to application of 0.4 mM Cd^{2+} to establish the background current level at 0 mV. Peak $I_{Ca,L}$ was measured as the background current level minus inward peak level. The Hill equation fitting the data obtained from myocytes held at −90 mV has an IC_{50} of 0.73±0.13 μM and a coefficient of 1.03. The fit of the data from the other group of myocytes has an IC_{50} of 0.08±0.01 μM and a coefficient of 1.07.

myocytes were depolarised by a prepulse to −40 mV for 200 ms to inactivate Na^+ current, and then pulsed to 0 mV at 0.1 Hz; they were also treated with 3 μM E4031 and exposed to Cs^{2+} after the effect of nisoldipine had reached a steady state.

The results obtained from the two groups of myocytes are summarised in Figure 7. The data from the group held at −90 mV are described by the Hill equation with IC_{50} of 0.73±0.13 μM and a coefficient of 1.03, whereas the data from the other group have an IC_{50} of 0.08±0.01 μM and a coefficient of 1.07. The difference in IC_{50} is an indication of the voltage dependence of nisoldipine action on L-type Ca^{2+} channels (Kass, 1982; McDonald *et al.*, 1994).

Discussion

The present study provides new information on the sensitivity of cardiac inward-rectifier and delayed-rectifier K^+ channels to inhibition by nisoldipine. The results are discussed and compared with findings from previous studies on interactions between nisoldipine and K^+ channels.

Effects on inwardly rectifying K^+ current

Nisoldipine inhibited guinea-pig ventricular I_{K1} with an IC_{50} of 80 μM. The inhibition appeared to be independent of voltage and direction of the current, and was at least partially reversible with prolonged washout of the drug. In an earlier study on nisoldipine, Kass (1982) observed that concentrations up to 100 μM had little effect on outward I_{K1} in cardiac Purkinje fibres. A possible reason for the discrepancy with the present findings is that the Purkinje fibres had been injected with tetrabutylammonium bromide to block I_{to} prior to the trials with nisoldipine. The pretreatment may have interfered with the measurements because the quaternary compound is itself a blocker of inwardly rectifying K^+ current (Barros *et al.*, 1992).

Inhibition of delayed-rectifier K^+ currents

Fast-activating currents Dihydropyridine Ca^{2+}-channel blockers have previously been shown to inhibit fast-activating types of delayed-rectifier K^+ currents in a variety of noncardiac cells. In most cases, nifedipine was the drug under investigation (e.g., Nishi *et al.*, 1983; Gola & Ducreux, 1985; Jacobs & DeCoursey, 1990; Tatsuta *et al.*, 1994). However, Nerbonne & Gurney (1987) included nisoldipine in their study on fast-activating inactivating K^+ current in *Aplysia* bag cell neurons, and reported that the IC_{50} of the drug was ca. 4 μM.

The fast-activating inactivating I_{to} in rabbit atrial myocytes has also been found to be quite sensitive to nisoldipine (IC_{50} 4.7 μM) (Gotoh *et al.*, 1991).

Slower-activating cardiac K^+ currents There have been two previous studies on the effects of nisoldipine on slower-activating global I_K in cardiac cells. In the first of these, Kass (1982) found that 10 μM nisoldipine had no effect on I_K (I_x) elicited by 1-s depolarisations of cardiac Purkinje fibres. Subsequently, Hume (1985) reported that 10 μM nisoldipine reduced I_K in frog atrial myocytes by about 40%. In relation to the present findings on I_{Kr} and I_{Ks}, it is important to note that frog atrial I_K has been characterised as a single K^+ conductance (Hume & Giles, 1983) that resembles I_{Ks} in some respects (slowly activating, noninactivating), but not in others (half-activation near 0 mV, saturation at ca. +40 mV) (see Hume, 1985). Thus, direct comparison of the findings on guinea-pig ventricular I_{Kr} (IC_{50} 23 μM) and I_{Ks} (IC_{50} 40 μM) with those on frog atrial I_K (IC_{50} 16 μM) may not be justified. Nevertheless, these determinations taken together indicate that nisoldipine has a weaker action on slower-activating types of cardiac delayed-rectifier K^+ channels than has been reported for fast-activating I_{to}-carrying channels by Gotoh *et al.* (1991).

Characteristics of I_{Ks} block

Block of I_{Ks} by nisoldipine had the following features: (i) the time course of nonblocked current in the presence of the drug was similar to that of current in the absence of the drug, (ii) block was fully established on the first pulse after a rest in the presence of the drug, and (iii) the degree of block was dependent on the voltage of the activating depolarisation. The first two features suggest (but do not prove) that, in contrast to dihydropyridine block of cardiac I_{to} (Gotoh *et al.*, 1991; Jahnel *et al.*, 1994) and hKv1.5 current (Zhang *et al.*, 1997), block of I_{Ks} by nisoldipine occurs *via* binding of drug molecules to closed rather than open channels.

