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Selective phenylalkylamine block of I_{Kr} over other K^+ currents in guinea-pig ventricular myocytes

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1 Previous studies on verapamil and D600 have established that the Ca^{2+}-channel blockers also inhibit delayed-rectifier K^+ currents in cardiac tissues and myocytes. However, estimated IC_{50} values range over two to three orders of concentration, and it is unclear whether this reflects a high selectivity by one or both of the phenylalkylamines for particular K^+ channels. The purpose of the present study was to determine the concentration-dependent actions of verapamil and D600 on three defined cardiac K^+ currents.

2 Guinea-pig ventricular myocytes in the conventional whole-cell configuration were bathed with normal Tyrode's or K^+-free solution, and pulsed from −80 mV for measurement of the effects of 0.01 μM to 3 mM verapamil and D600 on the inwardly-rectifying K^+ current (I_{K1}) and the two delayed-rectifier K^+ currents, rapidly-activating I_{Kr} and slowly-activating I_{Ks}.

3 The phenylalkylamines inhibited both inward- and outward-directed I_{K1}. The IC_{50} values for outward I_{K1} were approximately 220 μM.

4 Verapamil and D600 were approximately equipotent inhibitors of the delayed-rectifier K^+ currents. They inhibited I_{Kr} with IC_{50} near 3 μM, and I_{Ks} with IC_{50} $\geqslant$280 μM. These results are discussed in relation to previous findings on K^+ currents and to the clinical actions of the drugs.
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Keywords: Guinea-pig ventricular myocytes; delayed-rectifier K^+currents; inward-rectifying K^+ current; verapamil; D600; nisoldipine

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

Early voltage-clamp studies on the effects of D600 on membrane currents in multicellular cardiac preparations indicated that in addition to a primary inhibitory action on inward Ca^{2+} current, the phenylalkylamine inhibited the delayed-rectifier K^+ current (I_K) (Kass & Tsien, 1975; Nawrath *et al*., 1977; McDonald *et al*., 1984a,b). Subsequently, Hume (1985) examined the inhibitory actions of D600 on L-type Ca^{2+} current ($I_{Ca,L}$) and I_K in frog atrial myocytes, and reported that both currents were inhibited by the drug. However, the IC_{50} for I_K was remarkably high (820 μM) considering that the earlier findings of substantial block of I_K (I_x) in calf Purkinje fibres (Kass & Tsien, 1975) and I_K in cat ventricular muscles (Nawrath *et al*., 1977; McDonald *et al*., 1984a) were obtained with concentrations $\leqslant$10 μM. One plausible explanation for the divergent results is that the delayed-rectifier K^+ currents comprising global I_K in the mammalian cardiac tissues are different than the current(s) that makes up global I_K in frog atrial myocytes. In that regard, other types of cardiac and non-cardiac delayed-rectifier K^+ current differ widely in their sensitivities to the closely-related phenylalkylamine, verapamil (IC_{50} values ranging from 4 to 200 μM: see Discussion).

The present study on guinea-pig ventricular myocytes was performed to evaluate the effects of verapamil and D600 on three K^+ currents: the two components, rapidly-activating I_{Kr} and slowly-activating I_{Ks}, of the global delayed-rectifier I_K that is responsible for terminating the plateau of the action potential (Sanguinetti & Jurkiewicz, 1990; Luo & Rudy, 1994), and the inwardly-rectifying K^+ current (I_{K1}) responsible for setting the resting potential (Imaizumi & Giles, 1987). The results show that verapamil and D600 are approximately equipotent in their inhibitory actions on these currents, and that they are much more potent inhibitors of I_{Kr} than of I_{Ks} and I_{K1}.

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Methods

All procedures were carried out in accordance with 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 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 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 digitized 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

Verapamil and D600 were obtained from Sigma-Aldrich (Oakville, ON, Canada), and dissolved in water. Nisoldipine was kindly provided by Bayer Inc. (Etobicoke, ON, Canada), and dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St. Louis MO, U.S.A.) (0.1 M stock solution). Stock solutions were stored in the dark at −20°C, freshly added to bathing solutions, and protected from the light during experiments. E4031 was obtained from Eisai (Tokyo, Japan) and dissolved in the bathing solution.

Statistics

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

Results

Effects of verapamil and D600 on net membrane currents

Figure 1a shows records obtained from myocytes before and approximately 10 min after exposures to verapamil. The myocytes were bathed in normal Tyrode's solution, held at −80 mV, and depolarized for 500 ms from prepulse −40 mV to potentials up to +70 mV. The control records indicate that the depolarizations elicited inward $I_{Ca,L}$ that reached maximal amplitude near 0 mV, and time-dependent outward I_K that increased with positive potential and deactivated on subsequent repolarizations to −40 mV. Exposure to 10 μM verapamil resulted in a strong inhibition of $I_{Ca,L}$, but had little effect on time-dependent I_K activated at positive potentials. However, the drug had a marked inhibitory effect on the amplitude of tail currents ($I_{K,tail}$) at −40 mV. The

