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SD-3212, a new class I and IV antiarrhythmic drug: a potent inhibitor of the muscarinic acetylcholine-receptor-operated potassium current in guinea-pig atrial cells

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1 By use of patch-clamp techniques, the effects of SD-3212, a novel antiarrhythmic drug, on the calcium current (I_{Ca}), the sodium current (I_{Na}) and the muscarinic acetylcholine-receptor-operated potassium current ($I_{K.ACh}$) were examined and compared with those of bepridil in guinea-pig single atrial cells.

2 SD-3212 inhibited I_{Ca} and I_{Na} in a concentration-dependent manner. The IC_{50} values of SD-3212 for inhibition of I_{Ca} and I_{Na} were 1.29 μM and 3.92 μM, respectively. The steady state inactivation curves of I_{Ca} and I_{Na} were shifted in the hyperpolarizing direction in the presence of 1μM SD-3212. Similar inhibition of I_{Ca} and I_{Na} was also observed with bepridil. The IC_{50} values of bepridil for depression of I_{Ca} and I_{Na} were 1.55 μM and 4.43 μM, respectively.

3 The muscarinic acetylcholine-receptor-operated potassium current ($I_{K.ACh}$) was activated by the extracellular application of 1 μM carbachol in the GTP-loaded cells or by the intracellular loading of GTPγS, a nonhydrolysable GTP analogue. SD-3212 potently inhibited the carbachol- and GTPγS-induced $I_{K.ACh}$ and the IC_{50} values were 0.38 μM and 0.20 μM, respectively. These IC_{50} values were very close and about 10 times lower than those for inhibiting I_{Ca} and I_{Na}. Bepridil also suppressed the carbachol- and GTPγS-induced $I_{K.ACh}$ with the IC_{50} values of 0.69 μM and 0.84 μM, respectively.

4 In guinea-pig atrial cells stimulated at 0.2 Hz, carbachol at a concentration of 1 μM markedly shortened action potential duration. Both SD-3212 (0.1-1μM) and bepridil (1-10 μM) reversed the action potential shortening in a concentration-dependent manner. The antagonizing effect of SD-3212 on the carbachol-induced action potential shortening was more potent than that of bepridil.

5 These results suggest that SD-3212 inhibits $I_{K.ACh}$ by depressing the function of the potassium channel itself and/or associated GTP-binding proteins. SD-3212 is a unique antiarrhythmic drug, which potently inhibits $I_{K.ACh}$ in addition to its class I and IV effects. SD-3212 and bepridil may be useful for the termination and prevention of vagally-induced atrial flutter and fibrillation.

Keywords: Antiarrhythmic agent; SD-3212; bepridil; sodium current; calcium current; muscarinic acetylcholine-receptor-operated potassium current

Introduction

SD-3212 ((−)-(S)-2-[5 methoxy-2-[3-[methyl[2-[3,4(methylenedioxy) phenoxy] ethyl] amino] propoxy] phenyl]-4-methyl-2H-1,4-benzothiazine-3(4H)-one hydrogen fumarate) is a stereoisomer of semotiadil fumarate (SD-3211), a recently developed non-dihydropyridine type calcium antagonist (Miyawaki *et al.*, 1990; Fukuchi *et al.*, 1990; Teramoto, 1993). This agent is reported to possess antiarrhythmic properties with vasodilator action (Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992; Hirasawa *et al.*, 1992). In experimental animals, SD-3212 exerted a potent and long-lasting inhibitory action against ventricular tachyarrhythmias induced by chloroform, ouabain, adrenaline and ischaemia/reperfusion (Fukuchi *et al.*, 1990; Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992). In vascular smooth muscle, SD-3212 exerted a calcium antagonistic vasodilator action (Nakayama *et al.*, 1992). In addition, SD-3212 decreased the maximum upstroke velocity ($\dot{V}_{max}$) of action potential in a concentration-dependent manner in guinea-pig papillary muscles (Miyawaki *et al.*, 1991). These experimental results suggest that SD-3212 acts as an antiarrhythmic drug having class I and IV properties like bepridil (Dangman, 1985; Yatani *et al.*, 1986).

It is well-known that vagal stimulation can easily elicit atrial flutter and fibrillation (Allessie *et al.*, 1984; Euler & Scanlon, 1987). The muscarinic acetylcholine-receptor-operated potassium current ($I_{K.ACh}$) plays an important role in the repolarization of the action potential as well as the maintenance of the resting potential in atrial cells (Kaibara *et al.*, 1991). Patch-clamp studies have demonstrated the direct activation of the muscarinic acetylcholine-receptor-operated potassium channel via muscarinic receptor-coupled G proteins in mammalian atrial myocytes (Pfaffinger *et al.*, 1985; Breitwieser & Szabo, 1985; Kurachi *et al.*, 1986). Some class I or IV antiarrhythmic drugs inhibit $I_{K.ACh}$ by blocking muscarinic acetylcholine receptors or by blocking the potassium channel itself and/or GTP-binding proteins (Nakajima *et al.*, 1989; Ito *et al.*, 1989; Inomata *et al.*, 1993; Wu *et al.*, 1994). In addition, we have recently demonstrated that class III antiarrhythmic drugs inhibit $I_{K.ACh}$ by different molecular mechanisms; sotalol blocks muscarinic receptors while E-4031 and MS-551 inhibit not only muscarinic receptors but also the muscarinic potassium channel itself and/or GTP-binding proteins (Mori *et al.*, 1995). The inhibition of $I_{K.ACh}$ may be useful in the termination and prevention of atrial flutter and fibrillation (Coumel *et al.*, 1979). On the other hand, anticholinergic activity of antiarrhythmic drugs may cause untoward effects such as dry mouth, constipation and urinary retention in extracardiac tissues (Bigger & Hoffman, 1990). However, effects of antiarrhythmic drugs having class I and IV properties on $I_{K.ACh}$ have not been evaluated. Therefore, the effect of SD-3212 on $I_{K.ACh}$ was examined and compared with that of bepridil in this study. The findings of the present study, obtained by patch clamp techniques, indicate that SD-3212, possessing class I and IV properties, potently inhibits $I_{K.ACh}$ by blocking the potassium channel itself and/or GTP-binding proteins.

