British Journal of Pharmacology (1995) 116, 1775-1780 © 1995 Stockton Press All rights reserved 0007-1188/95 $12.00

Mechanism of inhibition of the sodium current by bepridil in guinea-pig isolated ventricular cells

[1]Takahiro Nawada, Yasunori Tanaka, Ichiro Hisatome, Norihito Sasaki, Akira Ohtahara, Hiroshi Kotake, Hiroto Mashiba & *Ryoichi Sato

First Department of Internal Medicine, Tottori University, Yonago, Japan and *First Department of Internal Medicine, Kinki University, Osaka, Japan

1 Effects of bepridil, a sodium-, calcium-, and potassium-antagonistic agent, on the Na^+ current were studied by the whole cell voltage clamp technique (tip resistance = 0.5 MOhm, $[Na]_i$ and $[Na]_o$ 10 mmol l^{-1} at 20°C).

2 Bepridil produced tonic block (K_{drest} = 295.44 μmol l^{-1}, K_{di} = 1.41 μmol l^{-1}; n = 4).

3 Bepridil (100 μmol l^{-1}) shifted the inactivation curve in the hyperpolarization direction by 13.4 ± 2.7 mV (n = 4) without change in the slope factor.

4 In the presence of 50 μmol l^{-1} bepridil, bepridil showed use-dependent block at 2 Hz, whereas changes in pulse duration did not significantly effect this use-dependent block (81% ± 2% at 10 ms, 84% ± 3% at 30 ms, 86% ± 3% at 100 ms; n = 4).

5 After removal of fast inactivation of the Na^+ current by 3 mmol l^{-1} tosylchloramide sodium, bepridil (50 μmol l^{-1}) still showed use-dependent block which was independent of the holding potential.

6 The recovery time constant from the bepridil-induced use-dependent block was 0.48 s at holding potential of −100 mV and 0.51 s at holding potential of −140 mV.

7 These results indicate that bepridil could bind to the receptor in the sodium channel through the hydrophobic and the hydrophilic pathway and leave the receptor through the hydrophobic pathway in the lipid bilayer. The binding and dissociation kinetics of this drug were shown to be fast, and the accumulation of the drug in the sodium channel appeared to be small. Bepridil is presumed to be safe in terms of adverse effects that result from drug-accumulation in the sodium channel.

Keywords: Sodium channel; bepridil; modulated receptor hypothesis; guinea-pig ventricular cells

Introduction

Bepridil was developed as an agent for the treatment of angina pectoris (Cosnier *et al.*, 1977; Singh 1991) and has also been used for the treatment of supraventricular and ventricular arrhythmias (Kane & Winslow 1980). Experimental results have shown (1) that bepridil decreases the upstroke velocity of depolarization of the action potential (V_{max}) and action potential amplitude in all cardiac tissues (Kane & Winslow 1980); (2) that both the binding and dissociation kinetics of bepridil with sodium channels are relatively fast (Anno *et al.*, 1984); (3) that bepridil acts preferentially upon inactivated Ca and Na channels (Yatani *et al.*, 1986); (4) that bepridil has curative effects on paroxysmal atrial tachycardia and on atrial extrasystolies. On the other hand, the action of bepridil on ventricular rhythm disorders induced by ligation of the anterior interventricular artery in the dog and the excellent prevention exerted against dysrhythmia and ventricular fibrillation induced by aconitine perfusion in the anaesthetized guinea-pig, tend to prove that bepridil should be able to suppress idiopathic ventricular extrasystolies and to reduce the risk of ventricular fibrillation occurring (Labrid *et al.*, 1981).

The effects of bepridil on I_{Na} and I_{Ca} have been investigated by Labrid *et al.* (1979) and Yatani *et al.* (1986). Yatani *et al.* (1986) showed that bepridil produced little if any block of open Ca and Na channels. In the present study, we employed the voltage-clamp protocol in which various clamp pulse durations and holding potentials were used when measuring the use-dependent block in order to evaluate the Na channel blocking mechanism of bepridil. We also used tosylchloramide sodium, which prevents fast inactivation of Na channels in cardiac ventricular myocytes, in order to demonstrate that the fast inactivation process is not necessary to show bepridil-induced use-dependent block.

[1]Author for correspondence at: First Department of Internal Medicine, Tottori University School of Medicine, 36-1 Nishimachi, Yonago 683, Japan.