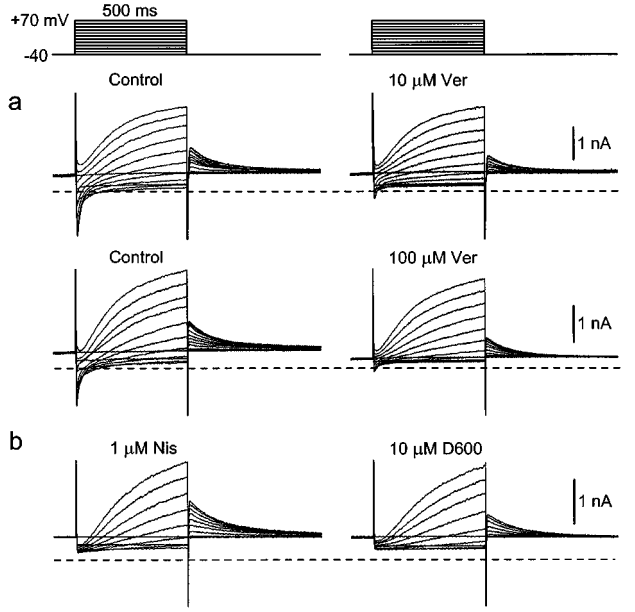

Figure 1 Effects of verapamil and D600 on membrane currents in guinea-pig ventricular myocytes. The myocytes were bathed in normal Tyrode's solution, held at −80 mV, depolarized to prepulse −40 mV for 200 ms, and then depolarized to more positive potentials for 500 ms at 0.1 Hz; tail currents ($I_{K,tail}$) were recorded on repolarizations to −40 mV. (a) Near-complete inhibition of inward $I_{Ca,L}$ and partial inhibition of $I_{K,tail}$ after 10-min exposures of myocytes to 10 μM (top) and 100 μM (bottom) verapamil. (b) Effect of 10 μM D600 on membrane currents in a myocyte pretreated with 1 μM nisoldipine to suppress $I_{Ca,L}$. The dashed lines on the records here and in other figures indicate zero-current levels.

only obvious additional effect of an exposure to a 10 fold higher concentration of the drug was a reduction in the outward current amplitude at prepulse −40 mV. The actions of D600 (10 and 100 μM) were similar to those of verapamil (see below).

The dependence of the inhibition of $I_{K,tail}$ on the concentration of phenylalkylamine was not unlike that for inhibition of $I_{Ca,L}$. To establish whether inhibition of $I_{Ca,L}$ was involved in the inhibition of $I_{K,tail}$, myocytes were treated with 1 μM nisoldipine prior to application of phenylalkylamine. Nisoldipine pretreatment had no significant effect on tail current amplitude (not shown), but largely abolished $I_{Ca,L}$ (Figure 1b). However, this action did not occlude inhibition of $I_{K,tail}$ by 10 μM D600 in this (Figure 1b), or other experiments (see below). Thus, inhibition of $I_{K,tail}$ by phenylalkylamines was not causally related to drug-induced changes in $I_{Ca,L}$.

The inhibitory effects of phenylalkylamines on $I_{K,tail}$ after 500-ms depolarizations were evident at concentrations considerably lower than those cited above, and were slowly reversed after removal of the drug. These points are illustrated by the families of tail currents (Figure 2a, top) recorded from a myocyte that was sequentially exposed to solutions that contained 1, 5 and 20 μM verapamil, and then washed with normal Tyrode's solution. Plots of $I_{K,tail}$ amplitude versus the voltage of the I_K-activating depolarization ($I_{K,tail}$–V) indicate that these concentrations of verapamil primarily depressed $I_{K,tail}$ elicited after depolarizations to voltages below +20 mV, i.e., they had little effect on the large increment in $I_{K,tail}$ amplitude elicited by depolarizations to more positive test voltages (Figure 2a, bottom). The selective inhibition of the low-voltage phase of the $I_{K,tail}$–V relationship was also observed in five experiments on