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Methods

Cell preparations

Guinea-pigs, weighing 200–350 g, were used in these experiments. Single atrial cells were isolated by an enzymatic dispersion, as previously described (Tohse *et al.*, 1992). Briefly, the heart was removed from the open-chest guinea-pig anaesthetized with pentobarbitone sodium, and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal HEPES-Tyrode solution. The perfusion medium was then changed to a nominally calcium-free Tyrode solution and then to a solution containing 0.02% w/v collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with a high K^+, low Cl^- solution (modified Kraft-Brühe (KB) solution) (Isenberg & Klockner, 1982; Nakaya *et al.*, 1993). Atrial tissue was cut into small pieces in the modified KB solution and was gently shaken to isolate cells. The cell suspension was stored in a refrigerator (4°C) for later use. The composition of the normal HEPES-Tyrode solution was (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES-NaOH buffer (pH 7.4) 5.0. The composition of the modified KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 1.0 and HEPES-KOH buffer (pH 7.4) 10.

Recording techniques

Whole-cell membrane currents were recorded by the patch clamp method (Hamill *et al.*, 1981). Single atrial cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES-Tyrode solution at a rate of 3 ml min^{-1}. Glass pipettes with a diameter of 1.5 mm were filled with an internal solution. The composition of the standard pipette solution was (mM): K-aspartate 110, KCl 20, $MgCl_2$ 1.0, ATP-K_2 5.0, phosphocreatine-K_2 5.0, EGTA 10 and HEPES-KOH buffer (pH 7.4) 5.0. The free calcium concentration in the pipette solution was adjusted to pCa 8 according to the calculation by Fabiato & Fabiato (1979) with the correction of Tsien & Rink (1980). The resistance of the patch pipette filled with the internal solution was 2–3 MΩ. The tight-seal, whole-cell voltage clamp technique was used. After a gigaohm-seal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by applying more negative pressure to make the whole cell voltage-clamp mode. The electrode was connected to a patch-clamp amplifier (Nihon Kohden CEZ-2300, Tokyo, Japan). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pClamp software (Axon Instruments, Inc., Foster City, CA, U.S.A.). Current signals were digitized and stored on the hard disk of an IBM compatible computer. A liquid junctional potential between the internal solution and the bath solution of −8 mV was corrected.

The $I_{K.ACh}$ was activated by the extracellular application of 1 μM carbachol in the GTP(100 μM)-loaded cells or by the intracellular loading of GTPγS (100 μM), a nonhydrolysable GTP analogue, in atrial cells held at −50 mV. Effects of various concentrations of SD-3212 and bepridil on $I_{K.ACh}$ activated in these two different ways were examined. To calculate percentage inhibition of $I_{K.ACh}$ by the drugs, the difference between the steady-state current in the solution containing 1 μM carbachol and the current level in the absence of the agent was taken as 100% in the GTP-loaded cells. In the GTPγS-loaded cells, the difference between the persistent outward current in the absence of agonist and the initial current level just after the break of the patch membrane in the pipette was taken as 100%.

In order to record the calcium current (I_{Ca}) the cells were depolarized to 0 mV from a holding potential of −40 mV by depolarization pulses of 300 ms at 0.1 Hz. The amplitude of the calcium current was obtained by subtracting the late current from the peak of the initial inward current. In some experiments the calcium current was isolated from other membrane currents by using Cs^+-rich external and pipette solutions. The Cs^+-rich external solution was prepared by replacing KCl of the normal HEPES-Tyrode solution with equimolar CsCl. The composition of the Cs^+-rich pipette solution was (mM): L-aspartate 110, CsOH 110, CsCl 20, $MgCl_2$ 1.0, ATP-K_2 5.0, EGTA 10 and HEPES-CsOH-buffer (pH 7.4) 5.0.

In order to record the sodium current (I_{Na}), the following external and pipette solutions were used. The external solution contained (mM): NaCl 30.0, tetramethylammonium chloride 110.0, $CoCl_2$ 1.0, CsCl 5.0, $MgCl_2$ 1.2, glucose 11.0, HEPES 20.0, $CaCl_2$ 1.8 (pH 7.3 adjusted by tetramethylammonium hydroxide). The pipette solution was composed of (mM): NaF 5.0, CsF 125.0, ATP-K_2 5.0, phosphocreatine-K_2 5.0, EGTA 5.0, HEPES 5.0 (pH 7.2 adjusted with CsOH). A train of 30 depolarizing pulses of 50 ms duration was applied from a holding potential of −120 mV to −30 mV at 1 Hz. Only the experiments to record I_{Na} were performed at room temperature; the others were at 36±1°C.

