Br. J. Pharmac. (1986), **87**, 705–711

Inhibition of the myocardial Ca^{2+} inward current by the class 1 antiarrhythmic agent, cibenzoline

Mark Holck & Wolfgang Osterrieder[1]

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland

1 Cibenzoline, a class 1 (local anaesthetic-type) antiarrhythmic drug, was investigated for possible effects upon the myocardial Ca^{2+} inward current.

2 In voltage-clamp experiments with isolated cardiac myocytes of guinea-pig, cibenzoline caused a concentration-dependent inhibition of the Ca^{2+} current, with an IC_{50} of 14 μM.

3 Inhibition of the Ca^{2+} current by cibenzoline (2 μM) was dependent upon stimulation frequency, with a greater block occurring at 2 Hz ($\sim$ 50%) than at 0.2 Hz ($\sim$ 15%).

4 The magnitude of Ca^{2+} current block was also potential-dependent. A markedly greater inhibition by cibenzoline (20 μM) was recorded when myocytes were depolarized (to + 20 mV) from a holding potential of − 35 mV than of − 80 mV. At the less negative potential, cibenzoline also caused a reduction in the level of the holding current, which suggests a decrease in the inwardly rectifying K^+ current.

5 Cibenzoline also caused a concentration-dependent inhibition of KCl-induced contractures of isolated aortic strips of the rat (IC_{50} = 55 μM) and a reduction in contractile force of isolated, electrically-stimulated papillary muscles of the guinea-pig (IC_{50} = 35 μM).

6 Thus, cibenzoline possesses Ca^{2+} channel blocking (class 4) properties in addition to its local anaesthetic actions.

Introduction

Cibenzoline is a new antiarrhythmic drug that is under clinical development in several countries. It is chemically unrelated to any currently available antiarrhythmic drugs. Electrophysiological studies in the rabbit heart have shown that its antiarrhythmic action is due mainly to an inhibition of the fast inward Na^+ current, as evidenced by a concentration-dependent decrease in the maximum rate of depolarization of transmembrane action potentials as well as a reduction in conduction velocity (Millar & Vaughan Williams, 1982; 1983; Dangman, 1984). Thus, cibenzoline can be labelled as a class 1 (local anaesthetic-type) antiarrhythmic, according to the classification scheme of Vaughan Williams (1980). In addition, cibenzoline prolonged action potential duration, indicating that it also possesses a class 3 component (Millar & Vaughan Williams, 1982; 1983). It also increased A-H conduction time in rabbit heart and suppressed the contractile rate in isolated, spontaneously-beating rabbit right atria (Millar & Vaughan Williams, 1982). The last findings suggest that cibenzoline might also cause a decrease in the slow inward Ca^{2+} current (I_{Ca}). It was, therefore, of interest to investigate the effects of cibenzoline on the myocardial Ca^{2+} current in voltage-clamp experiments. Additional information on a possible interaction of cibenzoline with Ca^{2+} channels was obtained from contractility measurements in isolated aortic strips of the rat and papillary muscles of the guinea-pig. The data described in this study indicate that cibenzoline possesses significant class 4 properties.

[1] Author for correspondence.

Methods

Voltage-clamp experiments with guinea-pig isolated ventricular myocytes

Adult guinea-pigs were anaesthetized with ether and the hearts were quickly excised. The hearts were retrogradely perfused in a Langendorff apparatus with solutions (warmed to 36°C and gassed with 95% O_2/5% CO_2) in the following sequence: (a) 3 min with Krebs-Henseleit solution (composition in mM:

NaCl 120, KCl 5.9, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15, glucose 11 and $CaCl_2$ 1.8); (b) 3 min with (nominally) Ca^{2+}-free Krebs-Henseleit solution: (c) 30 min recirculation with collagenase in Ca^{2+}-free Krebs-Henseleit solution (30 mg collagenase per 100 ml): and (d) washout of collagenase from the heart by perfusion with 50 ml of a 'storage solution'. The 'storage solution' contained (in mM): glutamic acid 70, taurine 10, KCl 25, KH_2PO_4 10, glucose 11, EGTA 0.5 and HEPES 10. The pH was adjusted to a value of 7.3 (with KOH). In order to dissociate the tissue, small pieces of the ventricles were shaken in a beaker containing 'storage solution'. The cells were filtered through a cell sieve and incubated for at least 1 h at room temperature before use. Incubation in 'storage solution' increased the percentage of Ca^{2+}-tolerant (viable) cells upon reperfusion with Ca^{2+}-containing Krebs-Henseleit solution (Isenberg & Klöckner, 1982).

