Br. J. Pharmac. (1982), **75**, 293–300

STUDIES ON THE BRADYCARDIA INDUCED BY BEPRIDIL

M. BEAUGHARD, M. FERRIER, C. LABRID, J.C. LAMAR, J. LEBOEUF & P. PIRIS

Pharmacodynamics Dept., CERM, 63203 R10M Cedex, France

1 Bepridil, a novel active compound for prophylactic treatment of anginal attacks, induced persistent bradycardia and a non-specific anti-tachycardial effect, the mechanisms of which were investigated *in vitro* and *in vivo*.

2 *In vitro* perfusion of bepridil in the life-support medium for isolated sino-atrial tissue from rabbit heart, caused a reduction in action potential (AP) spike frequency (recorded by KCl microelectrodes) starting at doses of 5×10^{-6} M. This effect was dose-dependent up to concentrations of 5×10^{-5} M, whereupon blockade of sinus activity set in.

3 Bepridil at a dose of 5×10^{-6} M, induced a concomitant reduction in AP amplitude (falling from 71 ± 8 mV to 47 ± 6 mV), maximum systolic depolarization velocity (phase 0) which fell from 1.85 ± 0.35 V/s to 0.84 ± 0.28 V/s, together with maximum diastolic depolarization velocity (phase 4) which fell from 38 ± 3 mV/s to 24 ± 5 mV/s.

4 *In vivo* injection of bepridil at a dose of 5 mg/kg (i.v.) into 6 anaesthetized dogs which had undergone ablation of all the extrinsic cardiac afferent nerve supply, together with a bilateral medullo-adrenalectomy, caused a marked reduction in heart rate which fell from $98{\cdot}7 \pm 4.2$ beats/min to 76 ± 5.3 beats/min sustained for more than 45 min.

5 It is concluded that bepridil reduces heart rate by acting directly on the sinus node. This effect, which results in a flattening of the phase 0 and phase 4 slope, together with a longer AP duration, may be due to an increase in the time constants of slow inward ionic currents (already demonstrated elsewhere), but also to an increased time constant for deactivation of the outward potassium current (Ip).

Introduction

Bepridil (β[(2-methylpropoxy)-methyl] -N-phenyl-N-phenyl methyl-1-1 pyrrolidine ethanamine monohydrochloride monohydrate) is a novel active compound for prophylactic treatment of anginal attacks (Lanney de Courten, Martin & Richalet, 1979; Julien, Luccioni & Gerard, 1979; Canicave, Deu, Jacq & Paillet, 1980) the pharmacological properties of which were initially described by Cosnier, Duchene-Marullaz, Rispat & Streichenberger (1977). These workers have shown that an intravenous injection of bepridil in anaesthetized dogs, apart from transitory haemodynamic effects, mainly caused mild but very persistent bradycardia associated with a reduction in myocardial oxygen consumption. These effects were also evident after cardiac denervation or pretreatment of the animal with propranolol.

In addition, Piris, Beaughard, Cosnier & Labrid (1978) have found that the elevation in heart rate engendered by conditioned anxiety in conscious dogs was clearly decreased after treatment with bepridil. Similarly, isoprenaline-induced tachycardia in anaesthetized dogs was inhibited (but only partially) following an injection of bepridil. The same applies to tachycardia induced by glucagon, theophylline or by electrical stimulation of the right stellate ganglion (Cosnier *et al.*, 1977). Bepridil therefore does not seem to act specifically or competitively against adrenergic stimulation, thus confirming other studies carried out *in vitro* (Labrid, Grosset, Dureng, Mirroneau & Duchene-Marullaz, 1979) and *in vivo* (Constantin, Beaughard, Piris & Labrid, 1981). Finally, a depressed idioventricular rhythm was observed in dogs with chronic atrioventricular block after treatment with bepridil (Boucher & Duchene-Marullaz, 1978). This effect of bepridil on heart rate, so often seen in pharmacological studies with animals, is corroborated by evidence from clinical studies in man (Canicave *et al.*, 1980).

Two types of experimental systems were used to investigate the mechanisms underlying the bradycardial and anti-tachycardial activity of bepridil. First, the effects of bepridil on the different parameters governing sino-atrial action potentials in rabbit heart, were investigated *in vitro* by means of the classical technique using KCl microelectrodes. Sec-

0007-1188/82/020293-08 $01.00

ond, variations in heart rate were investigated in anaesthetized dogs subjected to cardiac denervation and bilateral medullo-adrenalectomy, after administration of bepridil.