Current clamp experiments were also performed in the whole-cell recording mode at 36±1°C. External and pipette solutions were the same as those used to record the carbachol-induced $I_{K.ACh}$. The cells were stimulated by passing 2 ms currents through the pipette at a rate of 0.2 Hz. After stabilization of the action potential configuration, effects of various drugs on the action potential were evaluated.

Drugs

Drugs used in this study were as follows: SD-3212 (Daiichi Pharmaceutical Co., Tokyo, Japan), bepridil (Sigma Chemical Co., St. Louis, U.S.A.), carbachol chloride (Wako, Osaka, Japan), GTP-Na_3 (Sigma Chemical Co.) and GTPγS-Li_4 (Sigma Chemical Co.). SD-3212 and bepridil were dissolved in dimethyl sulphoxide as a stock solution of 10 mM. The final concentration of dimethyl sulphoxide was less than 0.1%. This concentration of dimethyl sulphoxide had no influence on the membrane currents and the action potential parameters.

Statistics

All values are presented in terms of mean±s.e. Student's test for paired and unpaired observations and ANOVA were used for statistical analyses. P value of less than 0.05 was considered significant. IC_{50} values (the concentrations required to produce 50% of the maximal inhibitory effect) were obtained with a Macintosh computer (Apple Computer Inc., CA, U.S.A.) and Sigma Plot programme (Jandel Corporation, CA, U.S.A.).

Results

Effects of SD-3212 and bepridil on the calcium current in guinea-pig atrial cells

Effects of SD-3212 on the calcium current (I_{Ca}) were examined and compared with those of bepridil in guinea-pig atrial myocytes. Changes in I_{Ca} after application of 10 μM SD-3212 or 10 μM bepridil are shown in Figure 1. Membrane currents were elicited by 300 ms test pulses to 0 mV from a holding potential of −40 mV at 0.1 Hz. Both SD-3212 and bepridil decreased the amplitude of I_{Ca}. SD-3212 in concentrations of 0.1, 0.3, 1, 3, and 10 μM decreased the amplitude of I_{Ca} by 28.8±25.7%, 34.8±2.4%, 53.6±4.0%, 81.3±10.6%, and 91.2±2.4% (n=3–10), respectively. Bepridil in concentrations of 0.1, 1, 3, and 10 μM reduced the amplitude of I_{Ca} by 37.8±16.6%, 50.9±4.8%, 81.1±1.2%, and 89.0±3.9% (n=3–5), respectively. The calculated IC_{50} values of SD-3212 and bepridil for inhibiting I_{Ca} were 1.29 μM and 1.55 μM, respectively. In some experiments we tried to wash out the drug

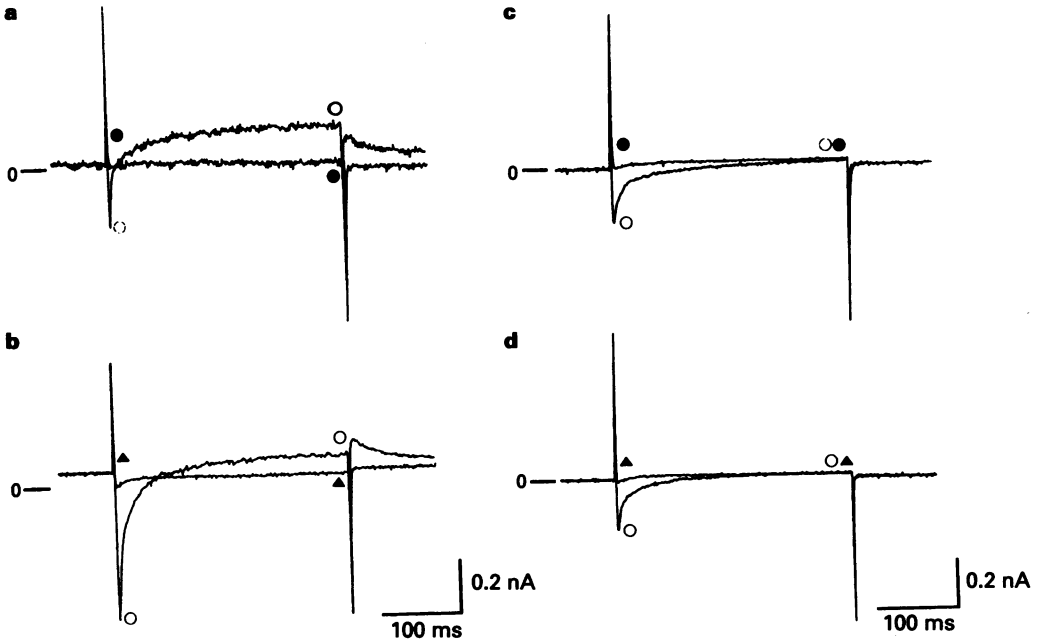

Figure 1 Effects of SD-3212 (a, c) and bepridil (b, d) on the calcium current in single atrial cells. Currents induced by depolarizing pulses to 0 mV from a holding potential of −40 mV in the absence (control, ○) and the presence of 10 μM SD-3212 (●) or 10 μM bepridil (▲) are superimposed. The currents recorded in normal HEPES-Tyrode solution are shown in the left panels (a and b), and the calcium currents isolated from other membrane currents by using Cs^+-rich external and pipette solutions are depicted in the right panels (c and d). Note that both SD-3212 and bepridil at a concentration of 10 μM inhibited the calcium current almost to the same extent in both conditions.

effect by superfusing a drug-free solution. The decrease in I_{Ca} after SD-3212 or bepridil very slowly reverted toward the control on changing to the drug-free solution.