For the voltage-clamp recordings, myocytes were allowed to settle to the glass bottom of a perspex recording chamber which was rapidly perfused with Krebs-Henseleit solution (37°C). Measurement of the whole-cell Ca^{2+} current (I_{Ca}) was made using the one-electrode voltage clamp technique. Patch clamp pipettes (resistance 2–3 MΩ) were pulled from borosilicate glass. The electrodes were filled with (in mM): KCl 30, K-aspartate 100, Na_2ATP 3, $MgCl_2$ 1, KH_2PO_4 10, glucose 5.5, EGTA 0.5 and HEPES 5 (pH 7.3). A 'GOhm seal' (Hamill *et al.*, 1981) was formed on the membrane surface and, then, the membrane under the electrode tip was disrupted by strong suction. The electrode was connected to the head-stage of a potential follower with current injection, the output of which was connected to a voltage clamp amplifier (Ehrler, Homburg/Saar, FRG) for recording of membrane currents. Stimulation of the myocytes through the electrode elicited action potentials which closely resemble those recorded in multicellular preparations. The original data were stored on an 8-channel PCM-tape (Heim, Bergisch-Gladbach, FRG) and evaluated by transferring to a DATALAB 4000 B (Data Laboratories, Mitcham, UK) microprocessor system.

Contractility measurements in guinea-pig papillary muscles

Papillary muscles were obtained from the right ventricle of guinea-pig (200–300 g) hearts. The muscles were mounted vertically in a perspex chamber and were bathed in Krebs-Henseleit solution (37°C) gassed with 95% O_2/5% CO_2. The muscles were stimulated electrically (Grass, S88) at a frequency of 1 Hz by square-wave pulses of 6 ms duration and intensity 20% above threshold. Contractile force was recorded isometrically with a Swema force transducer and displayed on a chart recorder (Hellige, Freiburg i. Br., FRG). The resting tension was adjusted to 150 mg at the beginning of the experiment. The control developed tension ranged from 50 to 200 mg. An equilibration period of at least 90 min preceded cumulative drug applications. Only one cumulative concentration-response relationship was determined on each papillary muscle.

Contractility measurements in rat aortic strips

The thoracic aorta of rats (200 g) was rapidly removed, cleaned of adhering connective tissue, opened along its length and four rings, 1–2 mm wide, were cut parallel to the circular muscle coat. The vascular preparations were mounted in an organ bath containing Krebs-Henseleit solution (composition mM): NaCl 115, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10 and $CaCl_2$ 2.5. The solution (pH 7.3) was gassed with 95% O_2/5% CO_2 and the temperature was 37°C. The strips were connected to a Statham force transducer for isometric recording of contractions on a polygraph (Hellige, Freiburg i. Br., FRG). The vessels were placed under 3.5 g passive tension for a 60 min equilibration period. Thereafter, the strips were contracted by adding 50 mM KCl to the bath. Effects of cibenzoline upon KCl depolarization-induced contractures were determined by incubating the strips with appropriate concentrations of the drug for 15 min before addition of 50 mM KCl to the organ bath.

Drug solutions

Cibenzoline was prepared as 10 mM stock solution in distilled water and diluted in Krebs-Henseleit solution to the final concentrations. In the experiments described in Figure 3, 20μ M tetrodotoxin (Sigma, St. Louis, Missouri) was added to the Krebs-Henseleit solution.

