*Br. J. Pharmac.* (1984), **81**, 589–597

# Effects of bepridil on the electrophysiological properties of guinea-pig ventricular muscles

Takafumi Anno, Tatsuji Furuta*, Masanori Itho**, Itsuo Kodama, Junji Toyama & Kazuo Yamada

Department of Circulation and Respiration, The Research Institute of Environmental Medicine, Nagoya University, Nagoya, Department of Pharmacology*, Nagoya University, Nagoya, Japan and Nippon Organon KK**, Tokyo, Japan

**1** Effects of bepridil, a new antianginal and potential antiarrhythimic agent, on transmembrane action potentials of ventricular muscles were examined in isolated right ventricular papillary muscles of guinea-pig.

**2** Bepridil at concentrations above $5 \times 10^{-6}$ M caused a dose-dependent decrease in both the maximum upstroke velocity ($\dot{V}_{max}$) and the action potential duration from the upstroke to 30% repolarization ($APD_{30}$). On the other hand, the resting potential (RP), the amplitude of action potential (AMP), and the action potential duration from the upstroke to 90% repolarization ($APD_{90}$) were not affected even at the highest concentration applied ($10^{-5}$ M).

**3** The curves relating membrane potential and $\dot{V}_{max}$ were shifted by bepridil at $5 \times 10^{-6}$ M along the voltage axis in the direction of more negative potentials.

**4** The recovery kinetics of $\dot{V}_{max}$ assessed by premature stimuli were definitely slowed by bepridil at above $10^{-6}$ M. This effect was more pronounced with higher $[K^+]_o$ (10 mM) than normal $[K^+]_o$ (5 mM).

**5** Bepridil at $5 \times 10^{-6}$ M caused a rate-dependent decrease of $\dot{V}_{max}$ (use-dependent block) with rapid onset and offset, as did lidocaine.

**6** Slow responses, which had been induced by isoproenaline ($5 \times 10^{-6}$ M) in $K^+$-depolarized preparations, were suppressed significantly by additional application of bepridil at $10^{-5}$ M.

**7** These findings suggest that bepridil has electrophysiological characteristics similar to those both of Class Ib and Class IV antiarrhythmic drugs.

## Introduction

Bepridil (1-N-benzylanilino-2-pyrolidino- 3-isobutoxypropane) is a newly developed antianginal agent (Consnier *et al.*, 1977; Piris *et al.*, 1978). This substance has a potent vasodilator action accompanied by a direct cardiac action including negative chronotropic and inotropic effects (Michelin *et al.*, 1977; Steenberg *et al.*, 1978; Marshall & Muir, 1981). Bepridil was also shown to prevent supraventricular and ventricular arrhythmias induced by aconitine or coronary ligation (Kane & Winslow, 1980; Labrid *et al.*, 1981; Winslow & Kane, 1981; Marshall & Muir, 1981). This evidence and the drug's oral availability suggest a high therapeutic potential for this drug in the treatment of cardiac arrhythmias. Recent electrophysiological studies showed that bepridil prolongs the refractory period of atrial muscles and the atrioventricular node (Leinot *et al.*, 1979; Chassing *et al.*, 1977). As to the changes in transmembrane action potential, bepridil decreases the upstroke velocity in guinea-pig atrial and ventricular muscles as well as in sheep Purkinje fibres (Vogel *et al.*, 1979; Kane *et al.*, 1980; Winslow *et al.*, 1981). It was also indicated that bepridil reduces the slow inward current and the slow responses in guinea-pig ventricular muscles (Vogel *et al.*, 1979) and in frog sinoatrial muscles (Labrid *et al.*, 1979). These facts seem to indicate that bepridil has characteristics both of Class I and Class IV antiarrhythmic drugs (Hauswith & Singh, 1979). However, the precise mode of action of this drug still remains to be elucidated.

In the present experiments, the effects of bepridil on the transmembrane action potentials and on the voltage- and rate- dependency of their upstroke velocity ($\dot{V}_{max}$) were investigated in guinea-pig isolated ventricular muscles in order to clarify the cellular mechanism of its antiarrhythmic action.

## Methods

Twenty six guinea-pigs of either sex weighing 250 to 300 g were killed by a blow on the head. The hearts were quickly removed and the papillary muscles (2 to 3 mm in length and less than 1 mm in diameter) were isolated from the right ventricle. The preparation was fixed in a tissue bath and superfused continuously with Krebs-Ringer solution equilibrated with 95% $O_2$ and 5% $CO_2$. The composition of the solution was as follows (mM): NaCl 120.3, KCl 5.0, $CaCl_2$ 1.2, $MgSO_4$ $7H_2O$ 1.3, $NaH_2PO_4$ 1.2, $NaHCO_3$ 24.2 and glucose 5.5 (pH 7.4). In some experiments the $K^+$ concentration was raised to 20 mM by adding KCl so as to inactivate the fast sodium channels. The temperature of the solution in the tissue bath was maintained at 33°C. The preparations were stimulated by a pair of bipolar electrodes with an interpolar distance of 0.5 mm. Pulses used for stimulation were 2 ms in duration and twice the diastolic threshold in intensity unless otherwise specified. Transmembrane potentials were recorded through standard glass microelectrodes filled with 3 M KCl and having a resistance ranging 10 to 20 megohms. The maximum upstroke velocity ($\dot{V}_{max}$) of the action potential was obtained by electronic differentiation. When the relationship between membrane potential and $\dot{V}_{max}$ was examined, the resting potential of the preparation was depolarized in steps up to around −60 mV by adding aliquots of KCl to the Krebs-Ringer solution.

The recovery kinetics of $\dot{V}_{max}$ were determined by a method similar to that used by Gettes & Reuter (1974). The tissue was stimulated at a basal conditioning rate of 1.0 Hz. After every eighth basal stimulus, a test stimulus was introduced with various coupling intervals. The intensity of the test stimulus was adjusted to obtain a constant delay between the stimulus artifact and the initial upstroke of action potential. $\dot{V}_{max}$ of the test action potential was then plotted as a function of diastolic interval, which was defined as the time (ms) between the end of the conditioning action potential and the initial upstroke of the test action potential.