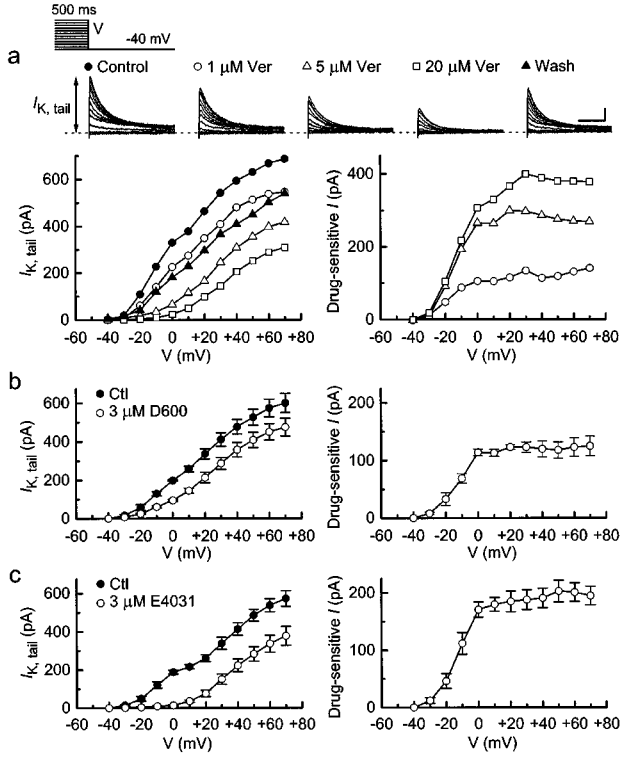

Figure 2 $I_{K,tail}-V$ relationships from myocytes treated with verapamil, D600, and E4031. The myocytes were bathed in normal Tyrode's solution, and tail currents on repolarizations to -40 mV after 500-ms pulses to potentials V were recorded before and 7–10 min after addition of drug. (a) Top: tail currents obtained from a myocyte that was sequentially exposed to 1, 5 and 20 μM verapamil, and then washed for 10 min with drug-free solution. The dotted line indicates steady current at -40 mV, and the calibration bars indicate 200 pA and 200 mS. Left hand plot: $I_{K,tail}-V$ relationships obtained from measurements of the $I_{K,tail}$ amplitudes. Right hand plot: verapamil-sensitive current obtained by subtracting drug data from control data. (b) Effects of 3 μM D600. Left: mean $I_{K,tail}-V$ relationships from five experiments. Right: D600-sensitive current. The latter data are significantly different than zero ($P<0.01$) at all potentials $\geqslant -10$ mV (one-way ANOVA and Dunnetts Multiple Comparison test). (c) Effects of the selective I_{Kr} inhibitor E4031 (3 μM) for comparison with those of the phenylalkylamines. Left: mean $I_{K,tail}-V$ relationships from five experiments. Right: E4031-sensitive current. The latter data are significantly different than zero ($P<0.01$) at all potentials $\geqslant -10$ mV.

myocytes that were pretreated with nisoldipine and then exposed to 3 μM D600 (Figure 2b). In these experiments, the amplitude of the D600-sensitive $I_{K,tail}$ was no larger after test depolarizations to $+70$ mV (125 ± 17 pA) than after test depolarizations to 0 mV (114 ± 6 pA). This pattern of inhibition of low-voltage $I_{K,tail}$ was similar to that observed in experiments with 3 μM E4031 (Figure 2c), a specific inhibitor of I_{Kr} (Sanguinetti & Jurkiewicz, 1990; Heath & Terrar, 1996).

Inhibition of I_{Kr}

Based on the voltage-dependence of the inhibition of $I_{K,tail}$ by E4031 (Figure 2C), the inhibition of I_{Kr} by phenylalkylamines was investigated by measuring the amplitudes of $I_{K,tail}$ elicited after depolarizations from prepulse -40 mV to 0 mV at 0.1 Hz. The depolarizations were of relatively short duration (200 ms) to minimize any concomitant activation of I_{Ks}. Figure 3a shows records and analysis of $I_{Kr,tail}$ from an experiment with 1 and 10 μM verapamil. The 1 μM concentration lowered $I_{Kr,tail}$ amplitude to 65% control within 2 min, and the higher concentration quickly reduced it to near 20% control. Subsequent washout for 5 min restored the amplitude to 75% of the predrug level.

Measurements of $I_{Kr,tail}$ from a myocyte pretreated with nisoldipine and exposed to 10 μM D600 (Figure 3b, left) indicate the drug was approximately equipotent with verapamil, and that its action was almost fully reversible. The records in Figure 3b (right) indicate that the outward current levels recorded on the I_{Kr}-activating pulses to 0 mV changed in a manner consistent with the changes in $I_{Kr,tail}$ amplitude.

The steady-state data from these and similar experiments on $I_{Kr,tail}$ are provided in Figure 3c. The phenylalkylamines had significant inhibitory effects at concentrations as low as 0.3 μM (e.g. verapamil: reduction to 89 ± 2% control ($n=9$), $P<0.001$, paired t-test), and almost completely abolished the current at concentrations $\geqslant 30$ μM. Verapamil and D600 were roughly equipotent, and the dependencies of inhibition on drug concentration were well described by the Hill equation with respective IC_{50} values of 2.6 ± 0.2 and 2.7 ± 0.2 μM, and Hill coefficients of 0.95 and 0.96.