In addition to the inhibitory action on the peak inward current, SD-3212 and bepridil also inhibited the late outward current at the end of 300-ms depolarizing pulse and the outward tail current elicited by the clamp back to −40 mV, as shown in Figure 1a and b. These findings suggest that both SD-3212 and bepridil depress the delayed rectifier potassium current (I_K) in similar concentrations. Therefore, in order to evaluate the effects of these drugs on I_{Ca} more accurately, further experiments were conducted with Cs^+-rich external and pipette solutions. Even if I_{Ca} were isolated from other membrane currents, both SD-3212 and bepridil suppressed the inward calcium current (Figure 1c and d). SD-3212 in concentrations of 0.1, 0.3, 1 and 10 μM decreased the isolated I_{Ca} by 18.7±8.4%, 36.8±11.1%, 66.3±6.8% and 92.7±1.0% (n=3–10), respectively. Bepridil in concentrations of 1 and 10 μM also inhibited the isolated I_{Ca} by 41.0±6.1% and 85.0±0.9% (n=5), respectively. The decreases in the isolated I_{Ca} by SD-3212 and bepridil were not significantly different from those in I_{Ca} measured with normal pipettes and external solutions after these drugs at the respective concentration.

In order to evaluate the voltage-dependence of the calcium channel blocking action of SD-3212, further experiments were conducted with Cs^+-rich pipette and external solutions. The voltage-dependence of I_{Ca} availability was examined by use of a double-pulse protocol: 1 s conditioning pulses of variable amplitude (from −60 to +20 mV in 10 mV steps) which produced steady state inactivation of I_{Ca} under control conditions were followed by a 5 ms return to the holding potential (−40 mV) and a 200-ms test pulse to 0 mV. This double-pulse sequence was applied once every 10 s. The steady-state inactivation curves before and after treatment of 1 μM SD-3212 were normalized to the maximum amplitude of I_{Ca} by test pulse and plotted against the conditioning potential. SD-3212 shifted the curve in the hyperpolarizing direction. The mean control value for the potential of half inactivation (V_h) in 7 cells was −21.6±1.5 mV, and it was significantly changed to −39.1±4.0 mV after 1 μM SD-3212 (P<0.05). No significant changes in the slope factor were observed (k=4.3±0.5 in control group and 5.0±1.1 in SD-3212 group).

Effects of SD-3212 and bepridil on the sodium current in guinea-pig atrial cells

Effects of SD-3212 and bepridil on the sodium current (I_{Na}) were also examined in guinea-pig isolated myocytes. The I_{Na} was recorded by applying 50-ms depolarizing pulses from a holding potential of −120 mV to −30 mV at 1 Hz. During a train of 30 pulses after a 60 s quiescent period, the peak I_{Na} showed almost no change in the absence of the drug. In the presence of 10 μM SD-3212 the peak I_{Na} was slightly depressed during the first pulse and decreased gradually with successive pulses (use-dependent block UDB), as shown in Figure 2. Decline of the peak I_{Na} during exposure to SD-3212 followed a single exponential. The onset rate of UDB, expressed in rate per depolarizing pulse, was calculated at 0.254 $pulse^{-1}$ and UDB was 68% in this cell. The tonic block, which was designated as a percentage decrease in I_{Na} of the first depolarizing pulse of the train after a 60 s pulse-free period, was about 22% in the cell. The UDB induced by 10 μM SD-3212 was 60.9±3.0% in 9 cells. Total decreases in the peak I_{Na} by 1, 3 and 10 μM SD-3212 were 28.9±3.3%, 46.9±13.8% and 67.7±4.2%, respectively. The IC_{50} value of SD-3212 for inhibition of I_{Na} at 1.0 Hz was 3.92 μM.

To study the affinity of SD-3212 for inactivated sodium channels, double-pulse protocol experiments were carried out by applying a 20-ms test pulse to −30 mV following a 1 s prepulse to various potential level ranging from −120 to −10 mV in 10 mV steps. The double-pulse was separated by a 1 ms return to the holding potential (−120 mV) and applied once every 10 s. I_{Na} was fully available at more negative potentials and was progressively decreased and finally inactivated at more positive potentials. The relation between the steady-state inactivation (h∞) and the membrane potential (Vm) was