To study rate-dependent effects of bepridil on $\dot{V}max$, the preparations were driven by trains of stimuli at varying rates for 20 to 100 s. Rest periods, which were sufficient to ensure full recovery from the rate-dependent decrease in $\dot{V}_{max}$ (use-dependent block), were interposed between the trains of stimuli. The recovery of $\dot{V}_{max}$ from the use-dependent block were studied by applying single extra-stimuli at varying coupling intervals after the end of a train.

Drugs employed were bepridil (Nippon Organon, Tokyo, Japan), lidocaine (Wyeth, Philadelphia, PA, U.S.A.) and procainamide (Lederle Lab. Wayne, NJ, U.S.A.). After one hour of equilibration, control measurements were performed, and then the preparations were superfused with Krebs-Ringer solution containing these drugs at various concentrations for 30 to 40 min.

Statistical analysis was performed using student's $t$ test, and significance was established at $P < 0.05$.

More details of procedure are given under Results.

## Results

### *Effects of bepridil on the membrane action potential*

Effects of bepridil at $10^{-6}$ to $10^{-5}$ M on the membrane action potential were examined in five preparations driven at 1.0 Hz (Table 1, Figure 1). After exposure for 30 min to bepridil at $10^{-6}$ M, no significant change in action potential characteristics was observed. Bepridil at $5 \times 10^{-6}$ M caused a significant decrease in the maximum upstroke velocity ($\dot{V}_{max}$) accompanied by a shortening of action potential duration at 30% repolarization ($APD_{30}$). The higher concentration of bepridil ($10^{-5}$ M) caused a further decrease in $\dot{V}_{max}$ and more remarkable shortening of $APD_{30}$. The resting potential (RP), the amplitude of action potential (AMP) and the action potential duration at 90% repolarization ($APD_{90}$) were unaffected even at the highest concentration ($10^{-5}$ M) of bepridil applied.

**Table 1** Effects of bepridil on the transmembrane action potential of guinea-pig ventricular muscles

| | *AMP* (mV) | *RP* (mV) | $\dot{V}_{max}$ ($Vs^{-1}$) | $APD_{30}$ (ms) | $APD_{90}$ (ms) |
|---|---|---|---|---|---|
| Control | 118.4 ± 3.3 | −82.5 ± 2.5 | 198.2 ± 15.7 | 161.1 ± 18.6 | 236.7 ± 19.1 |
| Bepridil $10^{-6}$ M | 117.0 ± 2.6 | −82.5 ± 2.1 | 174.5 ± 22.5 | 154.6 ± 18.3 | 241.2 ± 26.9 |
| $5 \times 10^{-6}$ M | 114.9 ± 3.3 | −81.4 ± 2.0 | 152.8 ± 34.5* | 146.0 ± 27.4* | 236.3 ± 29.0 |
| $10^{-5}$ M | 112.0 ± 4.6 | −81.1 ± 2.1 | 126.6 ± 37.0* | 124.2 ± 30.9* | 223.2 ± 37.6 |

Values are mean ± s.d. ($n = 5$). Abbreviations: AMP, action potential amplitude; RP, resting potential; $\dot{V}_{max}$, the maximum upstroke velocity; $APD_{30}$, action potential duration at 30% repolarization; $APD_{90}$, action potential duration at 90% repolarization. *Significantly different from the control at $P < 0.05$.

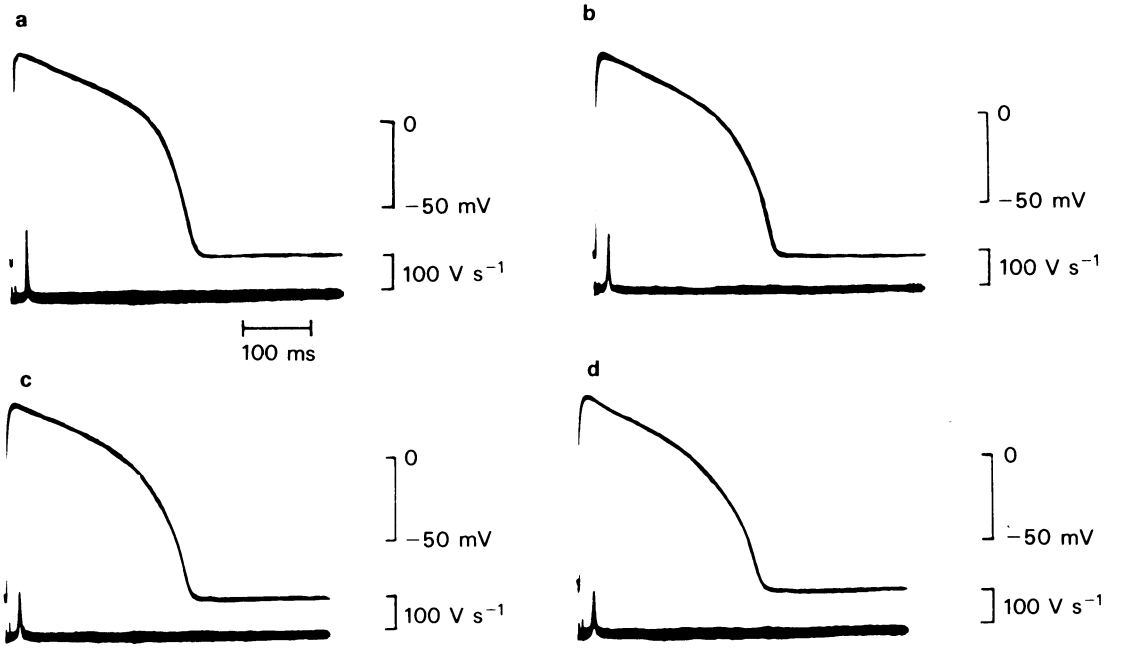


**Figure 1** Effects of bepridil on the transmembrane action potential of guinea-pig ventricular muscles: (a) control; (b) $10^{-6}$ M bepridil; (c) $5 \times 10^{-6}$ M bepridil; (d) $10^{-5}$ M bepridil. (b), (c) and (d) were recorded 30 min after application of bepridil at each concentration. Upper trace is membrane action potential and lower trace shows the differentiated upstroke spike of the action potential.