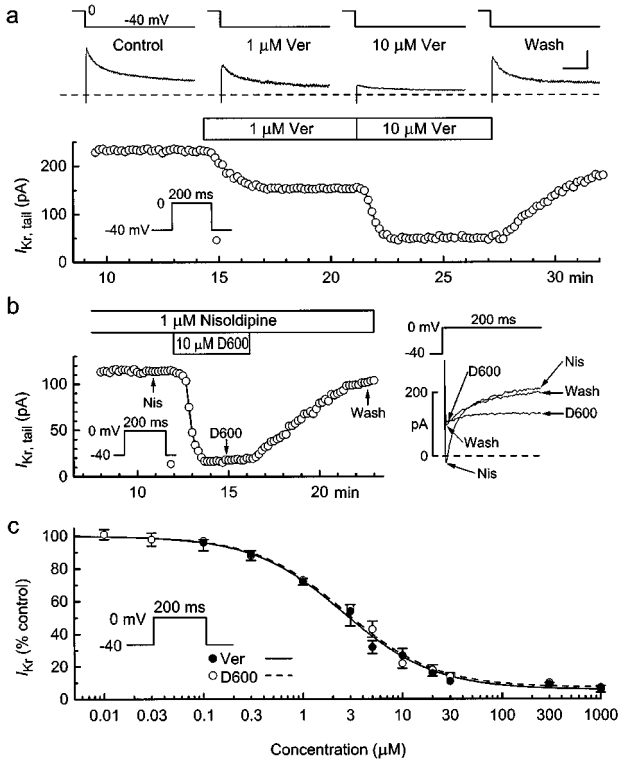

Figure 3 Inhibition of I_{Kr} by verapamil and D600. Myocytes bathed in Tyrode's solution were held at -80 mV and depolarized for 200 ms from prepulse -40 mV to 0 mV at 0.1 Hz; $I_{Kr,tail}$ was measured on the repolarizations to -40 mV. (a) Time course of changes in $I_{Kr,tail}$ amplitude before, during and after exposure of a myocyte to 1 and 10 μM verapamil. (b) Effects of 10 μM D600 in a myocyte pretreated with 1 μM nisoldipine. Left: time course of changes in $I_{Kr,tail}$. Right: superimposition of currents elicited by the 200-ms pulses to 0 mV at the times marked on the time plot. Note the inhibition and recovery of outward current at 200 ms. (c) Dependence of I_{Kr} inhibition on drug concentration. The Hill equation fitting the verapamil data has an IC_{50} of 2.6 ± 0.2 μM and a Hill coefficient of 0.95 (solid curve); the parameters describing the D600 data are 2.7 ± 0.2 μM and 0.96 (dashed curve), respectively. Myocytes were exposed to one or two concentrations of a drug for 6–10 min, and the numbers of observations ranged from 4 to 20.

Inhibition of I_{Ks}

The $I_{K,tail}-V$ data in Figure 2c indicate that the increments in $I_{K,tail}$ amplitude produced by pulses to potentials above 0 mV were little affected by E4031. Thus, the increments were primarily due to the activation of E4031-resistant I_{Ks}. This result suggested that phenylalkylamine action on I_{Ks} could be evaluated from measurements of the increment. As noted earlier in connection with Figure 2a,b, the increment was little affected by phenylalkylamine concentrations up to 20 μM. However, the $I_{K,tail}-V$ relationships in Figure 4a,b illustrate that increments were depressed by high concentrations such as 300 and 1000 μM.

The amplitude of I_{Ks} was taken as the amplitude of the increment obtained by subtraction of the amplitude of $I_{K,tail}$ on the test pulse to 0 mV from that on the test pulse to +70 mV (see Figure 4b). The data obtained from experiments with verapamil are well described by the Hill equation with an IC_{50} of 1080 ± 240 μM and a coefficient of 0.87, and similar parameters are likely to apply to D600 action (Figure 4c).

The inhibitory effects of phenylalkylamines on I_{Ks} were also evaluated under conditions where I_{Kr} was suppressed and I_{Ks} was enhanced (K^+-free Tyrode's solution that contained 0.2 mM Cd^{2+} (Sanguinetti & Jurkiewicz, 1992)). Figure 5a,b indicate that 200–500 μM concentrations of verapamil and D600 had large, rapid inhibitory effects on $I_{Ks,tail}$ activated by 500-ms pulses to +50 mV, and that these were largely reversed after removal of the drug. To evaluate the dependence of the inhibition on drug concentration, tail currents elicited after response to stronger activations of I_{Ks} (2-s depolarizations) were measured before and after steady-state drug action. Representative data obtained in experiments with 100 and 500 μM verapamil are shown in Figure 6a,b. These concentrations reduced the amplitude of $I_{Ks,tail}$ by approximately 25 and 67% respectively, and the degree of inhibition was independent of test voltage between +10 and +70 mV.

The dependence of inhibition on phenylalkylamine concentration was assessed from measurements of $I_{Ks,tail}$ amplitude following 2-s depolarizations to +70 mV. The data in Figure 6c indicate that 100-μM concentrations of phenylalkylamine caused significant ($P<0.001$) inhibition (e.g. $28\pm4\%$ ($n=7$) with verapamil), and that millimolar concentrations almost completely abolished the current (e.g., inhibition of $91\pm2\%$ ($n=3$) with 3 mM verapamil). The Hill equation describing the verapamil data has an IC_{50} of 280 ± 26 μM and a Hill coefficient of 0.96 (Figure 6c).

