Effect of Cibenzoline, a Class I Antiarrhythmic Drug, on Action Potential in Canine Ventricular Muscle

Hiroyasu SATOH, Masaaki ISHII and Keitaro HASHIMOTO
Department of Pharmacology, Yamanashi Medical College, Tamaho, Nakakoma-gun, Yamanashi 409-38, Japan
Accepted February 16, 1987

Abstract—Effect of cibenzoline, a class I (local anesthetic-type) antiarrhythmic drug, was investigated upon canine ventricular muscle using a conventional microelectrode method. In the presence of cibenzoline at the concentration of $3 \times 10^{-6}$ M or higher, the maximum rate of rise of the action potential was inhibited and the action potential duration was lengthened significantly in a concentration-dependent manner. The effective refractory period was also prolonged. From its effect on the action potential duration, cibenzoline should belong to la, according to the Vaughan Williams classification of antiarrhythmic agents. On the other hand, cibenzoline inhibition of the maximum rate of depolarization was greater with an increase in stimulation frequency (a use-dependent block). In the presence of cibenzoline concentrations of $3 \times 10^{-6}$ M and $8 \times 10^{-6}$ M, which blocked the maximum rate of depolarization by 36% and 67% at 180 beats/min, the rates of onset of inhibition of the maximum rate of depolarization were $0.109 \pm 0.027$ and $0.146 \pm 0.070$ AP$^{-1}$ (mean±S.D.), respectively. From the kinetics of inhibition of the maximum rate of depolarization, these results suggest that cibenzoline should be classified as an intermediate drug with the prolongation of the action potential duration.

Cibenzoline is a new antiarrhythmic agent chemically not related to known antiarrhythmic agents. Electrophysiological studies have already shown that the antiarrhythmic actions of cibenzoline are mainly due 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 potential as well as a reduction in conduction velocity (1-3). Thus, cibenzoline can be classified as a class I antiarrhythmic drug, according to the classification of Vaughan Williams (4). Furthermore, cibenzoline not only lengthened action potential duration (APD), but also increased A-H conduction time and depressed spontaneously-beating frequency in rabbit atria (1, 2). Voltage clamp experiments have shown that cibenzoline possessed a Ca$^{2+}$ channel blocking property in frog heart and cardiac myocytes of guinea-pig (5, 6). It is, therefore, considered that cibenzoline is an antiarrhythmic agent with a powerful class I type activity and additional properties in class III and IV.

Now there are two methods for classifying antiarrhythmic agents, which are due to the effect on APD and due to the speed of inhibition of the rate of depolarization of cardiac muscle action potentials. The former is divided into three subclasses. Antiarrhythmic agents causing the prolongation of APD are in subclass la, those causing the shortening of APD are in lb, and agents causing no change in APD are in lc. On the other hand, there are marked differences among drugs in the speed at which the maximum rate of depolarization fell to a new plateau level during exposure to an antiarrhythmic drug. Similarly, the class I drugs are now divided into three distinct subclasses by their interaction with fast Na$^+$ channels, i.e., fast, intermediate and slow kinetic drugs (7, 8). Although cibenzoline could belong to la from the former classification scheme since cibenzoline lengthens...
APD, on the speed of inhibition of the rate of depolarization, it is not yet clear to which subclass cibenzoline belongs. In the present experiments, we investigated the use-dependent inhibition of the maximum rate of depolarization and the rate of onset of inhibition at various stimulation frequencies in order to obtain more information about cibenzoline as a class I antiarrhythmic agent.

Materials and Methods

Mongrel dogs, weighing 7-10 kg, were anesthetized with 30 mg/kg pentobarbital sodium. The heart was quickly excised. The papillary muscles obtained from the right ventricle were placed in an organ bath and superfused with Tyrode’s solution at a temperature of 36°C, as described previously (9, 10). One end of the preparation was fixed on the paraffin base of the bath, and the other end was connected to a force displacement transducer (Nihon Kohden ST-1T) using a fine nylon thread. Field stimulation at frequencies between 30 and 180 beats/min with pulses of 5 msec duration and twice the voltage threshold in strength was used. Except for the experiments to examine the use-dependency of the maximum rate of depolarization, the specimens were usually stimulated at 60 beats/min. The action potential was recorded by using a standard glass microelectrode technique (resistance of the electrodes were 5-10 MΩ) on an oscilloscope (Nihon Kohden VC-10) and photographed (Nihon Kohden RI-G-6201). The refractory period was measured at the 11th pulse with shorter intervals by interrupting the constant stimulation interval of 1 sec. The composition of the Tyrode’s solution was (mM): NaCl, 137; KCl, 4.0; MgCl₂, 1.0; CaCl₂, 1.9; NaH₂PO₄, 0.4; NaHCO₃, 12.0; and glucose, 5.5; and it was bubbled with 95% O₂ and 5% CO₂. The pH was adjusted to 7.4.