Methods

Single guinea-pig ventricular myocytes were isolated by the enzymatic dissociation technique (Powell *et al.*, 1980; Ehara *et al.*, 1989). Sodium currents of single ventricular cells were recorded by the whole cell clamp technique. The chamber was perfused continuously at 20°C with a low sodium bathing solution of the following composition (mM); NaCl 10, CsCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $CoCl_2$ 1, D-glucose 11, HEPES 20 and tetramethylammonium (TMA) chloride 125, after which it was titrated to pH 7.4 with 1 mM TMA hydroxide. In the experiments with tosylchloramide sodium, bathing solution was used of the following composition (mM): NaCl 50, CsCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $CoCl_2$ 1, TMA chloride 90, HEPES 20 and glucose 11. The pH was adjusted to 7.4 with TMA hydroxide. The solution inside the suction pipette contained (mM): CsF 145, NaF 10, and HEPES 5; and was titrated to pH 7.2 with 1 mM CsOH. Use of this solution provided effective isolation of I_{Na} from the other ionic currents. The pipette had a tip resistance of less than 0.5 MOhm. Cell capacitance (Cm) was estimated from the current transient produced by a small (10 mV) voltage clamp step, and determined by integrating the current transient; Cm = 74 ± 6 pF (n = 5). Series resistance (Rs) was determined by fitting exponentials to the current transient which was well described by a single exponential. Rs was estimated from the time constant (t) of the capacitive transient on the assumption of t = RsCm. Mean time constant was 98 ± 8 μs (n = 5), and Rs was 1.3 ± 0.4 MOhm (n = 5). Several criteria have been established that permit indirect determina-

tion of the adequacy of space-clamp control in cardiac preparations. Under our experimental conditions (Hisatome *et al.*, 1987), the current recordings obtained from the isolated myocytes satisfied the criteria described by Colatsky & Tsien (1979). The membrane current signal was recorded on videotape (video recorder, Mitsubishi HV-F73, Mitsubishi, Japan) through a PCM converter (Shoshin EM, PCM-DP16, Shoshin, Japan) for later computer analysis (NEC PC9801XL, NEC, Japan).

The block induced by an antiarrhythmic drug is known to be composed of a tonic and a use-dependent block. In order to study tonic block, we defined the drug-induced decrease in I_{Na} at a low pulse frequency (0.01 Hz) sufficient to ensure full recovery from the use-dependent block of I_{Na} as tonic block. The amount of tonic block was calculated as the percentage decrease in I_{Na} after perfusion with the drug in comparison with the value for the control. To study the use-dependent block, we interposed a rest of 180 s between the trains of stimuli. I_{Na} decreased during the pulse train and reached a new steady state. The amount of use-dependent block was calculated as the percentage decrease in I_{Na} in the new steady state with respect to the value for the first pulse (Hisatome *et al.*, 1990).

To determine the decay time constant of I_{Na} or the time constant of the recovery kinetics from the use-dependent block of I_{Na}, we used a computer to fit the data to the sum of a double exponential function having the form:

$$I_{Na} = A \exp(-t/t_f) + B \exp(-t/t_s) + C \quad (1)$$

where A, B, and C are constants, and t_f and t_s are the respective time constants of the fast and slow phases.

In analysing the recovery time constant of the recovery kinetics, we focused only on the slow phase of the recovery kinetics. To summarize the multiple data, we used the arithmetical mean ± s.e.mean.

Drugs used

Tosylchloramide sodium (chloramie-T) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bepridil hydrochloride was supplied by Nippon Organon Co. (Tokyo, Japan).

Results

Tonic block by bepridil

Figure 1 shows the concentration-dependent effects of bepridil on I_{Na}. Figure 1(a) and (b) shows the original current traces of I_{Na} elicited by test pulses to −30 mV from the holding potential (HP) of −100 or −140 mV at 0.01 Hz before and after the administration of bepridil. Bepridil at concentrations from 1 to 1000 μmol l^{-1} elicited a concentration-dependent tonic block of I_{Na}. Figure 1c shows the semilogarithmic plot of the drug concentration and the tonic block of I_{Na} at HP of −100 mV and at HP of −140 mV. Each point represents the mean value of the block ratio of I_{Na} for each concentration ($n=4$). The sigmoidal curve was calculated from the empirical equation:

$$\% \text{ of tonic block} = [D]/([D] + K_d) \quad (2)$$

where [D] is the concentration of bepridil (μmol l^{-1}) and K_d the apparent dissociation constant. K_d is 25.9 μmol l^{-1} at HP of −100 mV (Hill coefficient 1.024) and 295.4 μmol l^{-1} at HP of −140 mV (Hill coefficient 1.034).