Methods

Study of changes in action potential of rabbit isolated sino-atrial node

Young male 'Burgundy fawn' rabbits weighing 1.0 to 1.4 kg were killed by medullary dislocation. The heart was immediately removed and the atrium cordis isolated and rapidly dissected out, as described by Paes de Carvalho, De Mello & Hoffman (1959). The preparation was then fixed in a flat-bottomed cuvette and superfused with McEwen's solution (3 ml/min) having the following composition (mM): NaCl 130, KCl 5.6, $CaCl_2$ 2.16, $MgCl_2$ 0.24, $NaH_2PO_4.6H_2O$ 0.66, $NaHCO_3$ 11.9, and glucose 11, bubbled with a 95% O_2 and 5% CO_2 mixture. The medium was buffered at pH 7.35, and was maintained at a temperature of 31°C.

Glass electrodes with a tip diameter of 0.5 μm were used. These were filled with 3.0 M KCl by capillarity under vacuum, giving a tip impedance of about 10 to 15 MΩ. The electrodes were then fixed in a microelectrode holder (Phymep EH 1 IR) and connected to the input of a differential amplifier (Grass P 16). The inert electrode consisted of a calomel half-cell connected to the organ bath via a McEwen solution agar bridge. The signals were displayed on an oscilloscope screen (Tektronix 5115) and photographed by means of a polaroid device (Tektronix C12). This set-up enabled the maximum diastolic potential, threshold potential, sinus rate, action potential (AP) amplitude, duration of AP at threshold potential level, maximum systolic depolarization velocity and maximum diastolic depolarization velocity to be measured or calculated. To establish that microelectrode recordings were being taken from dominant S-A nodal cells, the following criteria were used: maximum diastolic potential <65 mV, maximum systolic depolarization velocity <5 V/s and overshoot <10 mV.

Bepridil was initially dissolved in pure ethanol (10^{-2} M) then diluted in McEwen's solution. The final concentrations used for testing ranged from 10^{-7} M to 5×10^{-5} M. The drug was not added cumulatively to the tissue and each concentration was tested on 5 different preparations. Whenever possible, the recordings were taken from the same cell before and after addition of the drug. When the microelectrode was ejected from the preparation, recordings from 5 different cells were made and the mean value was taken. The test solution was perfused for 20 min, and any effects elicited by the drug were assessed 1 min, 5 min, 10 min and 20 min after starting the perfusion. Rapid alternation between control perfusion and bepridil-loaded perfusions were assured by means of a multiple-way stop cock. Statistical analysis was performed by use of Student's t test and differences are considered significant if probability is lower than 0.05.

Study of changes in heart rate in anaesthetized dogs with bilateral medullo-adrenalectomy and cardiac denervation

These experiments were carried out on 8 dogs of either sex, weighing 12 ± 2.5 kg. The animals were anaesthetized with chloralose (100 mg/kg i.v.) dissolved in 0.154 M NaCl (10 ml/kg).

The surgical procedure included setting up a Braun artificial respirator to ensure pulmonary ventilation, and opening the left and right sides of the rib cage in order to section the left and right vagal nerves. The left and right stellate ganglia were then removed together with a portion of the thoracolumbar sympathetic system in the immediate vicinity. After performing a left lateral laparotomy at the vertebrocostal arch, the adrenal medullo was ablated by means of puncture and curettage, followed by ligation of one of the main blood vessels to the adrenal, and sectioning of the left splanchnic nerve. Finally, a right lateral laparotomy was performed to allow completion of a total right adrenalectomy.

The pleural vacuum was then re-established and the animal allowed to breathe freely. Heart rate was subsequently recorded by means of a Beckman cardiotachometer, while arterial pressure was taken at the carotid artery by means of a Mikro-Tip gauge.

Bepridil (5 mg/kg) dissolved in 5 ml distilled water was injected intravenously (1 min) into 6 dogs. The effect of solvent was tested in two other animals.

Results

In vitro *studies on rabbit sino-atrial node*

The AP characteristics, recorded after 10 min of perfusion with bepridil at 10^{-7} M (Figure 1b(i)), did not significantly differ from those noted in the control situation where McEwen's medium was perfused alone (Figure 1a). Conversely, perfusing bepridil at a concentration of 5×10^{-6} M (Figure 1b(ii)), resulted in a slower sinus rate, a reduced maximum systolic and diastolic depolarization velocity, but an increase in AP duration.