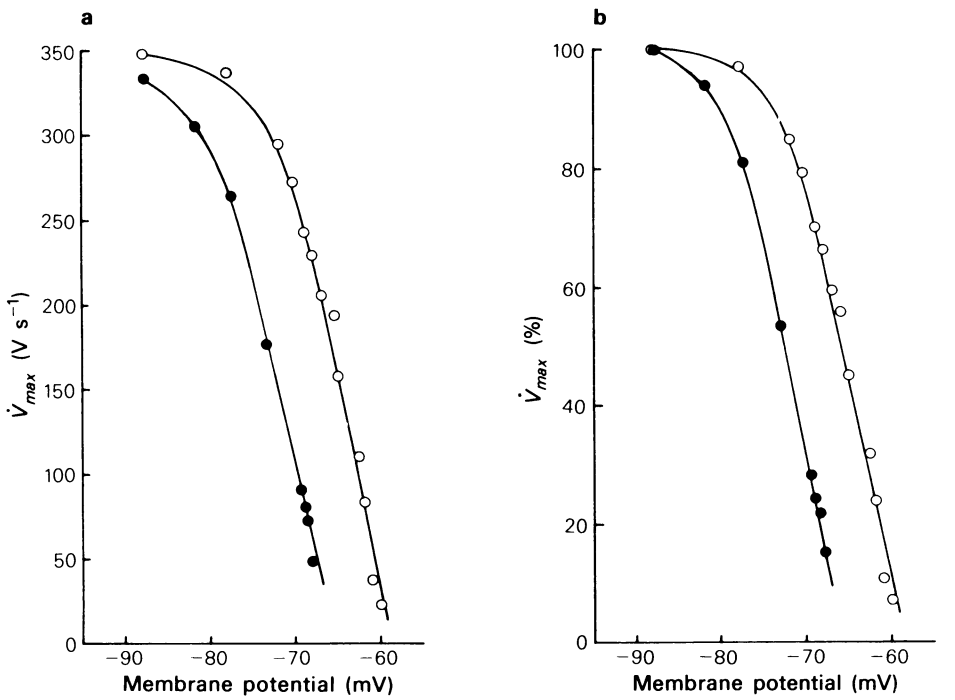


**Figure 2** Effects of bepridil ($5 \times 10^{-6}$ M) on the relationship between resting membrane potential and the maximum upstroke velocity ($\dot{V}_{max}$) in a fibre stimulated at 0.2 Hz. Values were obtained before (○) and 30 min after (●) application of bepridil. Absolute values are shown in (a), and normalized values (% $\dot{V}_{max}$) in (b).

*Effects of bepridil on the voltage dependency of* $\dot{V}_{max}$

The relationship between $\dot{V}_{max}$ and the membrane potential, from which the action potential takes off, was examined in three preparations driven at 0.2 Hz. The membrane potential was depolarized in steps from its original resting level to around −60 mV by increasing the $K^+$ concentration in the Krebs-Ringer solution. The results obtained from a preparation are shown in Figure 2. The decrease in $\dot{V}_{max}$ by bepridil ($5 \times 10^{-6}$ M) was more pronounced at less negative membrane potentials (Figure 2a). In normalized curves, plotting the percentage value of $\dot{V}_{max}$ (Figure 2b), the membrane potential associated with a 50% reduction in $\dot{V}_{max}$ is −66 mV before and −73 mV after application of the drug. Thus, in this case the normalized curve was shifted along the voltage axis in the hyperpolarizing direction by 7 mV. Similar shifts of the normalized curve after the drug application by 6 to 8 mV were observed in the other two preparations.

*Effects of bepridil on the recovery kinetics of* $\dot{V}_{max}$

The effects of bepridil on the recovery kinetics of $\dot{V}_{max}$ were examined under normal (5 mM) and high (10 mM) extracellular $K^+$ concentrations. The results obtained are shown in Figures 3 and 4. With 5 mM $[K^+]_o$, RP of the preparations before drug application was $-82.7 \pm 3.1$ mV (mean ± s.d., $n = 5$), and the time constant ($\tau_1$) with which $\dot{V}_{max}$ of the test action potential regained the same values as in the conditioning one, was $31.5 \pm 14.5$ ms. Thirty minutes after addition of bepridil at $10^{-6}$ M, the recovery time constant of $\dot{V}_{max}$ was largely prolonged. The higher concentrations of bepridil ($5 \times 10^{-6}$ M, $10^{-5}$ M) caused a further prolongation of $\tau_1$.

With 10 mM $[K^+]_o$, RP and $\tau_1$ before the drug application was $-71.4 \pm 2.6$ mV and $41.0 \pm 12.6$ ms respectively (mean ± s.d., $n = 4$). In such preparations, bepridil caused more prominent dose-dependent prolongation of $\tau_1$ than with 5 mM $[K^+]_o$. The values of RP in 10 mM $[K^+]_o$ as well as 5 mM $[K^+]_o$ were unaffected by bepridil even at $10^{-5}$ M.