The drug used was cibenzoline succinate (kindly supplied from Fujisawa Pharmaceutical Co., Ltd.), which was prepared as a 10⁻³ M stock solution in distilled water and diluted in Tyrode’s solution to the final concentrations. The Tyrode’s solution in the bath was completely exchanged with solutions having different concentrations of cibenzoline within 3 min. The drug was administrated cumulatively. The data were obtained 15 to 20 min after changing to a new solution.

The values were expressed as the mean±S.D. The differences of the mean values were analyzed by Student’s t-test for paired data, and P less than 0.05 was considered significant.

Results

At concentrations of 8×10⁻⁷ M or lower, cibenzoline did not cause any significant effect on the action potential in the canine ventricular muscle. Applications of cibenzoline at 3× and 8×10⁻⁶ M inhibited the maximum rate of depolarization by 13% and 44%, respectively; and they lengthened the action potential duration by 26% and 26% at 50%, by 25% and 32% at 75% and by 26% and 30% at 90% of repolarization, respectively (Fig. 1). Changes in the action potential parameters induced by cibenzoline are summarized in Table 1 (n=6). The resting membrane potential was unaffected by an increase in cibenzoline concentrations up to 8×10⁻⁶ M. The effect of refractory period was prolonged by 10% at 8×10⁻⁶ M (P<0.05), although significant increase in the action potential duration occurred at concentrations of 3×10⁻⁶ M or higher.

In order to examine the use-dependence of drug action, the experiments of the kind represented in Fig. 2 were performed (n=6). After the maximum rate of depolarization (the ordinate) was determined in the drug-free solution, cibenzoline was added in various concentrations, and the stimulation was stopped. Following a resting period of 90 sec, repetitive stimulation (0-20 sec, the abscissa) was resumed at the same rate as the control. As shown in Fig. 2, the maximum rate of depolarization at the first action potential was decreased (resting block) and declined during stimulation to a new steady-state (use-dependent or frequency-dependent block). In the presence of 8×10⁻⁷ M cibenzoline (Fig. 2, upper panel), the maximum rate of depolarization at the first action potential was decreased (resting block) and declined during stimulation to a new steady-state (use-dependent or frequency-dependent block). In the presence of 8×10⁻⁷ M cibenzoline (Fig. 2, upper panel), the maximum rate of depolarization at the first action potential was uniformly 257 V/sec, not different from that under drug free conditions (absence of resting block). The further decline of the maximum rate of
Fig. 1. Effect of cibenzoline on the action potential and the maximum rate of depolarization in the canine ventricular muscle stimulated at 60 beats/min. Cibenzoline suppressed the maximum rate of depolarization and lengthened the action potential duration. The brief horizontal lines represent the zero membrane potential.

Fig. 2. Cibenzoline-induced inhibition of the maximum rate of depolarization at different stimulation frequencies. The maximum rate of depolarization was depressed in a use-dependent fashion which was greater in the presence of $8 \times 10^{-6}$ M cibenzoline (lower panel) than in the presence of $8 \times 10^{-7}$ M cibenzoline (upper panel). The ordinate indicates the maximum rate of depolarization, and the abscissa indicates before and during administration and the time (0–20 sec) after onset of stimulations.

depolarization during repetitive activity depended on the stimulation frequency and became greater with a decrease in the inter-stimulus interval: at 30/min, it attained to 257 V/sec (ineffective); at 60/min, 236 V/sec; at 120/min, 229 V/sec; and at 180/min, 214 V/sec as a new steady-state. Although not shown in the figure, in the presence of $3 \times 10^{-6}$ M cibenzoline the maximum rate of depolarization attained to 236 V/sec at 30/
### Table 1. Effects of cibenzoline on the action potential in canine ventricular muscle (n=6)