These effects were increased with doses of 10^{-5} M (Figure 1b(iii)). At 5×10^{-5} M, the measurements were performed at 1, 5 and 10 min but not at 20 min,

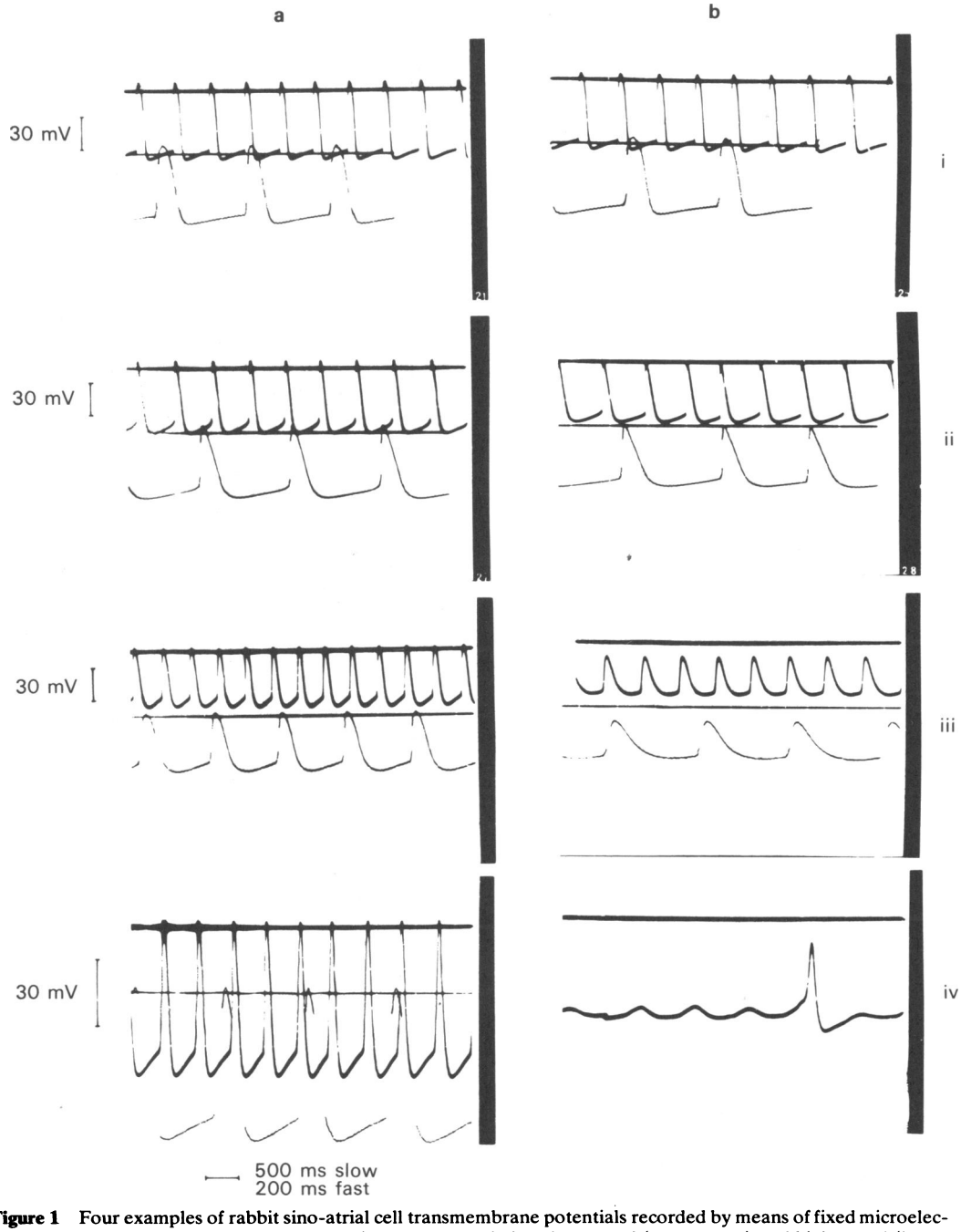

Figure 1 Four examples of rabbit sino-atrial cell transmembrane potentials recorded by means of fixed microelectrodes: in each case, transmembrane potentials were recorded at slow-speed (upper trace) and high-speed (lower trace) in the same preparation before adding bepridil (column a) and 10 min after adding bepridil (column b)). (a) Sinus pretreatment action potentials are characterized by a weak overshoot (the horizontal line indicates zero potential), a low amplitude (60–70 mV) and a low maximum diastolic potential (−60 mV). (b) Action potentials 10 min after adding bepridil at doses of 10^{-7} M (i), 5×10^{-6} M (ii), 10^{-5} M (iii) and 5×10^{-5} M (iv). Bepridil has little or no activity at 10^{-7} M. In contrast, doses of 5×10^{-6} M and above slow down sinus rate, decrease action potential amplitude as well as maximum systolic and diastolic depolarization velocity. It should also be noted that 10 min after adding bepridil at 5×10^{-5} M (ii) a blockade of rabbit sinus node pacemaker activity sets in.