Figure 2 shows the steady-state inactivation curve for I_{Na} under control conditions and after exposure to 100 μmol l^{-1} bepridil. The I_{Na} inactivation curve was assessed at selected membrane (prepulse) potentials using the standard two-pulse protocol. A 1000 ms prepulse to the designated level of membrane potential was followed by a 1 ms interpulse interval then by a 30 ms test pulse to −30 mV. This two-pulse sequence was applied once every 90 s. The curves drawn through the data points are described by the equation:

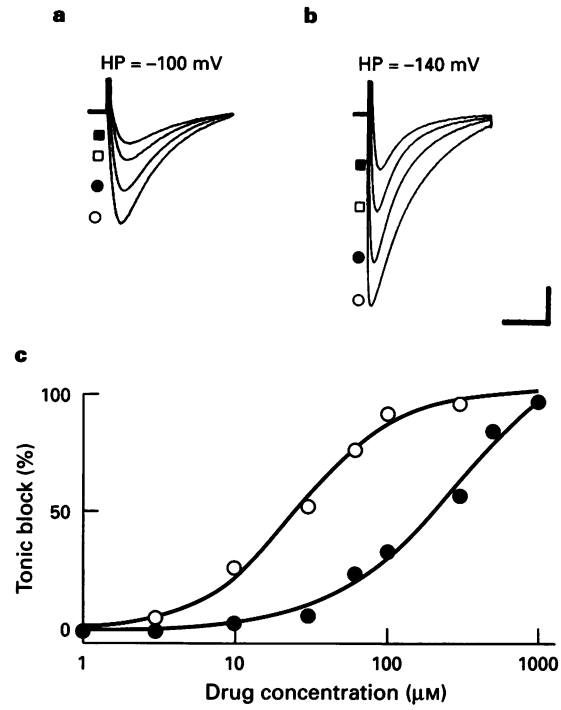

Figure 1 Relationship between the bepridil concentration and the percentage tonic block. (a) Original current traces of I_{Na} elicited by a test potential to −30 mV from HP of −100 mV under control conditions (○), and bepridil 10 μmol l^{-1} (●), 30 μmol l^{-1} (□) and 60 μmol l^{-1} (■) at 0.01 Hz. (b) Original current traces of I_{Na} elicited by a test potential to −30 mV from HP of −140 mV under control conditions (○), and bepridil 100 μmol l^{-1} (●), 300 μmol l^{-1} (□) and 500 μmol l^{-1} (■) at 0.01 Hz. The calibration shows a 1 nA current amplitude and 10 ms time scale. (c) The relationship between tonic block and drug concentration. Each point represents the mean value ($n=4$) of the tonic block at the concentrations tested at HP of −100 mV (○) or −140 mV (●). The sigmoid curve shown was the best fit to equation (2).

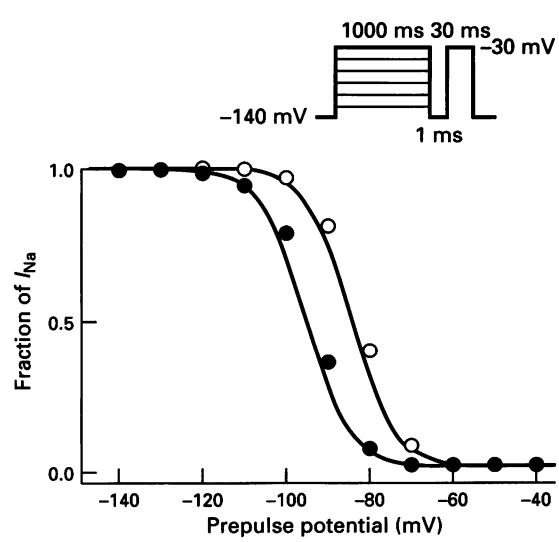

Figure 2 Effects of bepridil on the steady-state inactivation of I_{Na}. The inset shows the pulse protocol for evaluating the steady-state inactivation of I_{Na}. The prepulse potential where I_{Na} is one-half maximum (V_h) and the slope factor (k) were calculated using equation (3). V_h was −84.2 mV for the control (○) and −95.4 mV in the presence of 100 μmol l^{-1} bepridil (●).

$$h = (1 + \exp[(V_m - V_h)/k])^{-1} \quad (3)$$

where h is fraction of I_{Na}, V_m the prepulse potential, V_h the prepulse potential at which $h = 0.5$, that is, the midpotential and k the slope factor (Hodgkin & Huxley, 1952). Under control conditions, V_h was -84.2 mV and k was 5.05, and in the presence of 100 μmol l^{-1} bepridil, V_h was -95.4 mV and k was 5.05. The results of the 4 experiments show that 100 μmol l^{-1} bepridil shifted the I_{Na} inactivation curve by -11.2 ± 2.7 mV in the hyperpolarized direction along the voltage axis with no change in the slope factor.

