The electrophysiological effects of dicentrine on the conduction system of rabbit heart

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1 The electrophysiological effects of dicentrine, an aporphine alkaloid isolated from the root of *Lindera megaphylla*, were examined in the Langendorff perfused rabbit heart and rabbit isolated cardiac cells.

2 Standard electrophysiological characters were measured in the Langendorff perfused rabbit heart (control study) and after 5 min exposure to 1, 3 and 9 μM of dicentrine and during the subsequent recovery phase sequentially ($n = 7$). The same study protocols were performed in 0.5 to 4.5 μM quinidine ($n = 7$), 18 to 162 μM procainamide and N-acetylprocainamide ($n = 7$) for comparison.

3 The results showed that the spontaneously beating heart rate and the sinoatrial (SA) and atrioventricular nodal (AH) conduction time were not significantly affected by dicentrine but were significantly suppressed by the higher doses of quinidine (4.5 μM) and procainamide (162 μM).

4 The His-Purkinje conduction time was significantly increased by the higher dose of dicentrine, quinidine and procainamide.

5 The ventricular repolarization time and its effective refractory period were significantly increased by the higher dose of dicentrine and the other agents.

6 The effective refractory period of the atrium, AV node and His-Purkinje system were also significantly increased by dicentrine and the other agents.

7 A voltage clamp study revealed that the prolongation of atrial action potential duration by dicentrine (9 μM) was associated with a significant inhibition of the transient potassium outward current. As well as inhibition of the transient outward current, a significant inhibition of the sodium inward current by dicentrine was found.

8 We conclude that (1) dicentrine is potentially a useful antiarrhythmic agent with type I_a and type III antiarrhythmic action; (2) the relative potency of dicentrine on the electrophysiological function of cardiac tissue is 10–20 times more than that of procainamide.

Keywords: Dicentrine; quinidine; procainamide; conduction time; refractory period; transient outward current; sodium inward current; myocytes

Introduction

Medicinal plants have been used as traditional remedies in Taiwan for over hundreds of years. Among them, several aporphine alkaloids were isolated and reported to cause bradycardia and exert antiarrhythmic activity (Li *et al.*, 1984; 1989).

Dicentrine, a chemical principle isolated from the root of *Lindera megaphylla*, is an aporphine alkaloid found to have some antithrombotic activity and strong α -adrenoceptor blocking activity (Chen *et al.*, 1991; Teng *et al.*, 1991). In a study of the hypotensive effect on dogs, dicentrine when injected at 1 mg kg⁻¹ could effectively reverse the heart beats to normal sinus rhythm in dogs with recurrent ventricular tachycardia. In rat isolated ventricular cells, dicentrine prolongs the action potential duration (APD₅₀), and reduces the rate of action potential upstroke (Su *et al.*, 1994). These results indicate that dicentrine may possess antiarrhythmic activity. The effects of dicentrine on the conduction system of the rabbit heart were studied and compared with its effect on membrane currents of rabbit cardiac cells. We conclude that dicentrine is potentially a useful type I and type III antiarrhythmic agent.

Methods

Electrophysiological study of conduction in rabbit hearts

Hearts were removed from New Zealand white rabbits (≥ 2 kg) and retrogradely perfused with Tyrode solution at a

rate of 4 ml min⁻¹ g⁻¹ tissue. Tyrode solution was used, composition (mM): NaCl 120.3, NaH₂PO₄ 1.2, NaHCO₃ 24.2, KCl 5, MgCl₂ 1.3, CaCl₂ 1.2, and dextrose 5.5, which was saturated with 95% O₂ and 5% CO₂ to give a pH of 7.4 and warmed to 37°C.

For His bundle electrogram recording, a silver electrode connected to a tungsten spring was placed on the endocardium near the apex of the triangle of Koch. In order to obtain a recognizable T wave and a ventricular depolarization wave simultaneously on the ventricular electrogram, the tips of the ventricular recording electrode were separate and placed on the opposite sides of the ventricular epicardium near the ventricular apex.

High right atrial and ventricular pacing electrodes were placed near the junction of the superior vena cava and right atrium and on the epicardium near the right ventricular apex respectively. Pacing studies were performed utilizing a programmable stimulator (Bloom Ltd. DTU 215). A pacing stimulus of 1 ms in duration and twice the diastolic threshold voltage was applied to the preparation through the bipolar atrial or ventricular electrodes. The signals were continuously monitored on an oscilloscope (Hewlett Packard, 54503A) and pertinent data recorded on a two-channel physiological recorder (Gould, RS 3200) with a paper speed of 100 mm s⁻¹.