Results

Voltage-clamp experiments

The following experimental protocol was employed: the cell membrane potential was clamped at − 50 mV and, at 3 s intervals, transiently depolarized to + 10 mV. At a − 50 mV holding potential, the fast inward Na^+ current can be considered to be inactivated (Pappano, 1970) and the inward current elicited on depolarization is assumed to be the Ca^{2+} current. Figure 1a shows superimposed current traces in response to a 200 ms test pulse recorded from a cell before and during perfusion with various concentrations of cibenzoline. The Ca^{2+} current activated rapidly after depolarization and inactivated with a slower time course. The amplitude of I_{Ca} was deter-

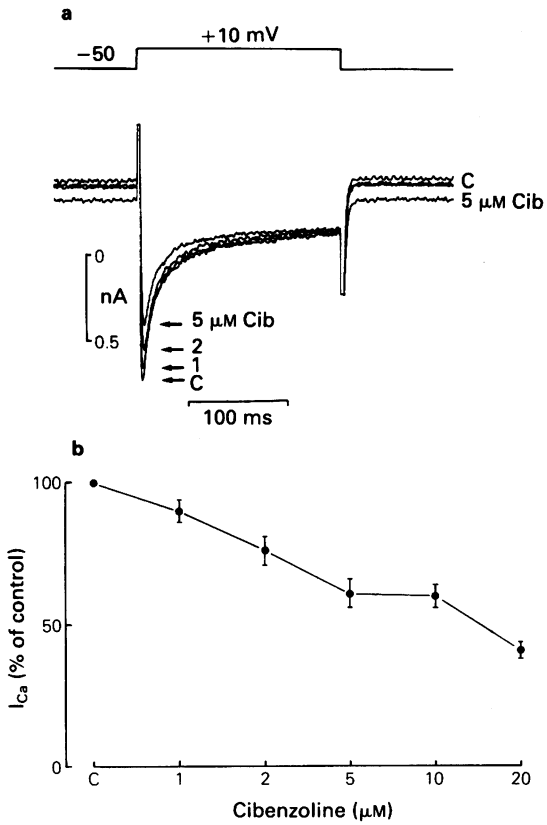

Figure 1 (a) Original current traces. The Ca^{2+} current (I_{Ca}) was elicited in a guinea-pig ventricular myocyte by voltage-clamp steps (duration 200 ms) from a − 50 mV holding potential to + 10 mV (indicated schematically at the top). The negative (downward) deflections of the traces correspond to I_{Ca}. Peak inward Ca^{2+} currents before (C) and during superfusion of the cell with 1, 2 and 5 μM cibenzoline (Cib) are marked by arrows. All currents were recorded in the steady state. The capacitative artifacts have been partially removed. Note the gradual decrease in amplitude of I_{Ca} caused by cibenzoline and the change in holding current observed with 5 μM of the drug. (b) Concentration-dependence of the decrease in Ca^{2+} current by cibenzoline. The amplitude of I_{Ca} was measured as the difference between peak inward current and the current amplitude 100 ms later, and is expressed as a percentage of control values. Each value is the mean of 4 observations; error bars indicate s.e.mean. The experiments with 10 and 20 μM cibenzoline were performed on different cells from those with 1, 2 and 5 μM.

mined as the difference between the peak inward current and the current amplitude 100 ms later. This procedure yields a good estimate of the magnitude of I_{Ca} (McDonald & Trautwein, 1978), since the inward Ca^{2+} current is virtually inactivated and the time-dependent outward K^+ current is not yet fully developed at this time. Cibenzoline (1, 2 and 5 μM) reduced the peak amplitude of I_{Ca} in a concentration-dependent manner (Figure 1a). The concentration of cibenzoline required for one-half maximal inhibition (IC_{50}) of I_{Ca} was estimated to be 14 μM (Figure 1b). It was further observed that at the highest concentration tested in this experiment (5 μM), cibenzoline caused a reduction in the current amplitude measured at the − 50 mV holding potential (Figure 1a). This effect, which indicates an inhibition of the inwardly rectifying K^+ outward current, was consistently seen at drug concentrations $\geq$ 5 μM.