Use-dependent block by bepridil

In addition to tonic block, bepridil showed use-dependent block of I_{Na}. Figure 3a (1–4) shows the I_{Na} elicited by the first depolarizing pulse at 2 Hz from a HP of -100 mV (1, 2) and of -140 mV (3, 4) to -30 mV, and by the second and the 20th pulses in the presence of 50 μmol l^{-1} bepridil, for which the pulse durations were varied from 10 to 300 ms. The 300 ms train pulse (Figure 3a; 2, 4) produced a somewhat greater degree of use-dependent block than the 10 ms pulse (1, 3). Figure 3b shows the percentage use-dependent block ($n = 5$) at 2 Hz for train pulses of 3, 10, 30, 100, 300 ms duration in the

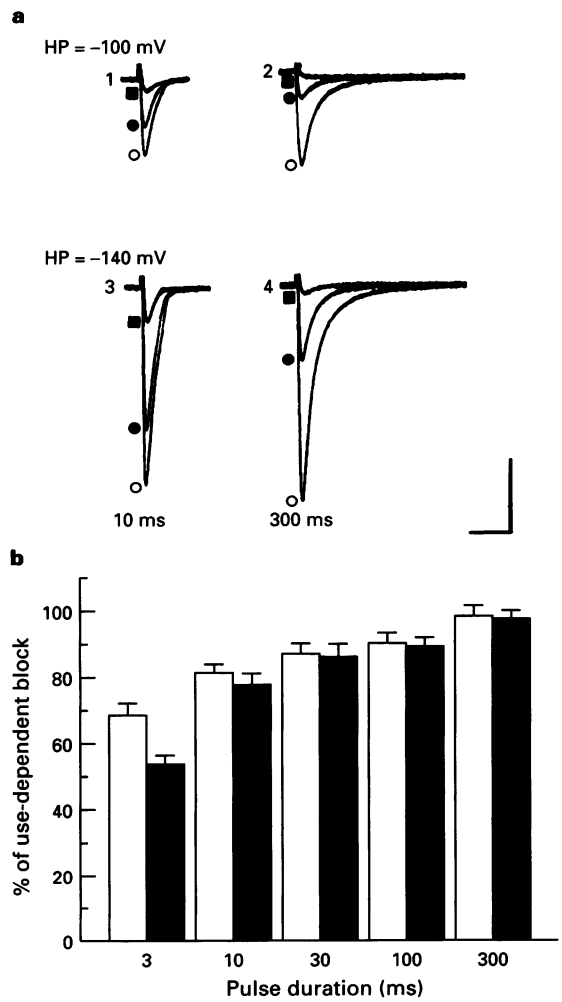

Figure 3 Relationships between pulse duration, holding potential, and use-dependent block at 2 Hz in the presence of 50 μmol l^{-1} bepridil. (a) I_{Na} elicited during a train of pulses to -30 mV from HP of -100 mV (1, 2) and of -140 mV (3, 4) at 2 Hz. Symbols show I_{Na} at the first pulse (○) at the second pulse (●), and at the 20th pulse (■) for the respective durations of 10 ms (1, 3) and 300 ms (2, 4). The calibration shows a 1 nA current amplitude and a 10 ms time scale. (b) Open columns indicate HP of -100 mV; solid columns indicate HP of -140 mV, ($n = 4$).

presence of 50 μmol l^{-1} bepridil. The use-dependent block elicited by the depolarizing pulse from a HP of -100 mV to -30 mV was almost as great as that elicited by the depolarizing pulse from a HP of -140 mV. Difference in HP did not affect the amount of use-dependent block. Pulse durations affected use-dependent block, but not significantly. The time course of the use-dependent block is shown in Figure 4.

These results suggest that the onset of the use-dependent block is rapid. That possibility was tested by use of a two-pulse protocol. A prepulse (the duration of which varied from 1 to 1000 ms) to -30 mV from a HP of -140 mV was followed by a 1.0 s interpulse interval and a test pulse to -30 mV from a HP of -140 mV to assess the onset block. The percentage of the onset of block after a prepulse that varied from 1 to 1000 ms in comparison with control I_{Na} values without a prepulse in the presence of 50 μmol l^{-1} bepridil, is shown in Figure 5. Even short prepulses of 3 and 10 ms produced the same use-dependent block as longer prepulses of 60 and 100 ms.

These results suggest that m-gate trapping may play an important role and the fast inactivation process is not needed to show the bepridil-induced use-dependent block. To determine if this were so, we examined whether use-dependent block could be induced by bepridil after the removal of fast inactivation by tosylchloramide sodium, which eliminates the fast inactivation of sodium channels in cardiac ventricular myocytes (Miyamoto *et al.*, 1989).

Figure 6a, b, and c shows the original current traces elicited by the first test pulse to -30 mV from a HP of -100 mV, -120 mV or -140 mV and by the 20th test pulse at 2 Hz after removal of the fast inactivation process by 3 mmol l^{-1} tosylchloramide sodium in the presence of 50 μmol l^{-1} bepridil. Even in the absence of fast inactivation, bepridil induced use-dependent block. Figure 6d shows the percentage of use-dependent block at 2 Hz induced by bepridil (50 and 30 μmol l^{-1}) after removal of fast inactivation ($n = 4$) at a HP of -100 mV, -120 mV and -140 mV. Bepridil gave almost the same amount of use-dependent block regardless of the holding potentials.