Electrophysiological studies were performed according to standard methods described previously (Josephson & Seides, 1979; Klitzner & Roberts, 1983; Young *et al.*, 1989).

The right atrium was paced at a constant rate that was slightly faster than the spontaneous heart rate and the following electrophysiological measurements made:

Intra-atrial conduction time (SA): the conduction interval

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measured from the beginning of the stimulation artifact to the first rapid deflection of the atrial depolarization wave. AV nodal conduction time (AH): the conduction interval measured from the first rapid deflection of the atrial depolarization wave to the beginning of the His bundle depolarization wave. His-Purkinje conduction time (HV): the conduction interval measured from the beginning of the His bundle depolarization to the earliest ventricular depolarization wave. Corrected ventricular repolarization time (QTc): the Q-T interval was defined as the interval measured from the beginning of the ventricular depolarization to the end of the T wave and corrected (QTc) by dividing the Q-T interval by the square root of the pacing cycle length.

The atrial pacing cycle length was then decrementally decreased (every 5–10 s) in steps of 10 or 20 ms, until a stable 1:1 AV nodal conduction pattern was lost. The longest pacing cycle length at which a 1:1 AV conduction pattern could not be maintained (either 2:1 conduction or Wenckebach cycle) was defined as the Wenckebach periodicity.

Refractory periods were determined using continuous premature stimulation techniques. Refractory periods were defined as follows: Atrial effective refractory period: the longest S_1 – S_2 interval that could not evoke an atrial depolarization (A_2) wave. AV nodal effective refractory period: the longest S_1 – S_2 interval in which the evoked A_2 failed to evoke a His bundle depolarization wave (H_2). His-Purkinje effective refractory period: the longest H_1 H_2 interval (measured from the His bundle spike of the last beat of constant rate pacing to the premature His bundle spike evoked by the conducted S_2 beat) that fails to evoke a premature ventricular depolarization (V_2).

The ventricular extrastimulation study protocol was similar to the atrial extrastimulation study. The ventricular effective refractory period was defined as the longest S_1 – S_2 interval in which the S_2 stimulation failed to evoke a premature ventricular depolarization (V_2).

Isolation of myocytes

Hearts were quickly removed from adult New Zealand white rabbits (≥ 2 kg) under anaesthesia (sodium pentobarbitone), 30 mg kg^{-1} and systemic heparinization (300 iu kg^{-1} , i.v.). The hearts were retrogradely perfused with a Langendorff apparatus by using prewarmed (37°C) calcium-free Tyrode solution for 3 min, followed by perfusing with the same solution containing 0.5 mg ml^{-1} collagenase (Type I, Sigma Chemical Co., St. Louis, MO, U.S.A.) and 0.075 mg ml^{-1} protease (Type VII, Sigma). The calcium paradox was prevented by perfusion with Kraftbruehe (KB) medium for 5 min after the enzymatic digestion (Isenberg & Klockner, 1981). Thereafter, the left and right ventricles were separated from the atria, dispersed and stored in KB medium at room temperature (25 – 27°C) for later use.

Whole-cell recording

Ionic currents were studied by a whole cell voltage clamp method (Hamill *et al.*, 1981) and pipette electrodes with a tip resistance of 2 – $5 \text{ M}\Omega$. A Dagon 8900 patch/whole cell clamp amplifier fitted with a $100 \text{ M}\Omega$ feedback resistor in the headstage was used to voltage clamp the myocyte. The formation of a high resistance seal was monitored by applying a 1 nA current from a digital pulse generator. A high resistance seal (5 to 10 gigaohm) was obtained before the disruption of the membrane patch. The cells were dialyzed with the electrode solution for 3 – 5 min to reach a state of equilibrium after disruption of the membrane patch. The total series resistance for the pathway between pipette interior and cell membrane was estimated from the cell capacitance and capacitance decay time constant. It was possible to compensate electronically for 60% of the voltage drop across the electrode produced by the current. The maximal sodium inward current elicited from adult myocytes in normal Tyrode solution

was usually higher than 20 nA and would escape effective voltage clamps. Therefore, Na currents were studied in low Na^+ Tyrode solution (80 mM NaCl was substituted with choline chloride) dialysis of the cell with Na containing (10 mM) Cs^+ pipette solution to reduce the membrane Na^+ concentration gradient and to prevent the contamination of potassium outward current.