Inhibition of I_{K1}

Figure 7a shows current traces obtained before and 9 min after the addition of 300 μM verapamil to a myocyte bathed in Tyrode's solution that contained 0.2 mM Cd^{2+}. The currents were elicited by 500-ms pulses from prepulse −40 mV to potentials between −120 and 0 mV. The $I-V$ plot in Figure 7a indicates that the voltage dependence of the current was typical of I_{K1}, and that the current had a reversal potential near −80 mV. Although verapamil had little effect on the reversal potential, it lowered the amplitude of the current at all other potentials. However, the inhibitory action

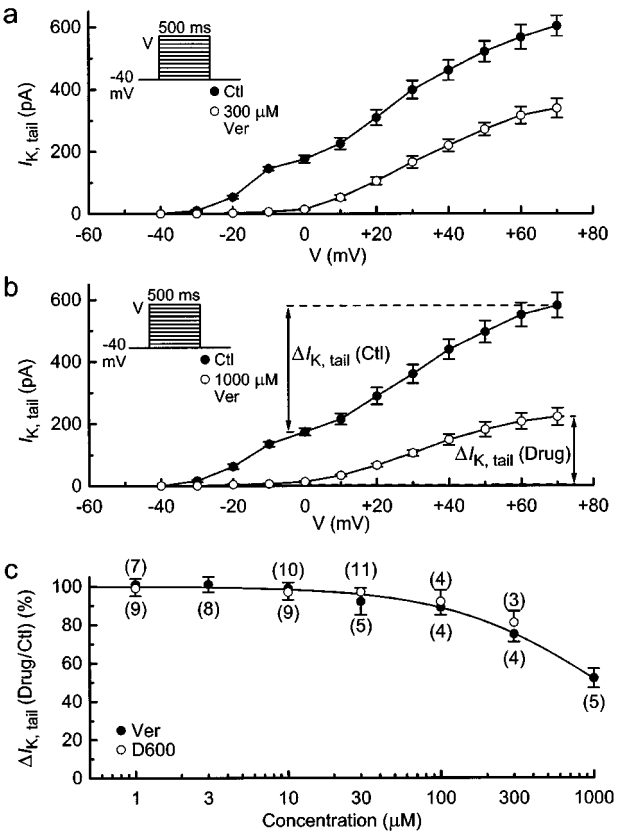

Figure 4 Phenylalkylamine-induced inhibition of I_{Ks} in myocytes bathed with normal Tyrode's solution. The myocytes were held at −80 mV and depolarized from prepulse −40 mV to more positive potentials V for 500 ms at 0.1 Hz for measurement of $I_{K,tail}$ on repolarizations to −40 mV. (a) The effect of 300 μM verapamil ($n=5$) on the $I_{K\text{-}tail}-V$ relationship. (b) The effect of 1000 μM on the $I_{K,tail}-V$ relationship. Also depicted are the measurements taken for calculation of the effect of drug on the amplitude of I_{Ks}. (c) Dependence of I_{Ks} inhibition on drug concentration. The Hill equation fitting the verapamil data has an IC_{50} of 1080 ± 240 μM and a coefficient of 0.87. Myocytes were exposed to one or two concentrations of a drug for 6–10 min, and the numbers of observations are shown in parentheses.

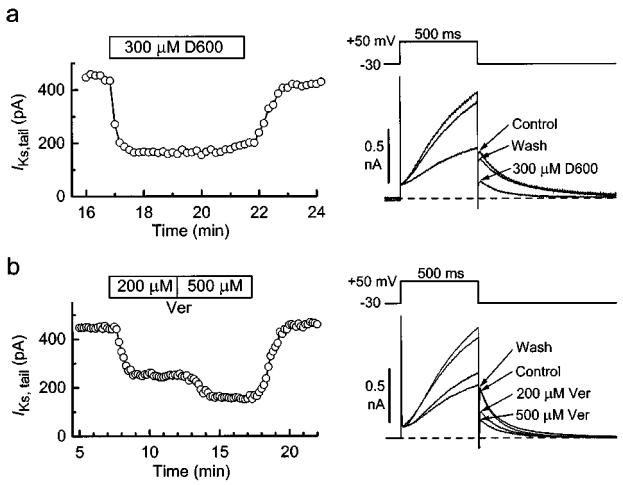

Figure 5 Time course and reversibility of phenylalkylamine-induced inhibition of I_{Ks} in myocytes bathed with K^+-, Ca^{2+}-free Cd^{2+} solution. (a) Inhibition by 300 μM D600. The myocytes were held at −30 mV and depolarized to +50 mV for 500 ms at 0.1 Hz. Left: changes in the amplitude of $I_{Ks,tail}$. Right: example records from this experiment; the records on the 500-ms pulses are (top to bottom) control, wash, and D600. (b) Inhibition by 200 and 500 μM verapamil. The records on the 500-ms pulses are (top to bottom) wash, control, 200 and 500 μM verapamil.