In the absence of tosylchloramide sodium, sodium channel inactivation process could be fitted to two exponentials, t_{fast} (7 ms) and t_{slow} (32 ms). After superfusing tosylchloramide sodium, sodium channel inactivation could also be fitted to

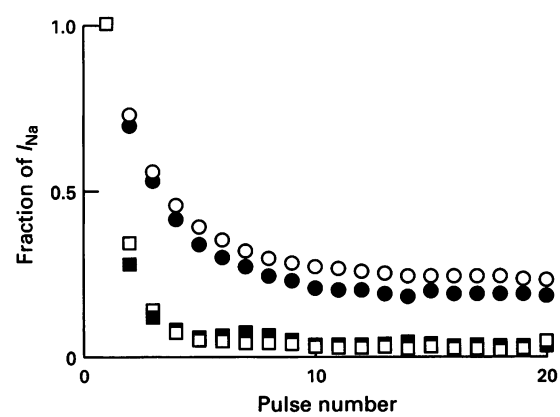

Figure 4 Use-dependent block of I_{Na} by bepridil. The graph represents beat after beat change of I_{Na} in the presence of 50 μmol l^{-1} bepridil. Ordinate scale indicates fraction of I_{Na} normalized by the value for the first depolarizing pulse of the train following a quiescent period. Abscissa scale indicates number of pulses. There were no significant differences in use-dependent block between two different holding potentials: (○) HP = -140 mV, PD = 10 ms; (●) HP = -100 mV, PD = 10 ms; (□) HP = -140 mV, PD = 300 ms; (■) HP = -100 mV, PD = 300 ms. With short pulse durations (10 ms; ○ and ●), the course of the inhibition of I_{Na} was slower and the rate of inhibition was somewhat less than with long pulse durations (300 ms; □ and ■).

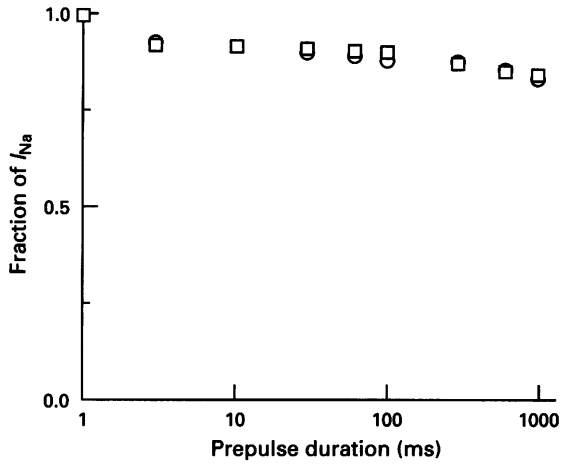

Figure 5 Onset of block of I_{Na} by bepridil examined with a two-pulse protocol. A prepulse to −30 mV from HP of −140 mV was followed by a 1.0 s interpulse interval and a test pulse to −30 mV from HP of −140 mV. Ordinate scale: fraction of I_{Na}. Abscissa scale: prepulse durations. (○) The first test; (●) the second test. Note that a shorter duration prepulse (3 and 10 ms) could produce the same amount of block as a longer duration prepulse (60 and 100 ms).

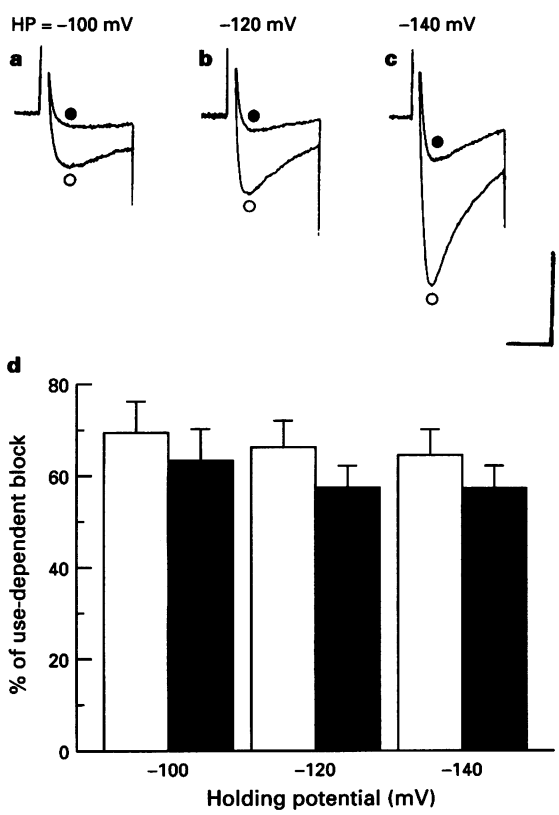

Figure 6 Use-dependent block of I_{Na} by bepridil after removal of fast inactivation. (a), (b) and (c): Original current traces elicited by the first test pulse (○) to −30 mV from HP of −100 mV, −120 mV, or −140 mV and by the 20th test pulse (●) at 20 Hz after the removal of fast inactivation by 3 mmol l^{-1} tosylchloramide sodium in the presence of 50 μmol l^{-1} bepridil. The calibration shows an amplitude of 1 nA and a duration of 10 ms. (d) Percentage of use-dependent block at 2 Hz by bepridil (50 μM open columns; 30 μM solid columns) after the removal of fast inactivation at HP of −100, −120 or −140 mV. Columns show mean with s.e.mean (n=4). Independent of the HP, bepridil gives almost the same amount of use-dependent block.