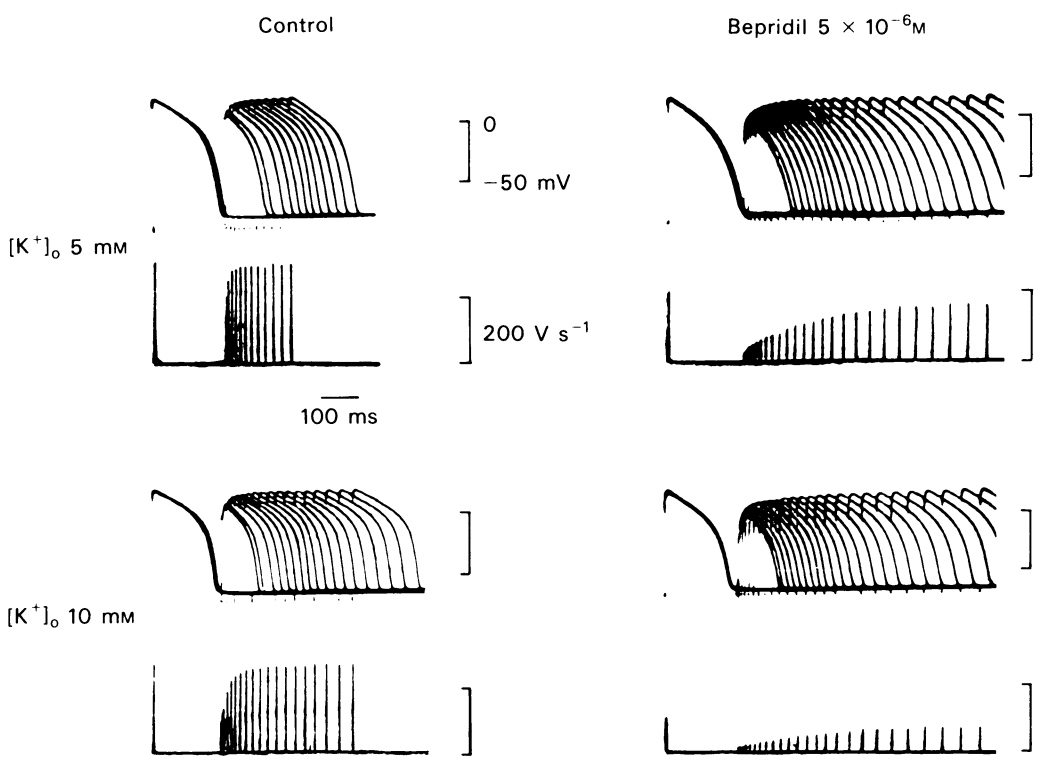


**Figure 3** Effects of bepridil on the recovery kinetics of $\dot{V}_{max}$. Upper trace in each record shows superimposed action potentials induced by conditioning stimuli at 1.0 Hz as well as a test stimuli with various coupling intervals. Lower trace shows differentiated upstroke spikes of the action potentials. Records were obtained before and 30 min after application of bepridil ($5 \times 10^{-6}$ M) in 5 mM or 10 mM $[K^+]_o$.

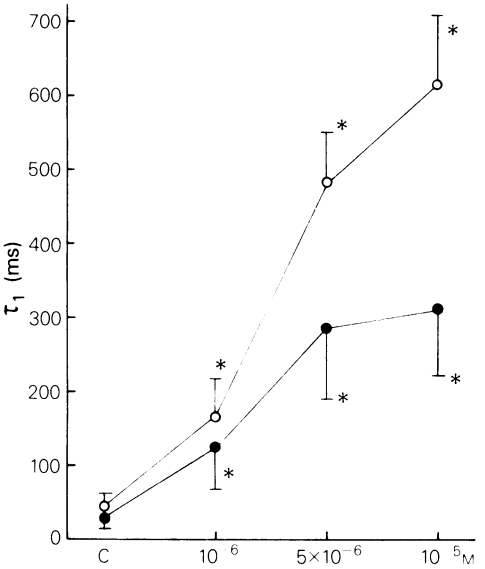


**Figure 4** Effects of bepridil on the recovery time constant ($\tau_1$) of $\dot{V}_{max}$ in 5 mM and 10 mM $[K^+]_o$. Values represent the mean of five preparations in 5 mM $[K^+]_o$ (●) and of four preparations in 10 mM $[K^+]_o$ (○); vertical lines show s.d.* Significantly different from the control value in the same $[K^+]_o$ at $P<0.05$.

*Rate-dependent effects of bepridil on* $\dot{V}_{max}$

The rate-dependent effects of bepridil on $\dot{V}_{max}$ were compared with those of lidocaine and procainamide. Under control condition, the value of $\dot{V}_{max}$ was almost unchanged during the stimulation trains at rates ranging from 0.2 Hz to 2.0 Hz (Figure 5). After treatment with bepridil ($5\times10^{-6}$ M) for 30 min, a rapid decrease in $\dot{V}_{max}$ approaching a new steady level within several beats was observed during the pulse trains at rates above 0.5 Hz (Figure 5). The decrease in $\dot{V}_{max}$ from the first to the 20th beats, which was measured as an index indicating the intensity of the use-dependent block, was $8.2\pm3.2\%$ at 0.5 Hz, $18.6\pm4.0\%$ at 1.0 Hz, and $35.3\pm5.6\%$ at 2.0 Hz (mean ± s.d., $n=4$).

In the presence of lidocaine ($6\times10^{-5}$ M), the rate-dependent decrease in $\dot{V}_{max}$ was observed at rates higher than 1.0 Hz (Figure 6). The onset of the use-dependent block was very rapid as in the case of bepridil. Thus, a steady state level of $\dot{V}_{max}$ was attained within several beats. The decrease in $\dot{V}_{max}$ from the first to the 20th beats was $4.2\pm1.8\%$ at 1.0 Hz and $25.3\pm5.6\%$ at 2.0 Hz (mean ± s.d., $n=4$). Procainamide ($3\times10^{-4}$ M) caused an apparent use-dependent block of $\dot{V}_{max}$ even at 0.2 Hz. In addition, the onset of the use-dependent block was much slower than with bepridil and lidocaine. Thus more than 20 beats were required for $\dot{V}_{max}$ to reach a steady state level. The decrease in $\dot{V}_{max}$ from the first to the 20th beats was $17.2\pm3.6\%$ at 0.2 Hz, $26.5\pm3.2\%$ at 0.5 Hz, $41.6\pm7.3\%$ at 1.0 Hz and $52.6\pm8.4\%$ at 2.0 Hz (mean ± s.d., $n=3$).