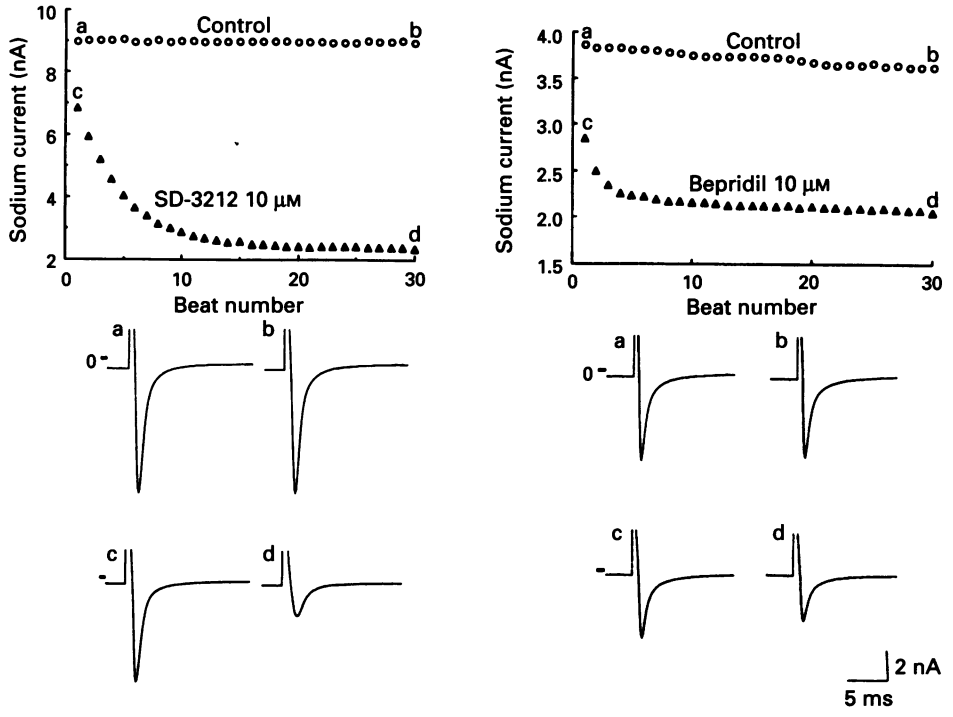

Figure 2 Inhibitory effects of SD-3212 (left) and bepridil (right) on the sodium current in single atrial cells. Depolarizing pulses to −30 mV were applied from a holding potential of −120 mV at 1 Hz. A train of 30 pulses of 50-ms duration was applied to the cell after a pulse-free period of 60 s in the absence (Control) and the presence of 10 μM SD-3212 and 10 μM bepridil. Actual current traces induced by the first (a and c) and the 30th (b and d) depolarizing pulses are shown in the lower panels. Note that both SD-3212 and bepridil produced the use-dependent block of the sodium current.

fitted to a Boltzmann function. SD-3212 at a concentration of 10 μM shifted the V_h from -72.4 ± 2.4 to -82.2 ± 3.0 ($n=5$, $P<0.05$) with the slope unaffected ($k=5.9\pm0.5$ during control and 4.7 ± 0.3 during SD-3212 superfusion).

The effect of bepridil on the I_{Na} was examined in guinea-pig isolated atrial cells in a similar manner. Bepridil produced a tonic block and UDB, as shown in Figure 2. The onset rate and the magnitude of UDB by 10 μM bepridil were 0.346 pulse^{-1} and $22.6\pm3.7\%$, respectively. Total decreases in the peak I_{Na} induced by 1, 3 and 10 μM bepridil were $14.3\pm13.1\%$, $25.3\pm6.8\%$ and $51.5\pm6.8\%$, respectively. The calculated IC_{50} value of bepridil for inhibiting I_{Na} at 1.0 Hz was 4.43 μM. The decrease in I_{Na} after SD-3212 or bepridil gradually reverted towards the control on change to a drug-free solution.

Effects of SD-3212 and bepridil on the muscarinic acetylcholine-receptor-operated potassium current in guinea-pig atrial cells

Effects of SD-3212 and bepridil on the carbachol-induced potassium current in the GTP (100 μM)-loaded cells were examined. Following application of 1 μM carbachol to the bath solution, an outward potassium current was rapidly activated at a holding potential of −50 mV. It then declined gradually in the continuous presence of carbachol, perhaps due to desensitization (Carmeliet & Mubagwa, 1986; Kurachi *et al.*, 1986). After the current almost reached a steady level, SD-3212 or bepridil was added to the bath solution. SD-3212 and bepridil depressed the carbachol-induced potassium current in a concentration-dependent manner (Figure 3). The outward current recovered gradually and partially upon the washout of the drugs. The IC_{50} values of SD-3212 and bepridil for depressing the carbachol-induced current were 0.38 μM and 0.69 μM, respectively (Figure 4).

Muscarinic receptors couple with a specific potassium channel through a pertussis toxin-sensitive class of GTP-binding proteins in atrial cells (Pfaffinger *et al.*, 1985). Intracellular application of a nonhydrolysable GTP analogue can directly activate the GTP-binding proteins and evoke antagonist-resistant, persistent activation of the muscarinic potassium channels (Breitwieser & Szabo, 1985). In these experiments, this potassium current was activated in a time-dependent fashion following intracellular loading of GTPγS