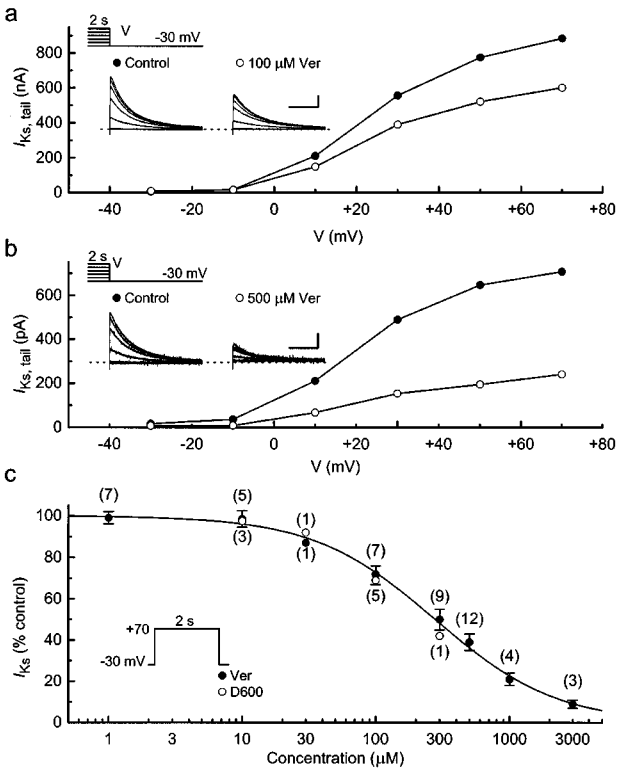

Figure 6 Phenylalkylamine concentration-dependent inhibition of I_{Ks} in myocytes bathed in K^+-, Ca^{2+}-free Cd^{2+} solution. The myocytes were held at -30 mV and depolarized to more positive potentials for 2-s at 0.1 Hz. (a,b) Records and $I_{K,tail}-V$ relationships from representative experiments. The calibration bars beside the records indicate 200 pA and 200 ms. (c) Dependence of inhibition on drug concentration. The amplitude of $I_{Ks,tail}$ elicited after 2-s depolarizations to $+70$ mV was measured before and 5 to 8 min after addition of drug. The Hill equation fitting the verapamil data has an IC_{50} of 280 ± 26 μM and a coefficient of 0.96. Myocytes were exposed to one or two concentrations of a drug for 6–10 min, and the numbers of observations are shown in parentheses.

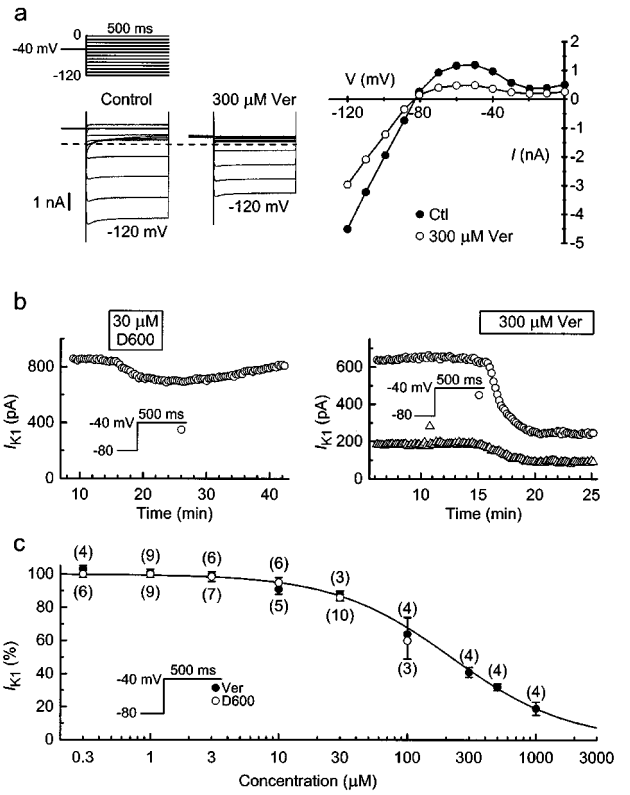

Figure 7 Inhibition of I_{K1} by phenylalkylamines. Myocytes were bathed in Tyrode's solution ±0.2 mM Cd^{2+}, and held at -80 mV. (a) Data from a myocyte treated with 300 μM verapamil for 9 min, Left: records obtained when the myocyte was pulsed from prepulse -40 mV to more positive and negative potentials. Right: end-of-pulse current amplitudes measured in this experiment. (b) Time courses of inhibition of outward I_{K1} in representative experiments. (c) Dependence of inhibition of outward I_{K1} at -40 mV on phenylalkylamine concentration. The Hill equation that describes the verapamil data has an IC_{50} of 220 ± 14 μM and a coefficient of 0.96. The numbers of myocytes are shown in parentheses.

on inward-directed I_{K1} (reduction to $71\pm4\%$ control at -120 mV) was significantly weaker than the action on outward-directed I_{K1} (reduction to $41\pm3\%$ at -40 mV) ($n=4$).

The dependence of inhibition of outward I_{K1} on phenylalkylamine concentration was evaluated over the range 0.3 to 1000 μM using 500-ms pulses from -80 to -40 mV. Example time courses of drug action are shown in Figure 7b, and the steady-state data are presented in Figure 7c. Both verapamil and D600 caused significant inhibitions at 30 μM and higher concentrations, and the Hill equation describing the verapamil data has an IC_{50} of 220 ± 14 μM and a coefficient of 0.96.