The value of $\dot{V}_{max}$ for the first action potential of the stimulation train following a rest period longer than 20 s was unaffected by bepridil ($5\times10^{-6}$ M), lidocaine ($6\times10^{-5}$ M), or procaineamide ($3\times10^{-4}$ M) indicating no 'resting block' (Campbell, 1983a) of $\dot{V}_{max}$ by these drugs.

The recovery process of $\dot{V}_{max}$ from the use-dependent block toward its initial (resting) value was studied by adding single extrastimuli at varying intervals after a series of trains of stimuli for 20 s at 1.0 Hz

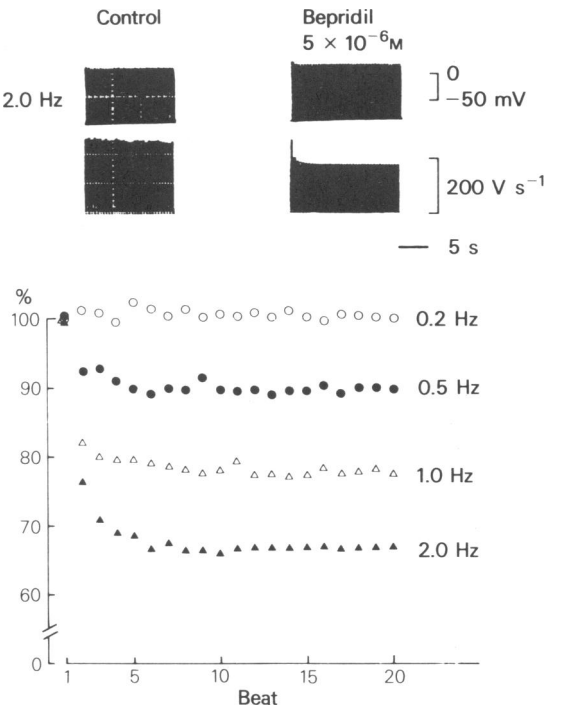


**Figure 5** Rate-dependent decrease of $\dot{V}_{max}$ (use-dependent block) by bepridil. Upper panel shows action potentials (upper trace) and their differentiated upstroke spikes (lower trace) during stimulation trains at 2.0 Hz in a previously quiescent tissue. The records were obtained before and 30 min after application of bepridil at $5\times10^{-6}$ M. Lower graph represents beat after beat change of $\dot{V}_{max}$ in the presence of bepridil ($5\times10^{-6}$ M). Ordinate scale indicates percentage of $\dot{V}_{max}$ normalized by the value for the first action potential of the train following a quiescent period. Abscissa scale indicates number of beats (action potentials). Frequences of the stimulation train were 0.2 Hz (○), 0.5 Hz (●), 1.0 Hz (△) and 2.0 Hz (▲).

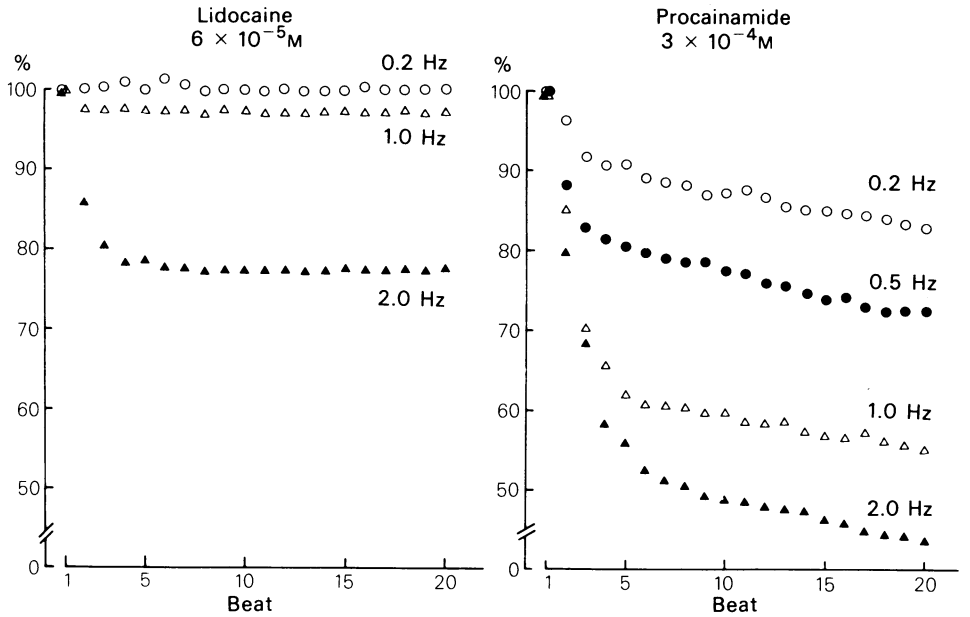


**Figure 6** Rate-dependent decrease of $\dot{V}_{max}$ (use-dependent block) by lidocaine ($6 \times 10^{-5}$ M) and by procainamide ($3 \times 10^{-4}$ M). Ordinate and abscissa scales are the same as in Figure 5. Frequencies of the stimulation train were 0.2 Hz (○), 0.5 Hz (●), 1.0 Hz (△), and 2.0 Hz (▲). Data were obtained from the preparations treated by these drugs for 30 min.

(Figure 7). The time course of this process for all three drugs was found to be fitted to single exponentials, though the time constant ($\tau_2$) for each drug was different. Recovery from the use-dependent block by bepridil at $5 \times 10^{-6}$ M was relatively rapid ($\tau_2 = 602 \pm 78$ ms, $n = 4$). $\tau_2$ for lidocaine ($6 \times 10^{-5}$ M) was somewhat shorter ($\tau_2 = 342 \pm 42$ ms, $n = 3$) than bepridil. In contrast, $\tau_2$ for procainamide ($3 \times 10^{-4}$ M) was much longer ($5.2 \pm 0.9$ s, $n = 3$) than the other two drugs.