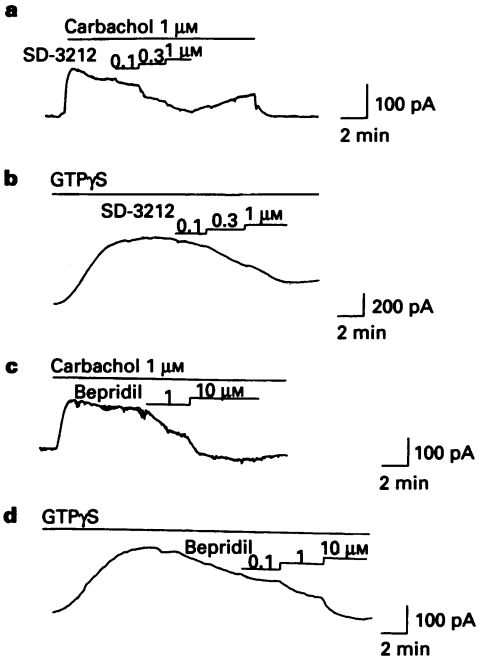

Figure 3 Effects of SD-3212 and bepridil on the muscarinic acetylcholine receptor-operated potassium current ($I_{K.ACh}$) in guinea-pig atrial cells. $I_{K.ACh}$ was activated by the extracellular application of 1 μM carbachol (traces a and c) or intracellular loading of 100 μM GTPγS (traces b and d). The holding potential was −50 mV. Intracellular loading of GTPγS and extracellular application of carbachol, SD-3212 and bepridil are shown by the lines above each original current trace.

(100 μM) at a holding potential of −50 mV, even in the absence of any agonist. Effects of SD-3212 and bepridil on this muscarinic potassium current after uncoupling from membrane receptors were examined in the GTPγS-loaded myocytes. Both drugs inhibited the GTPγS-induced potassium current in a concentration-dependent manner (Figure 4). The IC_{50} values of SD-3212 and bepridil for depressing the GTPγS-induced potassium current were 0.20 μM and 0.84 μM, respectively. Even with atropine (10 μM) in the external solution, intracellular loading of GTPγS (100 μM) induced the muscarinic potassium current at −50 mV. SD-3212 in concentrations of 0.01, 0.1, 1 and 10 μM inhibited the outward current by 12.4±5.0%, 32.8±6.4%, 86.0±1.9% and 100±0%, respectively. The IC_{50} value of SD-3212 for inhibition of the GTPγS-induced potassium current under these conditions was 0.26 μM, which was almost the same as that in the absence of atropine.

Effects of SD-3212 and bepridil on the action potential shortening induced by carbachol in guinea-pig atrial cells

Effects of SD-3212 and bepridil on muscarinic acetylcholine receptor-mediated action potential shortening were examined in the current clamp mode. The baseline characteristics of action potentials recorded from atrial myocytes stimulated at 0.2 Hz were as follows: resting membrane potential (RMP), −72.9±0.9 mV; action potential amplitude (APA), 106.4±1.5 mV; action potential duration at 20% repolarization level (APD_{20}), 12.1±1.1 ms; APD at 50% repolarization level (APD_{50}), 25.4±2.4 ms; APD at 90% repolarization level (APD_{90}), 55.9±3.2 ms ($n=12$). There were no significant differences in any of the baseline values of action potential parameters between the two subgroups. Carbachol at a concentration of 1 μM markedly shortened action potential duration, as shown in Figure 5a. Carbachol (1 μM) decreased APD_{90} from 55.9±3.2 ms to 15.4±1.5 ms ($P<0.05$) with a slight increase in RMP from −73.2±0.9 mV to

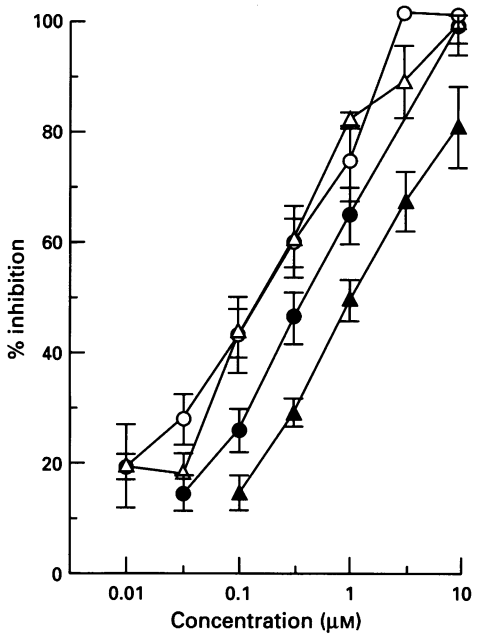

Figure 4 Concentration-response curves for the inhibitory effects of SD-3212 and bepridil on the muscarinic potassium channels current in isolated atrial myocytes. The muscarinic potassium channel current was activated by extracellular application of 1 μM carbachol (○, ●) or intracellular loading of 100 μM GTPγS (△, ▲). Percentage inhibition of the outward current is indicated on the ordinate scale and the concentration of SD-3212 (open symbols) and bepridil (closed symbols) are on the abscissa scale. Values are expressed as mean±s.e. of 3 to 26 experiments for each point.