The inhibitory effects of Ca^{2+} entry blockers, such as verapamil and diltiazem, upon I_{Ca} are known to depend upon the frequency of stimulation (McDonald *et al.* 1980; Lee & Tsien, 1983). This prompted us to investigate the dependence upon stimulation frequency of the cibenzoline-induced inhibition of I_{Ca}. Figure 2a shows current recordings from a voltage-clamp experiment in which the cell membrane was depolarized from − 50 mV to + 10 mV (duration of depolarization: 200 ms) at a rate of 0.2 and 2 Hz. At the lower stimulation rate, cibenzoline exerted only a weak inhibitory effect on I_{Ca}. At 2 Hz, however, the magnitude of I_{Ca} was reduced by about 50%. Figure 2b summarizes the results of 4 experiments in which the stimulation rate was varied between 0.2 and 3 Hz. Under control conditions, there was a small decrease in I_{Ca} current when the rate was increased from 0.2 to 2 Hz. A 44% reduction in amplitude was seen at 3 Hz, which is likely to be due to an incomplete recovery of the Ca^{2+} current from inactivation. At the lowest stimulation rate (0.2 Hz), cibenzoline reduced I_{Ca} by only 15%. The frequency-current relationship was steeper in the presence of cibenzoline and inhibition was approximately 50% at 3 Hz. Therefore, the block of Ca^{2+} current by cibenzoline was also dependent upon stimulation frequency.

Voltage clamp studies have revealed that the magnitude of I_{Ca} inhibition by Ca^{2+} entry blockers is also dependent upon the potential across the cell membrane (Kohlhardt & Mnich, 1978; McDonald *et al.*, 1980; Osterrieder *et al.*, 1981; Tung & Morad, 1983; Sanguinetti & Kass, 1984). In general, inhibition of I_{Ca} was found to be more pronounced at depolarized than at hyperpolarized membrane potentials. Experiments were undertaken to determine whether cibenzoline possesses similar properties. The cell membrane was first voltage-clamped from − 80 mV to + 20 mV (duration of the test pulse: 200 ms, stimulation frequency: 0.33 Hz) and, subsequently, to the same test potential, from a holding potential of − 35 mV. Tetrodotoxin (20 μM) was present throughout the experiment in order to eliminate the fast inward Na^+ current. This protocol was repeated during superfusion with a high concentration of cibenzoline