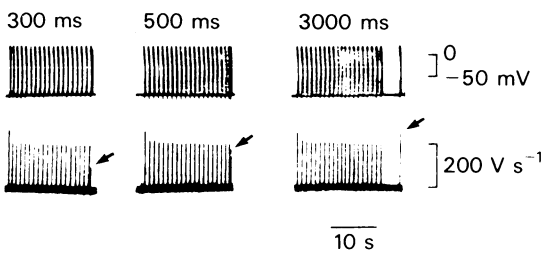


**Figure 7** Recovery of $\dot{V}_{max}$ from the rate-dependent block by bepridil. Upper traces are action potentials induced by stimulation trains at 1.0 Hz for 20 s and by an extra stimulus following the train. Lower traces are their differentiated upstroke spikes. The coupling interval from the last stimulus of the train to the extra stimulus was 300 ms, 500 ms and 3000 ms. Arrows indicate the $\dot{V}_{max}$ of test action potentials.

*Effects of bepridil on the slow responses*

In three preparations, the effects of bepridil on slow responses were examined in a high $K^+$ medium (Figure 8). When the $K^+$ concentration of Krebs-Ringer solution was increased from 5 mM (control) to 20 mM, the membrane was depolarized to a level around −50 mV, and the preparation became inexcitable because of the voltage-dependent inactivation of the fast sodium channels. In such prepara-

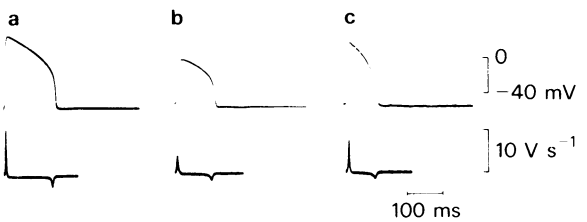


**Figure 8** Effects of bepridil on the slow responses induced by isoprenaline and high $[K^+]_o$. (a) Slow action potential induced by isoprenaline ($5 \times 10^{-6}$ M) in the medium with 20 mM $K^+$ concentration; (b) 30 min after application of bepridil ($10^{-5}$ M); (c) 15 min after the elevation of $Ca^+$ concentration in the medium from 1.2 mM (control) to 2.4 mM in the presence of both isoprenaline and bepridil. The preparation was constantly stimulated at 0.5 Hz.

tions, isoprenaline ($5 \times 10^{-6}$ M) restored the action potential, having very slow upstroke velocity (Figure 8a). Additional application of bepridil ($10^{-5}$ M) caused a significant suppression of these slow responses (Figure 8b). The average value (mean ± s.e.) of $\dot{V}_{max}$ in the three preparations driven at 0.5 Hz was $11.1 \pm 2.3\ Vs^{-1}$ before and $6.2 \pm 1.8\ Vs^{-1}$ after application of bepridil ($10^{-5}$ M) for 30 min ($P < 0.05$). The amplitude and the duration of the action potential were also reduced by bepridil. This inhibitory action of bepridil on the slow responses was partially reversed by elevating the $Ca^{2+}$ concentration of the medium from 1.2 mM (control) to 2.4 mM (Figure 8c).

## Discussion

The present study indicates that bepridil at concentrations above $5 \times 10^{-6}$ M caused a dose-dependent decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of action potential and a shortening of action potential duration (APD) at the early repolarization phase. However, the resting potential (RP), amplitude (AMP) and APD at a late repolarization phase were unaffected by the drug. Among these findings, the decrease in $\dot{V}_{max}$ is consistent with previous reports indicating the inhibitory action of bepridil on the fast sodium channels. Vogel *et al.* (1979) and Kane & Winslow (1980, 1981) reported that bepridil above $10^{-5}$ M reduced the $\dot{V}_{max}$ of action potentials in guinea-pig atrial and ventricular muscles as well as in sheep Purkinje fibres. In voltage clamp experiments on frog atrial muscles, it was demonstrated that bepridil at $8 \times 10^{-5}$ M decreased the peak amplitude of the fast sodium inward current by 27% (Labrid *et al.*, 1979).

As to the change in APD, there are some discrepancies between our data and those of other investigators. Kane & Winslow (1980) showed that bepridil caused a prolongation of APD in guinea-pig atrial muscle fibres as well as in ventricular muscle fibres. However, in sheep Purkinje fibres, they reported a shortening of APD at the early repolarization phase. In the present study on guinea-pig ventricular muscles, APD only at an early repolarization phase was shortened by bepridil. These discrepancies could be attributed to the tissue and species differences or to the different conditions of experiments.

We investigated the $\dot{V}_{max}$-membrane potential relationship in the presence and absence of bepridil by means of potassium depolarization. In the experiments, the decrease in $\dot{V}_{max}$ by bepridil was more pronounced at less negative membrane potentials resulting in a hyperpolarizing shift of the normalized curves relating $\dot{V}_{max}$ and RP. In this respect, bepridil is similar to lidocaine (Chen *et al.*, 1975; Chen & Gettes, 1976) or mexiletine, (Hohnloser *et al.*, 1982) but differs from quinidine which has been found to reduce $\dot{V}_{max}$ at all membrane potentials to about the same percentage extent resulting in no shift of the normalized curves (Chen *et al.*, 1975; 1976). Such an effect of bepridil could be described as a shift of steady state inactivation ($h\infty$) curve along the voltage axis to more negative membrane potentials, or as a higher affinity of this drug for the depolarized fast sodium channels.

Bepridil caused a marked prolongation of the recovery time constant of $\dot{V}_{max}$ ($\tau_1$) measured by the premature stimulus technique in the preparations driven at 1.0 Hz. The effect was more pronounced in the preparations depolarized by high (10 mM) $K^+$ concentration. These findings, which are similar to those for lidocaine (Chen *et al.*, 1975) and mexiletine (Hohnloser *et al.*, 1982), may be explained by an interference of the drug with the recovery kinetics of the fast sodium channels from inactivation (Chen *et al.*, 1975). It may also be interpreted by a modulated receptor hypothesis. According to the idea proposed by Hondeghem & Katzung (1977), Class I aniti-arrhythmic drugs can gain access (associate) to the receptor site mainly when the corresponding channel is open (activated) and leave (dissociate) gradually when the channel is closed (inactivated or rested). The drug-associated channels do not conduct sodium ions even when activated. i.e. they are blocked. The delayed recovery kinetics of $\dot{V}_{max}$ in the presence of bepridil may reflect a gradual increase in the fraction of unblocked channels during the diastolic interval of several hundred milliseconds.