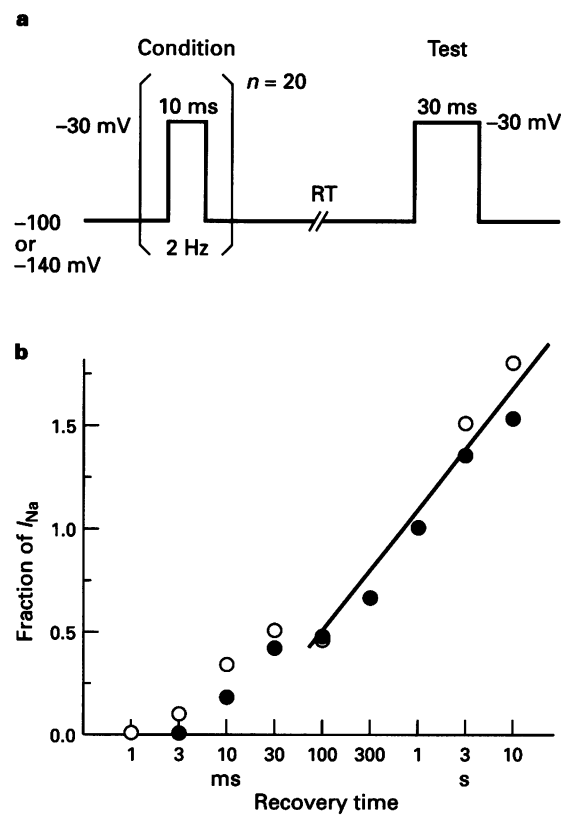

Figure 7 Recovery process from the use-dependent block of I_{Na} by bepridil. (a) The pulse protocol for assessing recovery from the use-dependent block. (b) Ordinate scale: the fraction of I_{Na} with respect to the value at 1 s. Abscissa scale: recovery time as the log function. (○) Recovery at HP of −100 mV; (●) recovery at HP of −140 mV in the presence of bepridil (50 μmol l^{-1}). Note that the time course of the recovery process is not affected by the HP.

single exponential, t_t (30 ms) and t_t was close to t_{slow}. Thus, tosylchloramide sodium removed the fast inactivation process. In addition to that, from these results, we can conclude that slow inactivation (t_{slow}) is a process independent of fast inactivation (t_{fast}).

Recovery from the use-dependent block of I_{Na} in the presence of 50 μmol l^{-1} bepridil was assessed by the protocol shown in Figure 7a. A train of 20 pulses of 10 ms duration at 2 Hz was followed by a recovery period and a test pulse to assess the amount of current recovered at HP of −100 or −140 mV. Pulse protocols were applied at 90 s intervals. Figure 7b shows each I_{Na} fraction with respect to the value at 1 s plotted against each recovery time at a HP of −100 mV and at a HP of −140 mV in the presence of 50 μmol l^{-1} bepridil. The recovery time course after 100 ms is described as a single exponential, for HP at −100 and −140 mV, the time constant being 0.48 ± 0.08 s and 0.51 ± 0.06 s, respectively (n=4).

Discussion

We found that bepridil decreased the I_{Na} of guinea-pig ventricular myocytes in the steady state (tonic block), or use-dependently (use-dependent block) and shifted the Na^+ inactivation curve in the hyperpolarizing direction. According to the modulated receptor theory (Hondegham & Katzung 1977): (1) Drugs bind to a receptor site in or very close to the sodium channel. (2) The affinity of the receptor for the drugs is modulated by the channel state; rested, inactivated or activated. (3) Drug-associated channels differ from drug-free channels in that they do not conduct and their ability to be

activated is shifted to a more negative potential. Tonic block is known to be composed of a rested and an inactivated state block. To determine K_{drest}, the dissociation constant for binding in the rested state, and K_{di}, the dissociation constant for binding in the inactivated state, we used the following equation (Bean *et al.*, 1983):

$$1/K_{dapp} = h/K_{drest} + (1\text{-}h)/K_{di} \quad (4)$$

where h is the fraction of channels in the rested state in the absence of the drug. Calculations show that K_{dapp} was 25.9 μmol l^{-1}, K_{drest} was 295.4 μmol l^{-1}, and K_{di} was 1.4 μmol l^{-1}. To confirm the calculations, we inserted our data in the following equation (Bean *et al.*, 1983):

$$dV_h = k\ \ln[(1+[D]/K_{drest})/(1+[D]/K_{di})] \quad (5)$$

where dV_h is the shift of the inactivation curve, k the slope factor of the inactivation curve, and [D] the drug concentration. The calculated hyperpolarizing shift in the presence of 100 μmol l^{-1} bepridil was 13.4 mV, near to the experimental value (10.8 mV). These results indicate that bepridil has a greater affinity for the inactivated than for the rested state, resulting in a decrease in the number of sodium channels available in the steady state condition.