A dependence of dihydropyridine block on voltage has been ruled out in some studies on noncardiac delayed-rectifier K^+ channels (e.g., Nerbonne & Gurney, 1987; Barros *et al.*, 1992; Pappone & Ortiz-Miranda, 1993), but has been detected in two previous studies on cardiac delayed-rectifier channels. Hume (1985) observed that inhibition of frog atrial I_K by 10 μM nisoldipine was weaker on depolarisations to positive potentials than to negative ones, and Zhang *et al.* (1997) reported that block of hKv1.5 channels by 30 μM nifedipine was weaker at potentials above +20 mV. In the present case, the block of I_{Ks} by 30 μM nisoldipine was approximately 40% weaker at $\geqslant$ +50 mV than at +10 mV. Since nisoldipine has a very low pK_a and is almost completely in the neutral form at pH 7.4, it is difficult to view the voltage dependency in terms of charged

drug molecules under the influence of the electrical field. However, Hume (1985) noted that this type of voltage-dependent block could be due to a drug-induced positive shift of the voltage dependence of current activation. It seems unlikely that a shift is the major cause of the voltage dependence of the I_{Ks} block studied here, because the time course of I_{Ks} at $+50$ mV was little affected by 100 μM nisoldipine. Rather, the voltage dependence may arise from a coupling of the binding site to a voltage-sensitive process such that unbinding is enhanced at more positive potentials. As proposed by Zhang *et al.* (1997) for nifedipine block of cardiac hKv1.5 channels, the dihydropyridine site on Ks channels may be near the external mouth of the pore, and relief of block may be linked to increased K^+ permeation at more positive voltages.

Selectivity of nisoldipine action on cardiac K^+ and Ca^{2+} channels

The IC_{50} values for nisoldipine block of I_{Kr}, I_{Ks}, and I_{K1} were 23, 40, and 80 μM, respectively. Thus, nisoldipine is far from being a potent blocker of these K^+ pathways. This is particularly the case with regard to I_{Kr} because there are numerous clinical and experimental drugs that inhibit this current with an $IC_{50} < 1\,\mu$M (e.g., Sanguinetti & Jurkiewicz, 1990; Carmeliet, 1992; Salata *et al.*, 1995; Roden, 1996; Yang *et al.*, 1997; Jones *et al.*, 1998; Zhang *et al.*, 1999). Nisoldipine is a potent blocker of L-type Ca^{2+} channels in cardiac cells, and is commonly used to abolish $I_{Ca,L}$ in electrophysiological studies on these cells. The present results indicate that any spillover effects on K^+ currents are likely to be negligible when the drug is used to abolish $I_{Ca,L}$ in studies on guinea-pig ventricular myocytes. The placing of a numerical value on the relative selectivity of nisoldipine for its binding site in L-type Ca^{2+} channels over those in delayed-rectifier K^+ channels is complicated by the fact that binding to the Ca^{2+} channels is strongly dependent on the holding potential (Sanguinetti & Kass, 1984; McDonald *et al.*, 1994). For this reason, the IC_{50} for $I_{Ca,L}$ in cardiac Purkinje fibres declined from 1.34 μM at a holding potential -80 mV, to ca. 0.2 μM at -50 mV, and a calculated 0.001 μM at ca. -20 mV (Sanguinetti & Kass, 1984).

In the present study, the IC_{50} for block of $I_{Ca,L}$ at 0 mV was $0.73 \pm 0.13\,\mu$M when myocytes were held at -90 mV, and a much lower $0.08 \pm 0.1\,\mu$M when myocytes were held at -80 mV and depolarised by a prepulse to -40 mV for 200 ms prior to the test pulses to 0 mV. Since the block of K^+ currents was not influenced by holding potential, the relative selectivity of nisoldipine for block of Ca^{2+} channels over delayed-rectifier channels is estimated to be about 30 when well-polarised myocytes undergo rapid depolarisation to a plateau potential (for example, during an action potential). The selectivity increases to near 400 when depolarisation to a plateau potential occurs after a prepulse from -80 to -40 mV (a protocol frequently used in electrophysiological studies). The latter estimate of relative selectivity is close to the 1000-fold value estimated by Hume (1985), whose reference IC_{50} for frog atrial $I_{Ca,L}$ was 0.016 μM (holding potential -60 mV).

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