The present experiments on the rate-dependent change in $\dot{V}_{max}$ demonstrated that bepridil and lidocaine caused a rapid rate-dependent decrease of $\dot{V}_{max}$ resulting in a new steady state level within several action potentials. This use-dependent block was observed during the stimulation trains at rates higher than 0.5 Hz for bepridil and 1.0 Hz for lidocaine. The recovery of $\dot{V}_{max}$ from the use-dependent block was attained quickly with a time constant ($\tau_2$) of $602 \pm 78$ ms for bepridil and $342 \pm 42$ ms for lidocaine. In the presence of procainamide, the use-dependent block of $\dot{V}_{max}$ with much slower onset was observed at rates above 0.2 Hz, and more than 20 action potentials were required to reach a steady state. The recovery of $\dot{V}_{max}$ from the use-dependent block by procainamide was also much slower ($\tau_2 = 5.2 \pm 0.9$ s) than those with bepridil and lodocaine. Based on Hondeghem & Katzung's model (1980), these facts can be explained by different rate constants for each drug to associate with and dissociate from the fast sodium channels. It is presumed that bepridil may have, like lidocaine, a relatively high association rate constant to the open channels accompanied by a high dissociation rate constant from the closed channels.

Courtney (1980, a,b,c), Sada & Ban (1981) found that the kinetics of onset and particulary recovery from the use-dependent block in a series of local anaesthetics and antiarrhythmic drugs correlate well with the molecular weights of the compounds. In terms of current concepts for the interaction of these drugs with the sodium channels, this could be interpreted as indicating that increasing molecular size somehow inhibits access to and egress from the receptor site which is thought to lie within the channel lumen (Hille, 1978). Our data are apparently inconsistent with this 'molecular size hypothesis'. The molecular weight of bepridil (366.5) is much larger than those of lidocaine (234.3) and procainamide (271.8), but is similar to those of disopyramide (339) and encainide (352). The latter two drugs were recently shown to cause a use-dependent block of $\dot{V}_{max}$ with very slow onset and offset (Campbell, 1983 a). Other characteristics in the chemical structure of bepridil including high lipid solubulity and/or low pKa (7.89) might explain why it is a kinetically fast drug despite its large molecular size.

Our results also showed that bepridil at $10^{-5}$ M significantly suppressed the slow responses which had been induced by isoprenaline in a high $K^+$ medium. This finding supports previous reports indicating inhibition of the slow channels by the drug (Labrid *et al.*, 1979). The shortening of $APD_{30}$ by bepridil in the medium with a normal $K^+$ concentration (Figure 1, Table 1) is most probably explained by such an inhibition of slow channels, because an activation of the slow inward current is of prime importance for the genesis of the early plateau phase of action potential in mammalian ventricular muscles (Carmeliet & Vereecke, 1979).

Based on the above discussion it is reasonable to conclude that bepridil has inhibitory effects on the fast sodium channels as well as on the slow channels of ventricular muscles. The inhibitory effect on the fast sodium channels is qualitatively similar to other Class Ib antiarrhythmic drugs such as lidocaine and mexiletine, but different from Class Ia antiarrhythmic drugs like quinidine or procainamide in terms of its voltage- and frequency-dependency (Chen *et al.*, 1975; 1976; Hondegehm *et al.*, 1977; 1980; Sada *et al.*, 1979; Courtney, 1980 c; Campbell, 1983 b). Bepridil-induced depression of $\dot{V}_{max}$ was augmented by shortening the interstimulus intervals, or by depolarizing the resting membrane potential. The former characteristic would be very important for the drug's action in preventing ventricular tachyarrhythmias or premature beats with shorter coupling intervals. The latter would induce the selective inhibition of excitability and conductivity in depolarized cardiac tissues due to ischaemia or other pathological conditions. Given this effect, bepridil may convert unidirectional into bidirectional block and thus prevent re-entrant arrhythmias. The high therapeutic potency of this drug for the arrhythimas induced by coronary ligation (Kane *et al.*, 1980; Marshall *et al.*, 1981) might be explained by such a mechanism.

Unlike lidocaine, however, bepridil was also shown to have a potent supraventricular antiarrhythmic activity in animal models (Winslow *et al.*, 1981). This could be related to the inhibitory action of this drug on the slow channels in sinoatrial nodes (Beaughard *et al.*, 1982) and in atrioventricular nodes, as in the case of verapamil (Wit & Cranefield, 1974; Rosen *et al.*, 1975). Nevertheless further experimental studies are required to confirm this interpretation.

## References


BEAUGHARD, M., FERRIER, M., LABRID, C., LAMAR, J.C., LEBOEUF, J. & PIRIS, P. (1982). Studies on the bradycardia induced by bepridil. *Br. J. Pharmac.*, **75**, 293–300.

CAMPBELL, T.J. (1983a). Resting and rate-depression of maximum rate of depolarization (Vmax) in guinea pig ventricular action potentials by mexiletine, disopyramide, and encainide. *J. cardiovasc. Pharmac.*, **5**, 291–296.

CAMPBELL, T.J. (1983b). Kinetics of onset of rate-dependent effect of Class I antiarrythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. *Cardiovasc. Res.*, **17**, 344–352.

CARMELIET, E. & VEREECKE, J. (1979). Electrogenesis of the action potential and automaticity. In *Handbook of Physiology, The Cardiovascular System*, Vol. 1, ed. Berne, R.M., Sperelakis, N. & Geiger, S.R. pp. 269–334, Bethesda, Maryland: American Physiology Society.