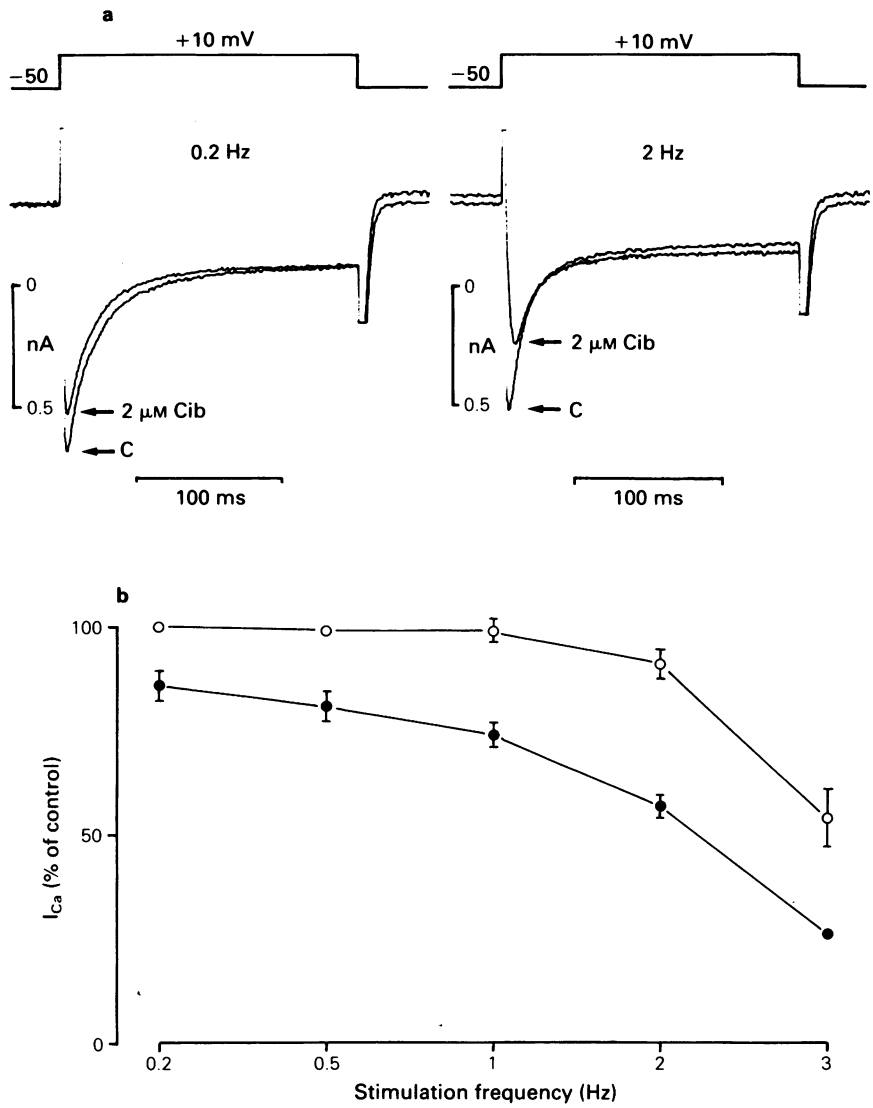

Figure 2 (a) Original recordings of the frequency-dependence of I_{Ca} block by cibenzoline. Cell membrane potential was voltage clamped at − 50 mV and transiently (200 ms) depolarized to + 10 mV at a frequency of 0.2 Hz (left) or 2 Hz (right). Note the stronger effect of 2 μM cibenzoline on the Ca^{2+} current at the higher stimulation frequency. (b) Relationship between the amplitude of I_{Ca} current and the stimulation frequency, measured under steady-state conditions before (○) and during administration of cibenzoline 2 μM (●).

(20 μM). At the − 80 mV holding potential (Figure 3a), I_{Ca} was reduced by 29%, while at the − 35 mV holding potential, I_{Ca} was reduced to a greater degree (60%, Figure 3b). It is probable that the remaining time-dependent component of the current during the depolarizing step represents a slowly activating K^+ outward current.

It is noteworthy that cibenzoline exerted opposite effects on the holding current at the two different holding potentials. At − 35 mV, cibenzoline caused this current to become less positive; this can be explained by a reduction of the inwardly rectifying K^+ outward current. At − 80 mV, however, the holding current became more positive during administration

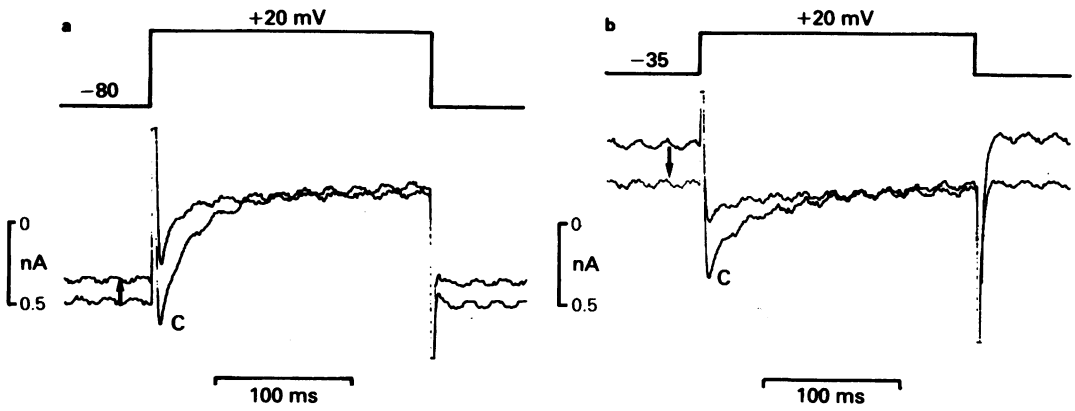

Figure 3 Influence of membrane potential on the inhibition of I_{Ca} by cibenzoline. (a) The Ca^{2+} inward current was elicited by 200 ms voltage steps from − 80 mV to + 20 mV (indicated schematically at the top) before (C) and during administration of cibenzoline 20 μM. The Krebs-Henseleit solution contained tetrodotoxin 20 μM in order to block the fast Na^+ inward current. The cell membrane was depolarized at a frequency of 0.33 Hz, and the current traces shown were recorded in the steady state. The capacitive artefacts have been partially removed. (b) Current recordings from the same cell as in (a), but depolarized to + 20 mV from a holding potential of − 35 mV. This experiment (two different holding potentials) was repeated in 3 other cells. The arrows indicate the direction of the change in holding currents caused by cibenzoline.

of cibenzoline. Such a result can be expected, when considering that − 80 mV was more negative than the membrane resting potential (− 70 mV in this experiment; Figure 3a) and, hence, the reversal potential for K^+ ions. At levels more negative to the reversal potential, the inwardly rectifying K^+ current flows into the cell rather than in an outward direction. Thus its inhibition results in a less negative holding current.