Like class I antiarrhythmic agents, bepridil produces use-dependent block of I_{Na}. In the presence of bepridil, a brief single pulse or a train of brief conditioning pulses which activate Na^+ channels with minimal inactivation, produced use-dependent I_{Na} block. These results indicate that m-gate trapping, activation block and/or fast inactivation process may play important roles in bepridil-induced I_{Na} block. We therefore tested whether bepridil has an affinity for the activated and fast inactivated states by removing fast inactivation with tosylchloramide sodium which inhibits the fast inactivation process of the Na^+ channel (Narahashi *et al.*, 1970; Wang, 1984; Schmidtmayer, 1985). Because tosylchloramide sodium has no proteolytic activity, no modification of the drug receptor or structural changes in it occurs in tosylchloramide sodium-treated cells (Wang *et al.*, 1987). Tosylchloramide sodium has been used to remove the fast inactivation process of I_{Na} in nerve preparations (Ulbricht & Stoye-Herzog, 1984) and cardiac myocytes (Miyamoto *et al.*, 1989). After the removal of fast inactivation, bepridil showed use-dependent block of I_{Na} that was independent of the holding potentials, evidence that bepridil blocks I_{Na} at least partially due to binding in the activated state and it is important that the neutral form of the drug blocks I_{Na}.

The time course of recovery from the use-dependent block may reflect the unbinding process of the agent from the receptor, either through the hydrophilic or hydrophobic pathway. The receptor site for local anaesthetics is considered to lie within the channel lumen (Hille, 1977) and the drug would leave the receptor through the hydrophilic pathway (i.e., through the pore) or the hydrophobic pathway (i.e., through the lipid layer). The hydrophilic pathway is used only by the charged form of the drug, favoured by its low molecular weight; whereas, the hydrophobic pathway is used only by the neutral form of the durg, favoured by its lipid solubility estimated by log P (log of the n-octanol: water partition coefficient (Hille, 1977; Bean, 1984). Bepridil has a high log P value (8.0) (Courtney, 1987). In our study, the time course of recovery from the use-dependent block was not affected by the membrane potential, indicating that bepridil may use the hydrophobic pathway, because dissociation of the charged form of the drug from the receptor via the hydrophilic pathway should be affected by the changes in the energy barrier height of the channel pore derived from changing the membrane potential. Dissociation of the neutral form of the drug from the receptor through the hydrophobic pathway could be calculated using the equation:

$$1/l_p = 10^{pka}/k_p \quad (6)$$

where $1/l_p$ is the life time of the charged form of the drug, k_p the protonation rate constant of 5×10^8 M^{-1} s^{-1} (Schwartz *et al.*, 1977), and pK_a the pH at which 50% of the drug molecules are in the charged form. The pK_a value is 7.9 for bepridil; therefore, the predicted lifetime value was 0.16 s, which is near to the experimental data (0.48 s). Moreover, the recovery time constant of I_{Na} in the presence of bepridil was voltage-independent, which is in favour of bepridil using the hydrophobic pathway because the protonation rate constant of the drug is voltage-indepenent.

In conclusion, bepridil could bind to the receptor during the activation process and/or the fast inactivation process, in addition to the slow inactivation process (through the channel pore and the lipid bilayer) resulting in a decrease of I_{Na} during frequent stimulation. Egress from the receptor was through the lipid bilayer, resulting in the drug being independent of changes in energy barrier height of the channel pore and the accumulation of the drug in the sodium channel appeared to be small.

References

ANNO, T., FURUTA, T., ITOH, M., KODAMA, I., TOYAMA, J. & YAMADA, K. (1984). Effects of bepridil on the electrophysiological properties of guinea-pig ventricular muscles. *Br. J. Pharmacol.*, **81**, 589–597.

BEAN, B.P. (1984). Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6388–6392.

BEAN, B.P., COHEN, A.J. & TSIEN, R.W. (1983). Lidocaine block of sodium channels. *J. Gen. Physiol.*, **81**, 613–642.

COLATSKY, T.J. & TSIEN, R.W. (1979). Sodium channels in rabbit cardiac Purkinje fibres. *Nature (Lond.)*, **278**, 265–268.