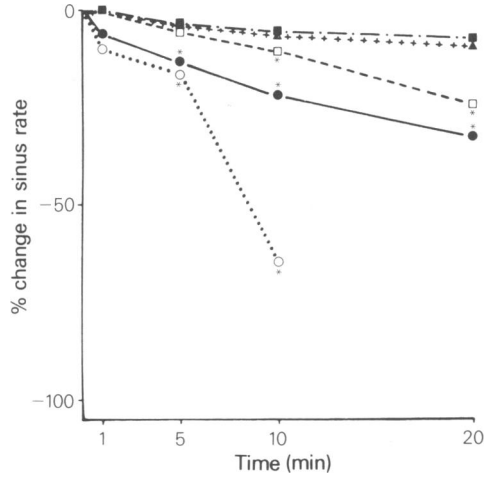

Figure 2 Change in sinus rate of isolated sinus node preparation from the rabbit, with doses of bepridil (from 10^{-7} M to 5×10^{-5} M): for each dose, $n = 5$ preparations. Bepridil: (■) 10^{-7} M; (▲) 5×10^{-7} M; (□) 5×10^{-6} M; (●) 10^{-5} M; (○) 5×10^{-5} M. *Significant changes from control values: $P < 0.05$.

since at that dose complete blockade of sino-atrial node pacemaker activity set in 12 ± 4 min after perfusing bepridil. The oscillations of the membrane potential (Figure 1b (iv)) could suggest a conduction block but even when the sinusal node is electrically stimulated the pacemaker activity is supressed at the dose of 5×10^{-5} M (unpublished observation). Recordings from cells outside the control area were not taken and so a shift of pacemaker site cannot be excluded.

Figure 2 shows the effect of bepridil on sinus rate as a function of the length of time the product is in contact with the preparation i.e. the dose-dependent bradycardia effect is only seen 5 min after perfusing the drug, and only becomes significant with doses of 5×10^{-6} M or above. Simultaneously, at this concentration heart rate fell from 128 ± 13 beats/min to 96 ± 14 beats/min 20 min after addition of bepridil.

The changes in AP amplitude were concomitant with changes in systolic depolarization velocity (phase 0) as shown in Figure 3. Thus for example, bepridil at doses of 5×10^{-6} M or above, caused AP amplitude to fall from 71 ± 8 mV to 47 ± 6 mV after being in contact with the organ for 20 min. At the same time, maximum systolic depolarization velocity was reduced from 1.85 ± 0.35 V/s to 0.84 ± 0.28 V/s.

Bepridil also induced a significant reduction in maximum diastolic depolarization velocity (spontaneous depolarization in phase 4) which went from 38 ± 3 mV/s to 24 ± 5 mV/s at a dose of 5×10^{-6} M. Higher doses induced a more pronounced effect (Figure 4). In addition, AP duration was increased at threshold potential level, since values rose to 220 ± 15 ms 20 min after injection of bepridil at 5×10^{-6} M, as compared to a maximum of 182 ± 12 ms in the controls.

On the other hand, neither the maximum diastolic potential (control values: 58 ± 3 mV) nor the threshold potential (control values: 48 ± 4 mV) were significantly modified by concentrations of bepridil up to 10^{-5} M. Only at higher concentrations