| Concentration (M) | Resting potential (mV) | Action potential amplitude (mV) | $V_{max}$ (V/sec) | 25% | 50% | 75% | 90% | Effective refractory period (msec) |
|-------------------|------------------------|---------------------------------|-------------------|-----|-----|-----|-----|-------------------------------|
| Control           | −86±2                  | 118±8                           | 248±59            | 119±23 | 116±16 | 195±16 | 213±19 | 209±17 |
| $3 \times 10^{-7}$ | −87±2                  | 120±6                           | 250±59            | 116±16 | 164±12 | 200±19 | 220±21 | 208±18 |
| $8 \times 10^{-7}$ | −87±2                  | 120±7                           | 234±57            | 130±33 | 171±14 | 207±15 | 229±20 | 211±8  |
| $3 \times 10^{-6}$ | −86±3                  | 120±7                           | 221±59*           | 132±28 | 192±28 | 221±22* | 241±25* | 213±13 |
| $8 \times 10^{-6}$ | −87±3                  | 118±10                          | 199±53*           | 126±28 | 181±23* | 220±26* | 244±26** | 228±17* |

Values are the mean±S.D. *P<0.05, **P<0.01, with respect to control values.

### Table 2. The rate of onset of inhibition of the maximum rate of depolarization in the presence of cibenzoline (n=6)

| 3×10⁻⁷ M | 8×10⁻⁷ M | 3×10⁻⁶ M | 8×10⁻⁶ M |
|----------|----------|----------|----------|
| 60 beats/min | —        | —        | 0.081±0.041 | 0.124±0.042 |
| 120       | —        | —        | 0.086±0.022 | 0.138±0.070 |
| 180       | 0.097±0.051 | 0.107±0.044 | 0.109±0.027 | 0.146±0.070 |

Values represent the mean±S.D. The unit of values is AP⁻¹.
min, 214 V/sec at 60/min, 186 V/sec at 120/min and 167 V/sec at 180/min, as a new steady-state. The resting block was not observed in the presence of \(3 \times 10^{-6}\) as well as \(8 \times 10^{-7}\) M cibenzoline. At \(8 \times 10^{-6}\) M (Fig. 2, lower panel), the maximum rate of rise of depolarization of the first action potential after the onset of stimulation decreased to 221 V/sec (resting block was 14%). Cibenzoline \((8 \times 10^{-6}\) M\) decreased the steady-state level of the maximum rate of depolarization at a rate of 30/min to 171 V/sec, at 60/min to 136 V/sec, at 120/min to 114 V/sec and at 180/min to 86 V/sec. These results reflect the use-dependence of drug action.

Campbell (8, 11) showed that antiarrhythmic agents can be classified by the rate of onset of blockade in the presence of a concentration which induced about 50% of the use-dependent depression of the maximum rate of depolarization at an interstimulus interval of 300 msec (equivalent to 180 beats/min approximately). Thus, in the present experiments at 180 beats/min of stimulation frequency and with cibenzoline concentrations of \(3 \times 10^{-6}\) M and \(8 \times 10^{-8}\) M, which induced 36% and 67% inhibition, respectively, the rates of onset of the use-dependent block were examined. Figure 2 (lower panel) also represents the decaying time course of the maximum rate of depolarization at 180 beats/min (closed circles) in the presence of \(8 \times 10^{-6}\) M cibenzoline. The rate of onset of blockade was 0.158 AP\(^{-1}\) (AP=action potential). At \(3 \times 10^{-6}\) M (not shown in the figure), it was 0.122 AP\(^{-1}\). When stimulation started, the upstroke velocities decline in an exponential fashion, action potential by action potential. As picking the maximum rate of depolarization during the train pulses, the logarithms of the decremental losses during the action potentials in the train were plotted against pulse number. Thus, the values (AP\(^{-1}\)) represent the reciprocal of the number of action potential which corresponds to the time constant for the decay of the rate of depolarization. The summarized mean values at 180 beats/min and at the lower stimulation frequencies in the various concentrations of cibenzoline are shown in Table 2 (n=6). The rate of onset of inhibition of the maximum rate of depolarization behaved in a concentration- and a frequency-dependent manners. The blanks in Table 2 indicate that at these concentrations cibenzoline did not cause significant decreases in the maximum rate of depolarization.

**Discussion**

A classification of antiarrhythmic drugs by Vaughan Williams (4) based on the drug-induced modification of configuration of action potential is widely accepted. Cibenzoline is classified as a class I antiarrhythmic drug. The present experiments showed that cibenzoline at concentrations of \(3 \times 10^{-6}\) M or higher caused a prolongation of the effective refractory period, a decrease in the rate of rise of the action potential and a prolongation in repolarization. However, cibenzoline had almost no effect on the resting membrane potential. These findings were consistent with other observations (1, 2, 5, 6, 12, 13). According to the effect of cibenzoline on APD, it should belong to the subclass Ia which causes APD prolongation.