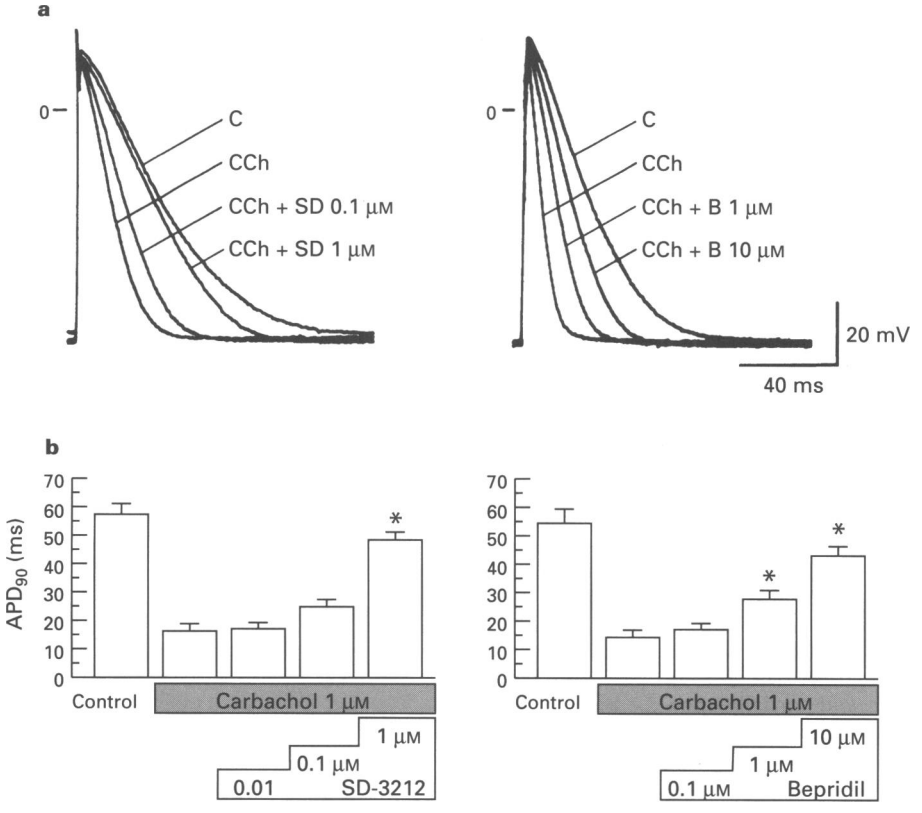

Figure 5 Effects of SD-3212 and bepridil on the carbachol-induced action potential shortening in guinea-pig atrial myocytes. (a) Superimposed records of action potentials obtained before (C) and after exposure to carbachol (CCh, 1 μM), and carbachol plus SD-3212 (SD) or bepridil (B). (b) Concentration-dependent antagonizing effects of SD-3212 (left) and bepridil (right) on the carbachol-induced action potential shortening in isolated atrial cells. Values are expressed as mean±s.e. of 6 experiments. *$P<0.05$ vs carbachol alone by ANOVA.

-75.4 ± 0.7 mV ($P<0.05$, $n=12$). SD-3212 and bepridil reversed the carbachol-induced action potential shortening in a concentration-dependent manner (Figure 5b). The carbachol-induced shortening of APD_{90} was reversed by $77.9\pm3.0\%$ and $72.4\pm9.8\%$ after 1 μM SD-3212 and 10 μM bepridil, respectively. The IC_{50} values of SD-3212 and bepridil for antagonizing the carbachol-induced action potential shortening were 0.66 μM and 2.90 μM, respectively. There were no significant changes in RMP and APA after these drugs. In the absence of carbachol, SD-3212 failed to produce a significant increase in APD in atrial myocytes (data not shown).

Discussion

SD-3212 is an (−)-(S)-enantiomer of semotiadil fumarate, a new calcium antagonist with a benzothiazine ring (Fukuchi *et al.*, 1990; Miyawaki *et al.*, 1990; Nagashima *et al.*, 1992). The antiarrhythmic action of the drug has been attributed to its sodium and calcium channel blocking properties from the profiles of the antiarrhythmic effect (Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992; Hirasawa *et al.*, 1992). SD-3212 was shown to be effective against various experimental arrhythmias such as those induced by ouabain-, chlorform, two-stage coronary ligation, and halothane plus adrenaline (Fukuchi *et al.*, 1990; Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992). In terms of the class I effect of SD-3212, two reports have indicated that SD-3212 inhibits $\dot{V}_{max}$ of the action potential in guinea-pig papillary muscle and ventricular cells (Miyawaki *et al.*, 1991; Kodama *et al.*, 1995). Consistent with these reports, SD-3212 inhibited I_{Na} in guinea-pig atrial cells in a use-dependent manner, and the IC_{50} value for inhibiting I_{Na} at 1.0 Hz was 3.92 μM. Kodama *et al.* (1995) have recently demonstrated that SD-3212 blocks inactivated sodium channels and has fast to intermediate onset and offset kinetics of UDB by use of $\dot{V}_{max}$ in guinea-pig ventricular cells. Although the mode of the sodium channel blocking action of SD-3212 was not fully evaluated, we observed a shift of the h_∞ curve in the hyperpolarizing direction by SD-3212 (10 μM) in the present study. These findings suggest that SD-3212 exhibits a higher affinity for the inactivated state than for the rested state of the sodium channels.