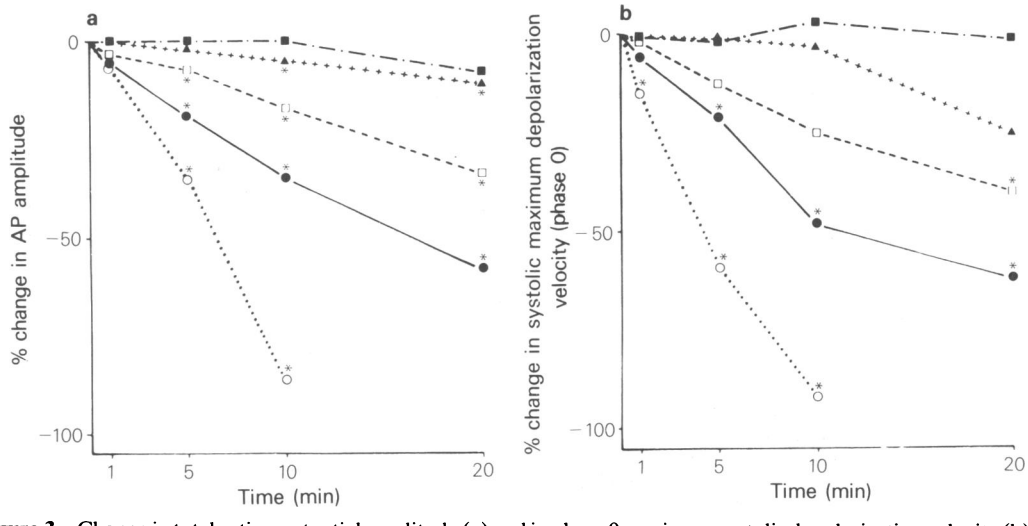

Figure 3 Change in total action potential amplitude (a) and in phase 0 maximum systolic depolarization velocity (b) with doses of bepridil (from 10^{-7} M to 5×10^{-5} M): for each dose $n = 5$ isolated sinus node preparations from rabbits. Bepridil: (■) 10^{-7} M; (▲) 5×10^{-7} M; (□) 5×10^{-6} M; (●) 10^{-5} M; (○) 5×10^{-5} M. *Significant changes from control values: $P < 0.05$.

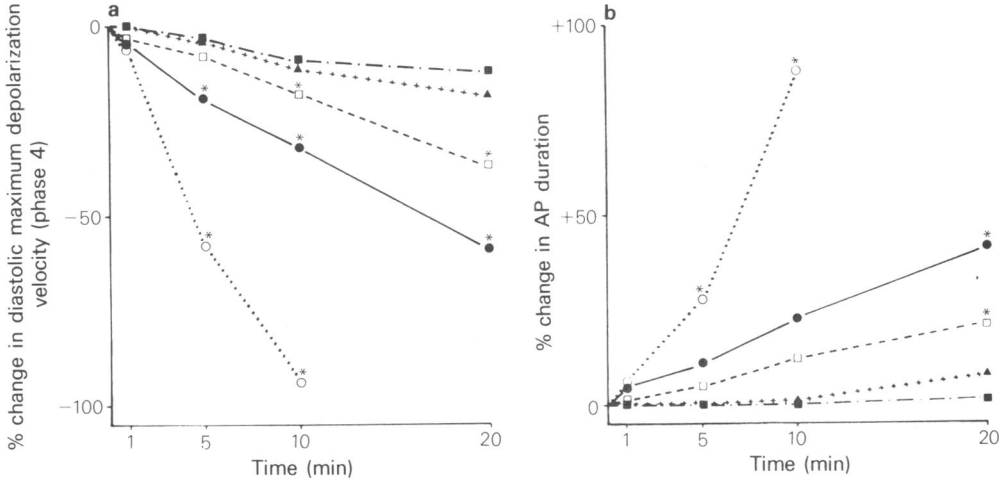

Figure 4 Change in phase 4, maximum diastolic depolarization velocity (a) and in action potential duration (b) with doses of bepridil (from 10^{-7} M to 5×10^{-5} M): for each dose $n=5$ isolated sinus node preparations from rabbits. Bepridil: (■) 10^{-7} M; (▲) 5×10^{-7} M; (□) 5×10^{-6} M; (●) 10^{-5} M; (○) 5×10^{-5} M. *Significant changes from control values: $P<0.05$.

(5×10^{-5} M) was a slight reduction in values for potential observed.

In the experimental protocol used, the test solution was perfused for only 20 min. In a few experiments, bepridil was perfused for approx. 1 h and even under such conditions, clear effects were seen only at a dose of 5×10^{-6} M. Since the drug was continuously perfused the effects increased with time.

Generally speaking, the effects of bepridil were not easily reversed. For example, if the compound was left in contact with the cardiac sino-atrial tissue for 20 min, then no amount of subsequent rinsing with the physiological solution alone, brought about a return to control levels of sinus activity.