Contractility measurements in guinea-pig papillary muscles and rat aortic strips

Cibenzoline has previously been shown to produce negative inotropic effects in rabbit atria (Millar & Vaughan Williams, 1982). These findings, as well as the voltage-clamp experiments in the present study, prompted us to investigate the effects of this drug on excitation-contraction coupling in isolated ventricular myocardium and vascular smooth muscle. Cibenzoline caused a concentration-dependent inhibition of the development of contractile force in guinea-pig isolated, electrically-stimulated papillary muscles. The concentration of cibenzoline required to cause a half-maximal inhibition of contractility (IC_{50}) was 35 μM (Figure 4a). In isolated strips of rat thoracic aorta, cibenzoline inhibited the development of contractile force in response to KCl (50 mM)-induced depolarization, with an IC_{50} of 55 μM (Figure 4b). These inhibitory effects occurred over a similar concentration range as was required to inhibit the myocardial inward Ca^{2+} current (Figures 1–3), suggesting that the Ca^{2+} channel blocking properties of cibenzoline were not selective for the heart.

Discussion

The purpose of this study was to demonstrate a direct inhibitory effect of cibenzoline on the transmembrane Ca^{2+} inward current, as previously suggested from action potential measurements recorded in isolated sino-atrial node preparations of the rabbit (Millar & Vaughan Williams, 1982; 1983). The data presented here clearly showed a reduction of I_{Ca} by cibenzoline at therapeutically-relevant concentrations (1–5 μM; Canal *et al.*, 1983; Van den Brand *et al.*, 1984). This result underlies the negative inotropic effects of cibenzoline in guinea-pig papillary muscles (this study) and in rabbit isolated atria (Millar & Vaughan Williams, 1982), and is in agreement with a recent report showing an inhibition of a slow inward current in frog atria (Masse *et al.*, 1984).

A particularly interesting observation of the present study was the strong influence exerted by the stimulation frequency and membrane holding potential upon the inhibitory effect of cibenzoline on I_{Ca}. The Ca^{2+} current blocking actions of single concentrations of cibenzoline could be significantly enhanced by increasing the frequency of stimulation as well as by holding the membrane potential at depolarized levels. A similar mode of action has been demonstrated for Ca^{2+} channel blockers such as diltiazem (Lee & Tsien,

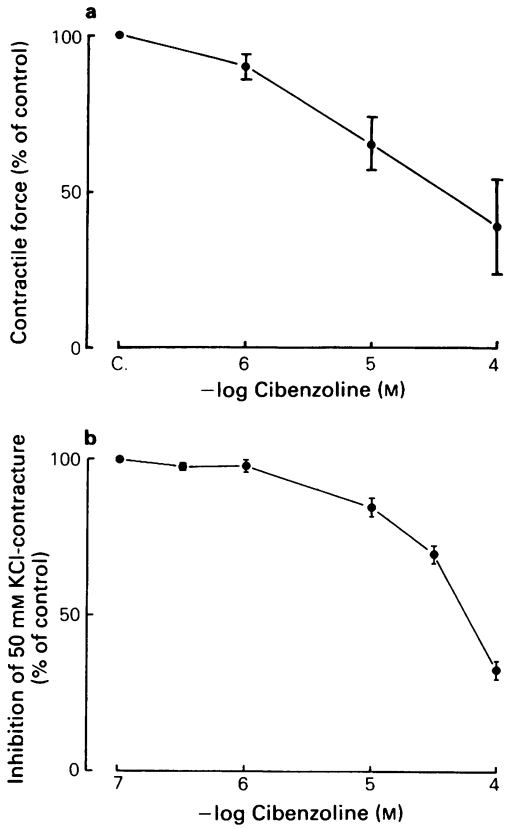

Figure 4 Concentration-dependent inhibition by cibenzoline of (a) contractile force development in isolated, electrically stimulated papillary muscles of guinea-pig and (b) in KCl (50 mM)-induced contractures of rat isolated aortic strips. The data are expressed as percentage of predrug values (= 100%). Each value is the mean of 4–6 determinations; vertical bars indicate s.e.mean.