Discussion

The results of the present study provide new information on the effects of phenylalkylamines on cardiac inward-rectifier I_{K1} and delayed-rectifier I_{Kr} and I_{Ks}. Verapamil and D600 inhibited all three current pathways, but were considerably less effective on I_{K1} (verapamil IC_{50} 220 μM) and I_{Ks} (verapamil $IC_{50}\geqslant280$ μM) than on I_{Kr} (verapamil IC_{50} 2.6 μM, D600 IC_{50} 2.7 μM). These findings and their implications are discussed in relation to observations in earlier studies of phenylalkylamine action on K^+ currents.

Effects on inward-rectifying K^+ current

To our knowledge, there have been no previous studies on the concentration-dependent effects of phenylalkylamines on cardiac I_{K1}. However, there have been several studies on non-cardiac cells that have included measurements on the effects of phenylalkylamines on Ba^{2+}-sensitive, inwardly-rectifying K^+ currents. Barros *et al.* (1992) found that the current in GH_3 anterior pituitary cells was highly sensitive to both verapamil (IC_{50} 1 μM) and D600 (IC_{50} 0.2 μM). In marked contrast, the inward-rectifier current in intermediate cochlea cells was relatively insensitive to verapamil (IC_{50} 56 μM) (Takeuchi & Ando, 1998), and the current in sheep parotid secretory cells was unaffected by 100 μM verapamil (Ishikawa & Cook, 1993). In the guinea-pig ventricular myocytes investigated here, neither verapamil nor D600 were potent inhibitors of outward I_{K1} (verapamil IC_{50} 220 μM). An interesting finding was that outward I_{K1} was more susceptible to inhibition than inward-directed I_{K1}. The inhalational anaesthetic sevoflurane has also been reported to exert a preferential inhibitory action on outward I_{K1} in guinea-pig ventricular myocytes (Stadnicka *et al.*, 1997). A possible explanation for the preferential block of outward I_{K1} by phenylalkylamine is that block is due to intracellular cationic drug molecules that enter channels more effectively when the cell interior is more positive.

Phenylalkylamines and delayed-rectifier K^+ currents in non-cardiac cells

Delayed-rectifier K^+ currents in non-cardiac cells are frequently of the fast-activating slowly-inactivating type that appear to be inhibited in a characteristic manner by micromolar concentrations of phenylalkylamines. As first reported by Kostyuk *et al.* (1975) for this type of current in *Helix* neurons, it is the amplitude of the late (e.g. 300 ms) current rather than the peak current that is most sensitive to drug, i.e. inhibition is characterized by a marked increase in the apparent rate of current inactivation, and this has been attributed to a time-dependent block of open channels (Jacobs & DeCoursey, 1990; Tatsuta *et al.*, 1994; DeCoursey, 1995; Trequattrini *et al.*, 1998). In regard to late current amplitude, phenylalkylamine IC_{50} values ranged from *ca.* 4 μM in guinea-pig villus enterocytes (Tatsuta *et al.*, 1994), embryonic chick ganglion neurons (Trequattrini *et al.*, 1998), and rat alveolar epithelial cells (Jacobs & DeCoursey, 1990; DeCoursey, 1995), to *ca.* 10 μM in lung tumour cells (Pancrazio *et al.*, 1991) and rat intracardiac ganglion neurons (Hogg *et al.*, 1999). Higher values of 48 μM (cochlea intermediate cells: Takeuchi & Ando, 1998) and 200 μM (cultured frog skeletal muscle cells: Lukyanenko *et al.*, 1995) have also been reported.

Comparison with previous results on delayed-rectifier K^+ currents in cardiac preparations

For the present purposes, it is convenient to classify cardiac delayed-rectifier K^+ currents into fast-activating types, and slower-activating types such as the I_{Kr} and I_{Ks} studied here.

Fast-activating currents Early studies on multicellular cardiac preparations suggested that the transient outward K^+ current (I_{to}) was inhibited by 2–10 μM D600 (Kass, 1982; McDonald *et al.*, 1984a), and subsequent investigations of I_{to} in rat ventricular myocytes established that both D600 (1–1000 μM) (Lefevre *et al.*, 1991) and verapamil (30 μM) (Jahnel *et al.*, 1994) partially inhibit the amplitude of the current and markedly increase its apparent rate of inactivation. Currents carried by cloned Kv1-class channels are also inhibited by verapamil (IC_{50} 45–120 μM) in this manner (Rampe *et al.*, 1993).

Slower-activating currents The first indication that slowly-activating cardiac delayed-rectifier current was sensitive to phenylalkylamines was provided by Kass & Tsien (1975) who found that 10 μM D600 caused a *ca.* 40% inhibition of I_K (I_x) in calf Purkinje fibres. Subsequent studies on cat papillary muscles showed that 2 μM D600 reduced I_K by *ca.* 50% (Nawrath *et al.*, 1977; McDonald *et al.*, 1984a). In retrospect, the latter result most likely reflected inhibition of I_{Kr}-like current by D600 because global I_K in cat ventricular myocytes appears to be predominantly comprised of I_{Kr}-like current (as judged by the finding that it is almost fully blocked by micromolar E4031 (Follmer & Colatsky, 1990)).