On the other hand, cibenzoline inhibited the maximum rate of rise of action potential in concentration- and a use-dependent manners. The rate of onset of blockade after the 90 sec quiescent state was similar to that of the intermediate or the slow kinetic drugs, according to the reports of Harrison et al. (7) and Campbell (8), who classified class I antiarrhythmic drugs into three subclasses. These three subclasses are fast, intermediate and slow kinetic drugs as judged by the rate of onset of inhibition of the maximum rate of depolarization in guinea-pig ventricle muscle. Although it is not yet clear how to divide drugs among these subclasses, the fast one was more than 0.277 AP\(^{-1}\), the intermediate one in the range from 0.113 to 0.055 AP\(^{-1}\), and the slow one less than 0.029 AP\(^{-1}\), according to their classification. They used drug concentrations to produce about 50% inhibition of the maximum rate of depolarization at a stimulation interval of 300 msec. The rate of onset of inhibition by cibenzoline was \(0.109 \pm 0.027\) and \(0.146 \pm 0.070\) AP\(^{-1}\) (n=6) at the concentrations to produce 36% and 67% inhibitions of the steady-state
maximum rate of depolarization. Thus, we can make a tentative conclusion that cibenzoline belongs to the subclass of intermediate drugs similar to disopyramide, quinidine and procainamide, according to Campbell’s observation in guinea-pig heart (8).

Cibenzoline greatly lengthened the action potential duration at ranges between 50–90% of repolarization and thereby prolonged the effective refractory period. Millar and Vaughan Williams (1) showed in rabbit Purkinje fiber that at higher concentrations cibenzoline 2.6×10⁻⁶ M did not lengthen the action potential duration at 90% repolarization, but did at 20% repolarization. In the present experiments, the early phase of the repolarization was rather unaffected, although it is impossible to explain the difference from the present experiments. As for the prolongation of the action potential duration, it would be considered that cibenzoline decreases a time-dependent outward current, increases the slow inward current or delays the inactivation of slow inward current. Several voltage clamp experiments (5, 6) have indicated that 2×10⁻⁶ M cibenzoline also inhibited the slow inward current in frog atrial muscle as well as in ventricular myocytes in guinea-pig heart. Furthermore, cibenzoline decreased the time-dependent outward current in mammalian heart (6), although Masse et al. (5) showed in frog atrial muscle that the outward current was conversely unaffected in the presence of cibenzoline. Thus, it is possible that cibenzoline possesses an action to decrease the time-dependent outward current, resulting in lengthening of action potential duration (although there would be a possibility that the delay of inactivation of the slow inward current might in part contribute to the APD prolongation).

It is important for an antiarrhythmic agent to increase the effective refractory period (ERP)/90% repolarization of action potential duration (APD₉₀) ratio. Cibenzoline at the concentration of 8×10⁻⁶ M decreased the ratio by approximately 5%, although it lengthened the effective refractory period and the action potential duration significantly. In the anesthetized dog, Sessine et al. (13) have observed that the ratio increased by 20% in the atrium and 30% in the ventricle.

In our laboratory cibenzoline suppressed all experimental canine ventricular arrhythmias induced by coronary ligation, digitalis or adrenaline (14). A particularly interesting thing is that the cibenzoline concentrations used were therapeutically relevant (1–5×10⁻⁶ M) (15, 16). Miura et al. (17) found that cibenzoline provided protection against ventricular tachycardia induction in 16 of 33 patients. The PR interval increased by 13%, QRS duration lengthened by 26%, and QTc interval was prolonged by 7%. It has also been shown that cibenzoline was effective in prolongation of the refractory period of an accessory pathway in a patient with Wolff-Parkinson-White syndrome (18). The clinical efficacy of cibenzoline has been also reported elsewhere (19–21). Cibenzoline, therefore, appears to be an effective antiarrhythmic agent in patients with life-threatening supraventricular and ventricular arrhythmias by inhibiting namely the Na⁺ channel and additionally with depressions of the Ca²⁺ and K⁺ channels.