1983; Tung & Morad, 1983), nitrendipine (Lee & Tsien, 1983; Bean, 1984; Sanguinetti & Kass, 1984), AQA 39, D600 and verapamil (Kohlhardt & Mnich, 1978; McDonald *et al.*, 1980; Osterrieder *et al.*, 1981; Trautwein *et al.*, 1981; Pelzer *et al.*, 1982; McDonald *et al.* 1984), where I_{Ca} blockade was most pronouced at high stimulation frequencies and/or depolarized membrane potentials. The frequency- and potential-dependence of I_{Ca} block by cibenzoline was similar to that observed with 'classical' Ca^{2+} channel blockers, in terms of both the magnitude of inhibition and the concentrations used. It is, therefore, reasonable to suggest that this property may be an important mechanism contributing to the antiarrhythmic actions of cibenzoline. Such a property is desirable for the treatment of tachyarrhythmic episodes, in which normal heart rate will be less affected than at increased rate or extrasystoles. Indeed, the effectiveness of cibenzoline in reducing or preventing tachyarrhythmic episodes has been documented (Herpin *et al.*, 1981).

The inhibition of the Ca^{2+} current by cibenzoline was not specific for cardiac muscle, as evidenced by the inhibitory effects on depolarization-induced contractures of rat aorta. This would indicate that cibenzoline is also capable of inhibiting potential-operated Ca^{2+} channels in vascular smooth muscle. In contrast to typical Ca^{2+} entry blockers such as nifedipine, verapamil or diltiazem, which are generally more potent inhibitors of excitation-contraction coupling in the vasculature than the heart (Fleckenstein, 1977), cibenzoline appeared to be slightly more potent in blocking myocardial Ca^{2+} channels.

An additional effect of cibenzoline, observed at concentrations $\geq 5\,\mu M$, was a reduction of the inwardly rectifying K^+ outward current, as evidenced by a reduction of the holding current at -35 mV, -50 mV and -80 mV. This membrane current is responsible for the fast terminal phase of repolarization of the myocardial action potential, and its inhibition by cibenzoline easily explains the prolongation of action potentials as previously reported (Millar & Vaughan Williams, 1982; 1983).

In summary, cibenzoline possesses a class 4 mode of action in addition to its local anaesthetic properties. This yields a pharmacological profile differing from that of other antiarrhythmic drugs such as disopyramide, quinidine and procainamide. The therapeutic value of this Ca^{2+} current inhibition remains to be determined. It must, however, be considered that depression of the Ca^{2+} current may result in undesirable negative inotropic effects. Indeed, in a recent study, such side effects were observed in patients under treatment with cibenzoline, and it was stated that the drug should be used cautiously in patients with severe ventricular dysfunction (Strom *et al.*, 1985).

We wish to acknowledge the technical assistance provided by Mrs B. Holck, Miss W. Kröger and Mr U. Wicki. We would also like to thank Prof. W. Haefely for critical reading of the manuscript.

References

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

CANAL, M., FLOUVAT, B., TREMBLAY, D. & DUFOUR, A. (1983). Pharmacokinetics in man of a new antiarrhythmic drug, cibenzoline. *Eur. J. clin. Pharmac.*, **24**, 509–515.

DANGMAN, K.H. (1984). Cardiac effects of cibenzoline. *J. cardiovasc. Pharmac.*, **6**, 300–311.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, pacemakers and vascular smooth muscle. *A. Rev. Pharmac. Tox.*, **17**, 147–166.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85-100.

HERPIN, D., GAUDEAU, B., BOUTAUD, P., AMIEL, A., TOURDIAS, B. & DEMANGE, J. (1981). Clinical trials of a new antiarrhythmic drug; Cibenzoline (Cipralan(R)). *Curr. Ther. Res.*, **30**, 742–752.