In vivo *studies in anaesthetized dogs with cardiac denervation and bilateral medullo-adrenalectomy*

No significant cardiovascular effect was observed after injection of solvent. Conversely, injection of bepridil at a dose of 5 mg/kg (i.v.) caused an abrupt fall in heart rate from a mean control value of $98{\cdot}7\pm4.2$ beats/min in 6 animals to 76 ± 5.3 beats/min within 10 min after treatment (Figure 5). The difference between the two means is statistically significant ($P<0.01$), giving a recorded reduction of $23.2\pm3.8\%$. This effect remained significant for more than 45 min. Furthermore, the same injection of bepridil only induced a brief (3 min) reduction in systolic and diastolic pressure of $39.23\pm4.1\%$ and $53.0\pm3.7\%$ respectively.

Discussion

Both phase 0 and the amplitude of the sinus action potential are due to activation of the slow inward current I_{si} (Reuter 1979). This inward current I_{si} is carried both by Ca and Na ions (Lenfant, Mironneau, Gargouil & Galand, 1968; Noma & Irisawa, 1976; Brown, Giles & Noble, 1977). Furthermore it seems that in the rabbit sinus node, the slow inward current consists mainly of Na^+ ions (Lenfant & Mironneau, 1970; Noma, Yangihara & Irisawa, 1978). The dose-dependent decrease in both maximum systolic depolarization velocity (phase 0) and AP amplitude in perfused rabbit sinus node following addition of bepridil, confirms the results of Vogel, Crampton & Sperelakis (1979) who found an increase in time constants of slow inward currents in guinea-pig heart in the presence of bepridil. Similarly, Labrid *et al.* (1979) using the technique of voltage clamp applied to frog sino-atrial trabecula, have shown that bepridil markedly reduces the Ca^{2+} and Na^+ slow inward current while simultaneously slowing both the activation and reactivation velocity for this slow current, thus explaining the modified phase 0 of AP recorded in the present study. It should be stressed that this mechanism is attributed to all the 'calcium antagonists' having known cardiac activity (Wit & Cranefield, 1974; Bayer, Kalusche, Kaufmann & Mannhold, 1975; Fleckenstein, 1977). The decrease in both phase 0 and AP amplitude in the present study confirms that bepridil markedly reduces Ca^{2+}/Na^+ slow inward current.

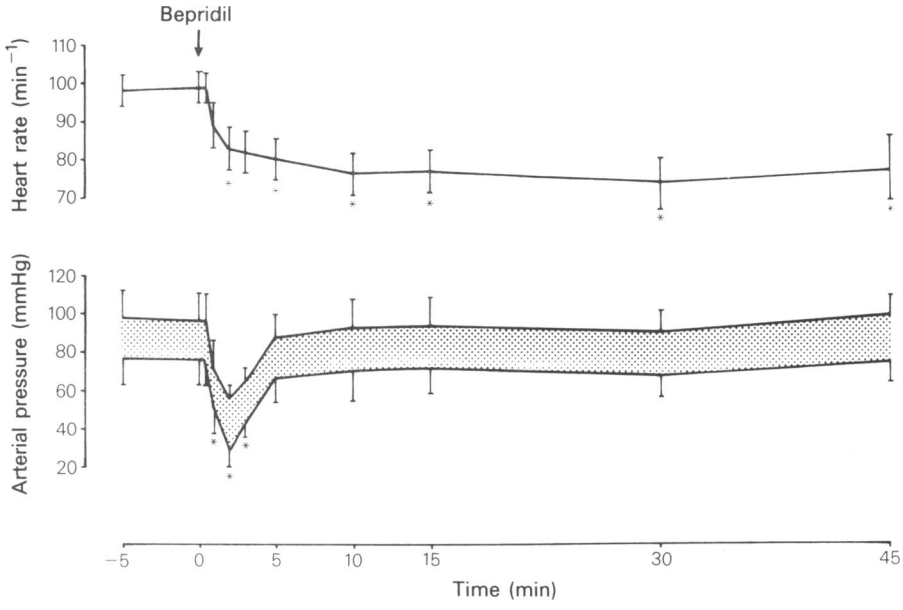

Figure 5 Effects of bepridil 5 mg/kg (i.v.) on heart rate, systolic and diastolic pressure in anaesthetized dogs with denervated hearts (bivagotomy, ablation of both stellate ganglia) and subtotal adrenalectomy (complete ablation of one adrenal gland and destruction of the second adrenal medulla). Values are mean of 6 observations; vertical lines show s.e. mean. *Significant changes from control values: $P < 0.05$.