CHASSING, C., MOINS, N., LAVARENNE, J. & DUCHENE-MARULLAZ, P. (1977). Comparative effects of bepridil, perhexiline, amiodarone and quinidine on atrial and atrio-ventricular refractory periods in the anesthetized dog. *J. Pharm.* (Paris) **8**, 503–514.

CHEN, C.M., GETTES, L.S. & KATZUNG, G. (1975). Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. *Circulation Res.*, **37**, 20–29.

CHEN, C.M. & GETTES, L.S. (1976). Combined effects of rate, membrane potential and drugs on maximum rate of rise (Vmax) of action potential upstroke of guinea pig papillary muscle. *Circulation Res.*, **38**, 464–469.

CONSNIER, D., DUCHENE-MARULLAZ, P., RISPAT, G. & STREICHENBERGER, G. (1977). Cardiovascular pharmacology of bepridil (1, 3-isobutoxy-2- (benzylphenyl) amino propyl pyrrolidine hydrochrolide), a new poten-

tial antianginal compound. *Archs int. Pharmacodyn. thér.*, **255,** 133–151.

COURTNEY, K.R. (1980a). Structure-activity relations for frequency-dependent sodium channel block in nerve by local anesthetics. *J. Pharmac. exp. Ther.*, **213,** 114–119.

COURTNEY, K.R. (1980b). Antiarrhythmic drug design: Frequency-dependent block in myocardium. In *Molecular Mechanisms of Anesthesia* (Progress in Anesthesiology, Vol. 2) ed. Fink, B.R., pp. 111–118. New York: Raven Press.

COURTNEY, K.R. (1980c). Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. *J. Mol. Cell. Cardiol.*, **12,** 1273–1286.

GETTES, L.S. & REUTER, H. (1974). Slow recovery from inactivation of inward currents in mammalian myocardial febers. *J. Physiol.*, **240,** 703–724.

HAUSWIRTH, O. & SINGH, B.N. (1979). Ionic mechanism in heart muscle in relation to the genesis and the pharmacologic control of cardiac arrhythmias. *Pharmac. Rev.*, **30,** 5–63.

HILLE, B. (1978). Local anesthetic action on inactivation of the sodium channel in nerve and skeletal muscle: Possible mechanisms for antiarrhythmic agents. In *Biophysical Aspects of Cardiac Muscle.* pp. 55–74. New York: Academic Press.

HOHNLOSER, S., WEIRICH, J. & ANTONI, H. (1982). Effects of mexiletine on steady-state characteristics and recovery kinetics of Vmax and conduction velocity in guinea pig myocardium. *J. cardiovasc. Pharmac.*, **4,** 232–239.

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

HONDEGHEM, L.M. & KATZUNG, B.G. (1980). Test of a model of antiarrhythmic drug action. Effects of quinidine and lidocaine on myocardial conduction. *Circulation*, **61,** 1217–1224.

KANE, K.A. & WINSLOW, E. (1980). Antidysrhythmic and electrophysiological effects of a new antianginal agent, bepridil. *J. cardiovasc. Pharmac.*, **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. Pharmac. exp. Ther.*, **211,** 546–554.

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

LEINOT, M., BASIEZ, M., TALVARD, J. & LABRID, C. (1979). Prolongation of the effective refractory period in isolated guinea pig atrium cordis by several antiarrhythmics: Interest in comparative potency. *C. R. Soc. Biol.*, **173,** 773–780.

MARSHALL, R.J. & MUIR, A.W. (1981). The beneficial actions of bepridil in acute myocardial infarction in anaesthetized dogs. *Br. J. Pharmac.*, **73,** 471–479.

MICHELIN, M.T., CHEUCLE, M. & DUCHENE-MARULLAZ, P. (1977). Comparative influence of bepridil, dipyridamole and propranolol on cardiac activity and coronary venous blood flow in the anesthetized dog. *Thérapie*, **32,** 485–490.

PIRIS, P., BEAUGHARD, M., COSNIER, D. & LABRID, C. (1978). Activity of bepridil and other anti-anginals on cardiovascular modifications engendered by conditioned anxiety in the dog. *Archs int. Pharmacodyn. Thér.*, **235,** 147–164.

ROSEN, M.R., WIT, A.L. & HOFFMAN, B.F. (1975). Electrophysiology and pharmacology of cardiac arrhythmias. VI. Cardiac effects of verapamil. *Am. Heart J.*, **89,** 665–673.

SADA, H. & BAN, T. (1981). Effects of various structurally related beta-adrenoceptor blocking agents on maximum upstroke velocity of action potential in guinea-pig papillary muscles. *Naunyn Schemiedebergs Arch. Pharmac.*, **317,** 245–251.

SADA. H., KOJIMA, M. & BAN, T. (1979). Effect of procainamide on transmembrane action potentials in guinea-pig papillary muscles as affected by external potassium concentration. *Naunyn-Schemiedebergs Arch. Pharmac.*, **309,** 179–190.

STEENBERG, M.L., BUCKLEY, J.P. & JANDHYLA, B.S. (1978). Effects of bepridil [3-isobutoxy-2-(benzyl phenyl) amino propyl pyrrolidine, HCL] on coronary and systemic hemodynamics of anesthetized dogs. *Pharmacologist*, **20,** 173.

VOGEL, S., CRAMPTON, R. & SPERELAKIS, N. (1979). Blockade of myocardial slow channels by bepridil (CERM-1978). *J. Pharmac. exp. Ther.*, **210,** 378–385.

WINSLOW, E. & KANE, A. (1981). Supraventricular antidysrhythmic and electrophysiological effects of bepridil, a new antianginal agent. *J. cardiovasc. Pharmac.*, **3,** 655–667.

WIT, A. & CRANEFIELD, P.F. (1974). Effect of verapamil on the sinoatrial and atrioventricular nodes of the rabbit and the mechanism by which it arrests reentrant atrioventricular nodal tachycardia. *Circulation Res.*, **35,** 413–425.

*(Received September 2, 1983.*
*Revised November 1, 1983.)*