ISENBERG, G. & KLÖCKNER, U. (1982). Calcium tolerant ventricular myocytes prepared by incubation in a "KB" medium. *Pflügers Arch.*, **395**, 6–18.

KOHLHARDT, M. & MNICH, Z. (1978). Studies on the inhibitory effect of verapamil on the slow inward current in mammalian ventricular myocardium. *J. molec. cell. Cardiol.*, **10**, 1037–1052.

LEE, K.S. & TSIEN, R.W. (1983). Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialyzed heart cells. *Nature*, **302**, 790–794.

MASSE, C., CAZES, M. & SASSINE, A. (1984). Effects of cibenzoline, a novel antiarrhythmic drug, on action potential and transmembrane currents in frog atrial muscle. *Archs int. Pharmacodyn.*, **269**, 219–235.

McDONALD, T.F. & TRAUTWEIN, W. (1978). Membrane currents in cat myocardium: separation of inward and outward components. *J. Physiol.*, **274**, 193–216.

McDONALD, T.F., PELZER, D. & TRAUTWEIN, W. (1980). On the mechanism of slow calcium channel block in heart. *Pflügers Arch.*, **385**, 175–179.

McDONALD, T.F., PELZER, D. & TRAUTWEIN, W. (1984). Cat ventricular muscle treated with D600: characteristics of calcium block and unblock. *J. Physiol.*, **352**, 217–241.

MILLAR, J.S. & VAUGHAN WILLIAMS, E.M. (1982). Effects on rabbit nodal, atrial, ventricular and Purkinje cell potentials of a new antiarrhythmic drug, cibenzoline, which protects against action potential shortening in hypoxia. *Br. J. Pharmac.*, **75**, 469–478.

MILLAR, J.S. & VAUGHAN WILLIAMS, E.M. (1983). Pharmacological mapping of regional effects in the rabbit heart of some new antiarrhythmic drugs. *Br. J. Pharmac.*, **79**, 701–709.

OSTERRIEDER, W., PELZER, D., YANG, Q.-f. & TRAUTWEIN, W. (1981). The electrophysiological basis of the bradycardic action of AQA 39 on the sinoatrial node. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 233–237.

PAPPANO, A.J. (1970). Calcium-dependent action potentials produced by catecholamines in guinea-pig atrial muscle fibers depolarized by potassium. *Circulation Res.*, **27**, 379–390.

PELZER, D., TRAUTWEIN, W. & McDONALD, T.F. (1982). Calcium channel block and recovery from block in mammalian ventricular muscle treated with organic channel inhibitors. *Pflügers Arch.*, **394**, 97–105.

SANGUINETTI, M.C. & KASS, R.S. (1984). Voltage-dependent block of calcium channel current in the calf Purkinje fiber by dihydropyridine calcium channel antagonists. *Circulation Res.*, **55**, 336–348.

STROM, J.A., MIURA, D., JORDAN, A. & LOOR, S. (1985). Effects of cibenzoline on left ventricular performance in patients with coronary artery disease. *J. Am. Coll. Cardiol.*, **5**, 451 (abstr.).

TUNG, L. & MORAD, M. (1983). Voltage- and frequency-dependent block of diltiazem on the slow inward current and generation of tension in frog ventricular muscle. *Pflügers Arch.*, **398**, 189–198.

TRAUTWEIN, W., PELZER, D., McDONALD, T.F. & OSTERRIEDER, W. (1981). AQA 39, a new bradycardiac agent which blocks myocardial calcium (Ca) channels in a frequency- and voltage-dependent manner. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 228–232.

VAN DEN BRAND, M., SERRUYS, P., DE ROON, Y., AYMARD, M.F. & DUFOUR, A. (1984). Haemodynamic effects of intravenous cibenzoline in patients with coronary heart disease. *Eur. J. clin. Pharmac.*, **26**, 297–302.

VAUGHAN WILLIAMS, E.M. (1980). *Antiarrhythmic Action.* London: Academic Press.

(Received October 14, 1985.
Accepted November 22, 1985.)