On the other hand, the marked decrease in maximum diastolic depolarization velocity together with the increase in AP duration observed with doses of 5×10^{-6} M or above, were the most characteristic electrophysiological effects of bepridil on this isolated preparation. It has been shown that the balance between the background sodium inward current, as discovered by Trautwein & Kasselbaum (1961) but more particularly by Seyama (1976) in rabbit sinus node, and the outward potassium current I_p (Brown, Clark & Noble, 1976), is responsible for the values of maximum diastolic potential. Deactivation of the potassium current (I_p) causes the inward going background current to predominate and therefore diastolic depolarization occurs (Irisawa, 1978). As a reduction in maximum depolarization velocity (phase 4) was recorded with bepridil, it is highly probable that this compound interferes with deactivation of the potassium current (I_p). Consequently, bepridil should logically increase the time constant for deactivation of this current.

The observed prolongation in AP duration following treatment of rabbit sinus node with bepridil at 5×10^{-6} M, is in marked contrast to the results with frog sino-atrial fibres, where no change in AP duration was observed up to doses of 5×10^{-5} M (Labrid *et al.*, 1979). However, it is now recognized that the effect of drugs on outward currents is highly variable, depending on the preparations used. In this respect, Kane & Winslow (1980) have demonstrated that AP duration in guinea-pig ventricular fibres is indeed prolonged under the influence of bepridil, while the opposite effect is observed in lamb Purkinje tissue where AP duration is shortened. Furthermore, it has also been shown that the cellular electrophysiological activity of bepridil is directly dependent on the electrical frequency used in stimulating the preparation. Consequently the increase in AP duration observed in rabbit heart sino-atrial node under normal pacemaker conditions, cannot be interpreted as conflicting with previous evidence from other organs. This prolongation can only result from a lowered amplitude of the outward potassium current specific to sinus tissue, and perhaps also from a slight increase in the time constant for inactivation of the slow inward current. In addition, this explains the slight depolarization of the maximum diastolic potential, as observed with the highest dose of bepridil (5×10^{-5} M).

This direct activity of bepridil at 5×10^{-6} M on isolated sinus node (decrease in maximum systolic depolarization velocity, prolongation of AP duration and decrease in maximum diastolic depolarization velocity), results in a slowing down of heart rate. This is exactly what is observed *in vitro* as well as *in vivo* with anaesthetized dogs subjected to cardiac denervation and bilateral medullo-adrenalectomy. These animals exhibit persistent (more than 45 min) but

moderate bradycardia (23.2%), exactly reflecting the results of Cosnier *et al.* (1977) with anaesthetized dogs having intact cardiac innervation, and of Michelin, Cheucle-Beaughard & Duchene-Marullaz (1980) with anaesthetized dogs that had undergone cardiac denervation. The results of these experiments clearly show that the bradycardial or anti-tachycardial activity of bepridil does not proceed from a change in cardiac sympathetic or parasympathetic tone, or from a modification in levels of catecholamines released by the adrenals.

The two mechanisms which govern its bradycardial activity i.e. prolongation of sino-atrial action potential duration, and reduction in diastolic depolarization slope, suggest that bepridil rather resembles amiodarone in this respect (Goupil & Lenfant, 1976). In fact, bepridil is differentiated by its action on the systolic depolarization slope which is unaffected by amiodarone. Similarly, both bepridil and verapamil share common effects on sinus pacemaker activity i.e. both drugs reduce action potential amplitude by prolonging the duration, and flattening the slope of diastolic depolarization. However, only verapamil is additionally capable at doses of 1×10^{-7} M and 1×10^{-6} M of rendering the maximum diastolic potential more negative (Yamaguchi, Obayashi & Mandel, 1978).

In conclusion, these experiments carried out *in vitro* on rabbit sino-atrial node, and *in vivo* with bilaterally adrenalectomized and cardiac denervated anaesthetized dogs, demonstrate that bepridil slows down heart rate by a direct action on the cardiac sinus pacemaker.

The authors are grateful to Professor P. Duchene-Marullaz for his interest and suggestions in the initial stages of this work. Reprint requests to J.C.L. please.

References

BAYER R., KALUSCHE D., KAUFMANN R. & MANNHOLD R. (1975). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. III. Effects of the optical isomers on transmembrane action potentials. *Naunyn-Schmiedebergs Arch. Pharmac.*, **290,** 81–97.

BOUCHER M. & DUCHENE-MARULLAZ P. (1978). Comparative effects of amiodarone, perhexiline and bepridil on the cardiac rhythm in the conscious dog under chronic heart block. *Archs int. Pharmacodyn. Thér.*, **223,** 65–75.

BROWN H.F., CLARK A. & NOBLE S.J. (1976). Identification of the pacemaker current in frog atrium. *J. Physiol.*, **258,** 521–545.