To our knowledge, there are only two previous reports on the actions of phenylalkylamines on I_K in cardiac myocytes. The earliest of these is the frequently-cited study of Hume (1985) on I_K in frog atrial myocytes. He found that the current was highly resistant to inhibition by D600 (IC_{50} of 820 μM). More recently, Zhang *et al.* (1997) noted that exposure of guinea-pig ventricular myocytes to 5 μM verapamil inhibited I_{Kr} by 87±24% and I_{Ks} by 39±14%. Taking the latter results first, there is reasonable accord with our findings on I_{Kr} (inhibition of 68±4% by 5 μM verapamil) but not with our findings on I_{Ks} (no inhibition by 5 μM verapamil). Rather, our results on the inhibition of I_{Ks} by verapamil (IC_{50} 280 μM for myocytes in Tyrode's solution; IC_{50} 1080 μM for myocytes in K^+-, Ca^{2+}-free Cd^{2+} solution) are in good agreement with those of Hume (1985) (who used 2.5 mM K^+ solution). In regard to the latter comparison, it is important to note that certain properties of single-pathway I_K in frog atrial myocytes (Hume & Giles, 1983) are I_{Ks}-like in nature (e.g., little inactivation (Hume, 1985) and cyclic AMP-dependent regulation (Duchatelle-Gourdon *et al.*, 1989)) whereas others are not (half-activation near 0 mV and saturation near +40 mV (Hume, 1985)).

There are a number of factors that might account for the *ca.* 3.5 fold difference in the sensitivity of I_{Ks} to inhibition when the myocytes investigated here were bathed in Tyrode's solution rather than K^+-, Ca^{2+}-free Cd^{2+} solution. These include differences in external divalent cations, a difference in the method of measuring I_{Ks}, and a difference in external K^+ concentration. An antagonistic action by external K^+ appears to be the most likely of these factors.

Comparison with results on cloned cardiac K^+ channels

Chouabe *et al.* (1998) and Zhang *et al.* (1999) have recently examined the inhibitory effects of verapamil on cloned cardiac HERG channels that carry I_{Kr}-like current (Sanguinetti *et al.*, 1995; Trudeau *et al.*, 1995). Chouabe *et al.* (1998) activated the HERG current with 2-s depolarizations to +60 mV, evaluated tail current amplitudes on repolarizations to −40 mV, and determined an IC_{50} of 0.83 μM. Zhang *et al.* (1999) used 4-s depolarizations to +20 mV, evaluated tail currents at −50 mV, and determined an IC_{50} of 0.15 μM. By comparison, we used 200-ms depolarizations to 0 mV and measured tail currents at −40 mV. This difference in pulsing protocol may be one reason for the lower IC_{50} values in the HERG studies than in the myocytes (IC_{50} 2.6 μM) because both Chouabe *et al.* (1998) and Zhang *et al.* (1999) reported that the inhibition of HERG current by verapamil increased with the duration of the depolarizing pulse. Whether a similar dependency of inhibition on pulse duration pertains to I_{Kr} in myocytes was not investigated in the present study. A second possible reason for the disparity in IC_{50} values is that the sensitivity of HERG channels to inhibitors is different than that of native Kr channels (for example, see McDonald *et al.*, 1997).

Chouabe *et al.* (1998) also studied the effect of verapamil on the I_{Ks}-like current carried by expressed KvQT1/Isk channels. They found that a 10 μM concentration of the drug had no effect on the current, a result that coincides with our findings on I_{Ks}.

Implications

It is well-established that although phenylalkylamines are effective inhibitors of L-type Ca^{2+} channels in cardiac cells, they are also capable of blocking other cardiac channels (Bayer *et al.*, 1975; Kass & Tsien, 1975; Nawrath *et al.*, 1977; McDonald *et al.*, 1984a; Lefevre *et al.*, 1991). The present study establishes that concentrations as low as 0.3 μM can inhibit cardiac I_{Kr}, a current that has an important role in terminating the cardiac action potential plateau (Sanguinetti & Jurkiewicz, 1990; Luo & Rudy, 1994; Jones *et al.*, 1998) (and in governing pacemaker activity (Ono & Ito, 1995)). In regard to the duration of the action

potential in ventricular cells, the net effect of micromolar concentrations of phenylalkylamines will be determined by the sum of the lengthening influence related to inhibition of I_{Kr} and the shortening influence related to inhibition of $I_{Ca,L}$. Since the inhibition of $I_{Ca,L}$ is use-dependent (weaker at higher resting potential and lower stimulation rate) (McDonald *et al*., 1984b), it is not surprising that the action potential in ventricular preparations treated with micromolar phenylalkylamine may be lengthened rather than shortened (Cranefield *et al*., 1974; Nawrath *et al*., 1977; McDonald *et al*., 1984a; Zhang *et al*., 1997). Thus, the antiarrhythmic action of these compounds may well include an I_{Kr}-mediated lengthening of the refractory period with consequent suppression of re-entry. A similar action for two members of the dihydropyridine class of Ca^{2+} channel inhibitors (nisoldipine, nifedipine) is unlikely because recent studies on guinea-pig ventricular myocytes indicate that relatively high concentrations are required for inhibition of I_{Kr} (nisoldipine IC_{50} 24 μM (unpublished); nifedipine IC_{50} 275 μM (Zhabyeyev, 2000)).

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