References

1 Millar, J.S. and Vaughan Williams, E.M.: 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. Pharmacol. 75, 469–478 (1982)
2 Millar, J.S. and Vaughan Williams, E.M.: Pharmacological mapping of regional effects in the rabbit heart of some new antiarrhythmic drugs. Br. J. Pharmacol. 79, 701–709 (1983)
3 Dangman, K.H.: Cardiac effects of cibenzoline. J. Cardiovasc. Pharmacol. 6, 300–311 (1984)
4 Vaughan Williams, E.M.: Antiarrhythmic Action. Academic Press. London (1980)
5 Masse, C., Cazes, M. and Sassine, A.: Effects of cibenzoline, a novel antiarrhythmic drug, on action potential and transmembrane currents in frog atrial muscle. Arch. Int. Pharmacodyn. Ther. 269, 219–235 (1984)
6 Holck, M. and Osterrieder, W.: Inhibition of the myocardial Ca²⁺ inward current by the class I antiarrhythmic agent, cibenzoline. Br. J. Pharmacol. 87, 705–711 (1986)
7 Harrison, D.C., Winkle, R.A., Sami, M. and Mason, J.W.: Encainide: a new and potent
antiarrhythmic agent. In Cardiac Arrhythmias: Decade of Progress. Edited by Harrison, D.C., p. 315–330. Boston GK Hall Medical Publishers, Boston (1981)

8 Campbell, T.J.: Resting and rate-dependent depression of maximum rate of depolarization ($V_{\text{max}}$) in guinea pig ventricular action potentials by mexiletine, disopyramide, and encainide. J. Cardiovasc. Pharmacol. 5, 291–296 (1983)

9 Hashimoto, K., Satoh, H. and Imai, S.: Effects of etafenone and antiarrhythmic drugs on Na and Ca channels of guinea pig atrial muscle. J. Cardiovasc. Pharmacol. 1, 561–570 (1979)

10 Satoh, H. and Hashimoto, K.: An electrophysiological study of amiloride on sino-atrial node cells and ventricular muscle of rabbit and dog. Naunyn Schmiedebergs Arch. Pharmacol. 333, 83–90 (1986)

11 Campbell, T.J.: Kinetics of onset of rate-dependent effects of class I antiarrhythmic drugs are important in determining their effects on refactoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. Cardiovasc. Res. 17, 344–352 (1983)

12 Verdouw, P.D., Hartog, J.M., Scheffer, M.G., Van Bremen, R.H. and Dufour, A.: The effects of cibenzoline, an imidazoline derivative with antiarrhythmic properties on systemic hemodynamics and regional myocardial performance. Drug Dev. Res. 2, 519–532 (1982)

13 Sassine, A., Masse, C., Dufour, A., Hirsch, J.L., Cazes, M. and Peuch, P.: Cardiac electrophysiological effects of cibenzoline by acute and chronic administration in the anesthetized dog. Arch. Int. Pharmacodyn. Ther. 269, 201–218 (1984)

14 Hashimoto, K., Akiyama, K. and Mitsuhashi, H.: Antiarrhythmic plasma concentrations of cibenzoline on canine ventricular arrhythmias. J. Cardiovasc. Pharmacol. 9, 148–153 (1987)

15 Canal, M., Flouvat, B., Tremblay, D. and Dufour, A.: Pharmacokinetics in man of a new antiarrhythmic drug, cibenzoline. Eur. J. Clin. Pharmacol. 24, 509–515 (1983)

16 Van den Brand, M., Serruya, P., De Roon, Y., Aymard, M.F. and Dufour, A.: Haemodynamic effects of intravenous cibenzoline in patients with coronary heart disease. Eur. J. Clin. Pharmacol. 26, 297–302 (1984)

17 Miura, D.S., Kerem, G., Torres, V., Butler, B., Aogaichi, K. and Somberg, J.C.: Antiarrhythmic effects of cibenzoline. Am. Heart J. 109, 827–833 (1985)

18 Thebaut, J.F., Achard, F. and de Langenhagen, B.: Étude électrophysiologique chez l’homme d’un nouvel antiarrhythmique, la cibenzoline, dans le syndrome de Wolff-Parkinson-White. L’Information Cardiologique 4, 393–402 (1980)

19 Herpin, D., Gaudeau, B., Amiel, A., Bauple, J.L., Boutaud, P.H. and Demange, J.: Étude de l’active et de la tolérance d’un nouvel antiarythmique, la cibenzoline (Cipralan), administré par voie orale. Acta Cardiol. 36, 131–148 (1981)

20 Brown, K.F., Heger, J.J., Zipes, D.P., Chilson, D.A. and Prystowsky, E.N.: Clinical and electrophysiologic effects of cibenzoline in patients with ventricular arrhythmias. J. Am. Coll. Cardiol. 3, 867–864 (1984)

21 Miura, D.S., Dangman, K.H., Berchin, B. and Sonberg, J.C.: New antiarrhythmic agents: Part II. The pharmacology and clinical use of cibenzoline. Pract. Cardiol. 11, 103–117 (1985)