BROWN H.F., GILES W. & NOBLE S.J. (1977). Membrane currents underlying activity in frog sinus venosus. *J. Physiol.*, **271,** 783–816.

CANICAVE J.C., DEU J., JACQ J. & PAILLET R. (1980). Un nouvel antiangoreux, le bépridil. Appréciation de son efficacité par l'épreuve d'effort au cours d'un essai à double issu contre placebo. *Thérapie*, **35,** 607–612.

CONSTANTIN M., BEAUGHARD M., PIRIS P. & LABRID C. (1981). Bepridil antagonism of β_1-stimulation in the anaesthetized dog: dissociation between cardiovascular and lipolytic effects. *J. Pharmac. (Paris)*, **12,** 239–254.

COSNIER D., DUCHENE-MARULLAZ P., RISPAT G. & STREICHENBERGER G. (1977). Cardiovascular pharmacology of bepridil (1, 3-isobutoxy-2-(benzylphenyl) amino propyl pyrrolidine hydrochloride) a new potential anti-anginal compound. *Archs int. Pharmacodyn. Thér.*, **225,** 133–151.

FLECKENSTEIN A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. *A. Rev. Pharmac. Tox.*, **17,** 149–166.

GOUPIL N. & LENFANT J. (1976). The effects of amiodarone on the sinus node activity of the rabbit heart. *Eur. J. Pharmac.*, **39,** 23–31.

IRISAWA H. (1978). Comparative physiology of the cardiac pacemaker mechanism. *Physiol. Rev.*, **58,** 461–498.

JULIEN G., LUCCIONI R. & GERARD R. (1979). Evaluation ergométrique des effets d'un nouvel anti-angineux: le bépridil. *Vie Médicale*, **60** (15), 1267–1270.

KANE D.A. & WINSLOW E. (1980). Antidysrhythmic and electrophysiological effects of a new anti-anginal 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 anti-anginal agent. *J. Pharmac. exp. Ther.*, **211,** 546–554.

LANNEY DE COURTEN J.F., MARTIN, J. & RICHALET A. (1979). Etude clinique d'un nouvel anti-angineux le bépridil (à propos de 92 cas). *Vie Médicale*, **20,** 1675–1676.

LENFANT J. & MIRONNEAU J. (1970). Transport du sodium par le système de cinétique lente de la membrane myocardique sinusale de Lapin. *J. Physiol., Paris*, **62,** 293.

LENFANT J., MIRONNEAU J., GARGOUIL Y.M. & GALAND F. (1968). Analyse de l'activité électrique spontanée du centre de l'automatisme cardiaque de lapin par les inhibiteurs de perméabilités membranaires. *C.R. Acad. Sc. Paris*, **266,** 901–904.

MICHELIN M.T., CHEUCLE-BEAUGHARD M. & DUCHENE-MARULLAZ P. (1980). Comparative effects of amiodarone, bepridil and perhexiline on coronary venous flow and several cardiovascular parameters. *Archs int. Pharmacodyn. Thér.*, **245,** 236–248.

NOMA A. & IRISAWA H. (1976). Effects of calcium ion on the rising phase of the action potential in rabbit sinoatrial node cells. *Jap. J. Physiol.*, **26,** 93–99.

NOMA A., YANAGIHARA K. & IRISAWA H. (1978). Ionic currents in rabbit sinoatrial node cells. In *The Sinus Node. Structure, Functions and Clinical Relevance.* ed. Bonke F.I.M. pp.301–310. The Hague: Martinus Nijhoff Publish. Co.

PAES DE CARVALHO A., DE MELLO W.C. & HOFFMAN B.F. (1959). Electrophysiological evidence for specialized fiber types in rabbit atrium. *Am. J. Physiol.*, **196,** 483–488.

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.

REUTER H. (1979). Properties of two inward membrane currents in the heart. *A. Rev. Physiol.*, **41,** 413–424.

SEYAMA I. (1976). Characteristics of the rectifying properties of the sino-atrial node cell of the rabbit. *J. Physiol.*, **255,** 379–397.

TRAUTWEIN W. & KASSELBAUM D.G. (1961). On the mechanism of spontaneous impulse generation in the pacemaker of the heart. *J. gen. Physiol.*, **45,** 317–330.

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

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

YAMAGUCHI I., OGAYASHI K. & MANDEL W.J. (1978). Electrophysiological effects of verapamil. *Cardiovasc. Res.*, **12,** 597–608.

(Received April 4, 1981
Revised July 27, 1981.)