In addition to the concentration-dependent sodium channel blocking action, SD-3212 inhibited I_{Ca} in guinea-pig atrial cells in this study. The IC_{50} value for inhibition of I_{Ca} was 1.29 μM, which was comparable with that for inhibition of I_{Na}. Since we observed that SD-3212 also inhibited the outward tail current, i.e., the delayed rectifier potassium current (I_K), we conducted additional experiments in which I_{Ca} was isolated from I_K by using Cs^+-rich external and pipette solutions. Again, SD-3212 inhibited the isolated I_{Ca} in a concentration-dependent manner, and the inhibition was comparable to that observed in control conditions. The steady state I_{Ca} inactivation was also examined under Cs^+-rich conditions in this study. SD-3212 shifted the steady-state I_{Ca} inactivation curve along the voltage axis in the hyperpolarizing direction, indicating that the block of I_{Ca} by SD-3212 is also modulated by membrane potential. The shift of the steady-state I_{Ca} inactivation curve by the drug suggests that SD-3212 exhibits a higher affinity for the inactivated state than for the resting state of the calcium channels. Thus SD-3212 is an antiarrhythmic drug possessing balanced class I and class IV properties.

We confirmed that bepridil also inhibited I_{Ca} and I_{Na} of guinea-pig atrial cells in a concentration-dependent manner, which is consistent with a previous report (Yatani *et al.*, 1986). The IC_{50} values of bepridil for inhibition of I_{Ca} and I_{Na} were 1.55 μM and 4.43 μM, respectively, values which were very close to those of SD-3212 determined in the same experimental protocol. Bepridil also inhibited the outward tail current of I_K, and the inhibition of the isolated I_{Ca} by bepridil under Cs^+-rich conditions was comparable with that in control conditions.

It has been reported that class I antiarrhythmic drugs inhibit the muscarinic acetylcholine-receptor-operated current ($I_{K.ACh}$) in guinea-pig atrial cells (Nakajima *et al.*, 1989; Inomata *et al.*, 1993; Wu *et al.*, 1994). Two mechanisms by which these antiarrhythmic drugs inhibit $I_{K.ACh}$ have been proposed; some drugs block the muscarinic receptors and others inhibit the muscarinic potassium channel itself and/or GTP-binding proteins. Disopyramide and pilsicainide belong to the former group whereas quinidine, flecainide, propafenone and cibenzoline belong to the latter group (Nakajima *et al.*, 1989; Inomata *et al.*, 1993; Wu *et al.*, 1994). The class IV antiarrhythmic drug, verapamil, inhibits $I_{K.ACh}$ by blocking the muscarinic receptors but also suppressing the function of the potassium channel itself and/or GTP-binding proteins (Ito *et al.*, 1989). The results obtained in this study have demonstrated that antiarrhythmic drugs having both class I and IV properties, bepridil and SD-3212, inhibit $I_{K.ACh}$ in guinea-pig atrial cells. Since SD-3212 inhibited the $I_{K.ACh}$ activated by extracellular application of carbachol and intracellular loading of GTPγS almost to the same extent, this drug would be expected to inhibit directly the muscarinic potassium channel itself and/or GTP-binding proteins. Support for this concept derives from the present findings that SD-3212 still inhibited the $I_{K.ACh}$ activated by intracellular loading of GTPγS even after pretreatment with atropine. The inhibition of the carbachol-induced $I_{K.ACh}$ by bepridil was slightly greater than that of the GTPγS-induced $I_{K.ACh}$ by this drug. Therefore, bepridil may interact with muscarinic M_2 receptors in atrial cells in addition to its direct inhibition of the muscarinic potassium channel itself and/or GTP-binding proteins.

Both SD-3212 and bepridil inhibited $I_{K.ACh}$ at concentrations lower than those required to inhibit I_{Na} or I_{Ca}. SD-3212 was 4–10 times more potent as an inhibitor of $I_{K.ACh}$ than I_{Na} or I_{Ca}. As a result, SD-3212 would be expected to exert anticholinergic activity in atrial cells. Our results (Figure 5) show that SD-3212 very effectively reversed the M_2-receptor-mediated action potential shortening in the current clamp experiments. At a concentration of 1 μM, SD-3212 reversed the carbachol (1 μM)-induced action potential shortening by 78% in single atrial cells. However, a higher concentration (10 μM) of bepridil was needed to reverse the action potential shortening to a similar extent (72%). One possible explanation may be that the inhibitory action of bepridil on $I_{K.ACh}$ was less potent than that of SD-3212 on a molar basis, and that the calcium channel blocking action, produced by slightly higher concentrations of bepridil, might partly offset the APD-prolonging effect.

Anticholinergic activity of antiarrhythmic drugs may produce both therapeutic and deleterious effects. Enhancement of vagal tone can result in the clinical occurrence of atrial fibrillation although the contribution of increased vagal tone may be variable (Coumel *et al.*, 1979). Therefore, these drugs may be effective for the prevention and termination of atrial flutter and fibrillation. In fact, it was reported that bepridil was effective against atrial flutter and fibrillation in human subjects (Perelman *et al.*, 1987). On the other hand, the anticholinergic activity in the heart may be unfavourable because it may increase the ventricular rate during atrial flutter or fibrillation. However, this increase in the ventricular rate may be counteracted by the inherent calcium channel blocking action of these drugs at the atrio-ventricular node.

In summary, SD-3212 is a unique antiarrhythmic drug which potently inhibits the muscarinic acetylcholine receptor-operated current in addition to its class I and IV effects. This drug may be useful for the prevention and termination of vagally-induced atrial arrhythmias.

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