COSNIER, D., DUCHENE-MARULLAZ, P., RISPAT, G. & STREICHENBERGER, G. (1977). Cardiovascular pharmacology of bepridil: a new potential antianginal compound. *Arch. Int. Pharmacodyn.*, **225**, 135–151.

COURTNEY, K.R. (1987). Quantitative structure/activity relations based on use-dependent block and repriming kinetics in myocardium. *J. Mol. Cell. Cardiol.*, **19**, 319–330.

EHARA, T., MATSUOKA, S. & NOMA, A. (1989). Measurement of reversal potential of Na^+-Ca^{2+} exchange current in single guinea-pig ventricular cells. *J. Physiol.*, **410**, 227–249.

HILLE, B. (1977). Local anaesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.*, **69**, 497–515.

HISATOME, I., MATSUOKA, S., MIYAMOTO, J., SAWAGUCHI, M., OMODANI, H., OSAKI, S., KOTAKE, H., MASHIBA, H. & SATO, R. (1990). Blocking effect of 1389-S on the sodium current in isolated guinea-pig ventricular myocytes. *Eur. J. Pharmacol.*, **179**, 447–451.

HISATOME, I., SATO, R., NISHIMURA, M. & SINGER, D.H. (1987). External proton blocks Na^+ current in isolated guinea-pig ventricular myocytes. *Circulation*, **76**, 110 (abstr.)

HODGKIN, A.L. & HUXLEY, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, **117**, 500–544.

HONDEGHEM, L.M. & KATZUNG, B.G. (1977). Time- and voltage-dependent interactions of antiarrhythmic drugs with sodium channel. *Biochem. Biophys. Acta*, **472**, 373–398.

KANE, K.A. & WINSLOW, E. (1980). Antidysrhythmic and electrophysiological effects of a new antianginal agent, bepridil. *J. Cardiovasc. Pharmacol.*, **2**, 193–203.

LABRID, C., GROSSET, A., DURENG, G., MIRONNEAU, J. & DUCHENE-MARULLAZ, P. (1979). Some membrane interactions with bepridil, a new antianginal agent. *J. Pharmacol. Exp. Ther.*, **211**, 546–554.

LABRID, C., LEINOT, M., BEAUGHARD, M., BASIEZ, M. & DUCHENE-MARULLAZ, P. (1981). Comparative antidysrhythmic profiles of bepridil, amiodarone and disopyramide in the guinea-pig and dog. *Arch. Int. Pharmacodyn.*, **249**, 87–97.

MIYAMOTO, J., SATO, R., YEH, J.Z. & SINGER, D.H. (1989). Amiodarone induced use dependent block of I_{Na} in the absence of the fast inactivation process in guinea-pig ventricular myocytes. *Circulation*, **80**, II-136 (abstr.)

NARAHASHI, T., FRAZIER, D.T. & YAMADA, M. (1970). The site of action and active form of local anaesthetics. 1. Theory and pH experiments with tertiary compounds. *J. Pharmacol. Exp. Ther.*, **171**, 32–44.

POWELL, T., TERRAR, D.A. & TWIST, V.W. (1980). Electrical properties of individual cells isolated from adult rat ventricular myocardium. *J. Physiol.*, **302**, 131–153.

SCHMIDTMAYER, J. (1985). Behavior of chemically modified sodium channels in frog nerves support a three-state model of inactivation. *Pflügers. Arch.*, **404**, 21–28.

SCHWARTZ, W., PALADE, P.T. & HILLE, B. (1977). Local anaesthetics: Effects of pH on use-dependent block of sodium channels in frog muscle. *Biophys. J.*, **20**, 343–368.

SINGH, B.N. (1991). Comparative efficacy and safety of bepridil and diltiazem in chronic stable angina pectoris refractory to diltiazem. The Bepridil Collaborative Study Group. *Am. J. Cardiol.*, **68**, 306–312.

ULBRICHT, W. & STOYLE-HERZOG, M. (1984). Distinctly different rates of benzocaine action on sodium channels of Ranvier nodes kept open by chloramine-T. *Pflügers. Arch.*, **402**, 439–445.

WANG, G.K. (1984). Irreversible modification of sodium channel inactivation in toad myelinated nerve fibres by the oxidant chloramine-T. *J. Physiol.*, **346**, 127–141.

WANG, G.K., BRODWICK, M.S., EATON, D.C. & STRICHARTZ, G.R. (1987). Inhibition of sodium currents by local anaesthetics in chloramine-T-treated squid axons. *J. Gen. Physiol.*, **89**, 645–667.

YATANI, A., BROWN, A.M. & SCHWARTZ, A. (1986). Bepridil block of cardiac calcium and sodium channels. *J. Pharmacol. Exp. Ther.*, **237**, 9–17.

(Received April 11, 1994
Revised May 22, 1995
Accepted May 30, 1995)