During measurement of the potassium outward current, contamination by the calcium inward current (I_{Ca}) was prevented by adding 0.5 to 1.0 mM Co^{2+} to the bathing medium. In this condition, 400 ms depolarization of the membrane potential results in a generation of a fast inward current followed by a transient outward current (I_{to}). Since most of the I_{Na} inactivates within 5 to 10 ms after the start of depolarizing pulses, the contamination of I_{to} by this current could be reduced if the magnitude of I_{to} was measured at 10 ms after the start of the depolarizing pulse.

The effective refractory period (ERP) of rabbit ventricular cells was determined by twin stimulation at 0.2 Hz . The interval between the twin stimulation was decrementally decreased in 20 ms steps until the action potential could not be elicited by the second stimulation.

Solutions

Five basic solutions were used with the following composition in mM : (1) Ca^{2+} -Tyrode: NaCl 137 , KCl 5.4 , MgCl_2 1.1 , CaCl_2 1.8 , HEPES 12 , titrated with NaOH to pH 7.4 ; (2) Calcium-free Tyrode: Tyrode solution without CaCl_2 ; (3) Cobalt containing Tyrode solution: Tyrode solution containing CoCl_2 1 mM ; (4) Internal solution for filling the suction

Figure 1 (a) His bundle electrograms showing effects of 1 , 3 and $9 \mu\text{M}$ dicentrine on the electrophysiological function of an adult rabbit heart. The right atrium near the superior vena cava was paced at a constant rate with a pacing cycle length of 350 ms . A: atrial depolarization. H: His bundle depolarization. S: stimulation artifact. T: ventricular repolarization. V: ventricular depolarization. Paper speed: 100 mm s^{-1} . Calibration line: 10 ms . The measurements of SA, AH, HV and QTc (see Text for definitions) are in ms. (b) Chemical structures of dicentrine and quinidine.

pipettes: KCl 120, NaCl 10, MgATP 5, K₂EGTA 11, CaCl₂, HEPES 10, titrated with KOH to pH 7.4. Internal solution containing 120 mM Cs⁺ instead of K⁺ was used for I_{Na} and I_{Ca} studies; (5) KB medium: taurine 10, glutamic acid 70, KCl 25, KH₂PO₄ 10, dextrose 22, EGTA 0.5, titrated with KOH to pH 7.3

Drugs

Quinidine, procainamide and N-acetylprocainamide were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Dicentrine (($\pm$)-form, 99% purity) was isolated by column chromatography of the ethanol extract of the roots of *Lindera megaphylla* on silica gel columns (Chen *et al.*, 1991). Both quinidine and dicentrine were dissolved in dimethylsulphoxide (DMSO) as a 10 mM stock solution and further dilutions made in Tyrode solution. Procainamide (18, 55 and 165 μ M) and N-acetylprocainamide (18, 55 and 165 μ M) were dissolved in Tyrode solution.

In each of the Langendorff preparations the entire electrophysiological study protocol was performed before and after each drug addition.

In order to test the stability of the preparations and the reversibility of the agent, the same electrophysiological study protocol was repeated after the heart was again perfused in Tyrode solution for a period of at least 50 min. If the control data suggested a dysfunction of the conduction system of the preparation, or the recovery data deviated from that of the control by 20% or more, the data were discarded.

Figure 1 shows example tracing of His bundle electrograms taken from one experiment in which the atrium of an adult rabbit heart was paced at a pacing cycle length of 350 ms.

The results obtained were expressed as mean $\pm$ s.e. for each parameter. Student's *t* test was used for the comparison of control data among drugs. A repeated-measures analysis of variance was used for data comparison of different drugs and doses. A value of $P < 0.05$ was taken to indicate statistical significance.

Figure 2 Comparison of the effect of dicentrine (○), quinidine (●), N-acetylprocainamide (△) and procainamide (▲) on intra-atrial conduction (S-A interval) and effective refractory period of atrial tissue. The method for the measurement of S-A interval and AERP was described in the text. * $P < 0.001$.

Results

Forty adult rabbit hearts divided into four groups (ten in each group) were sequentially exposed to increasing concentrations of dicentrine, quinidine, procainamide and N-acetylprocainamide respectively.

The control values for all parameters except the ventricular effective refractory period were not significantly different among drug testing groups. The recovery values for all parameters were not significantly different from the control values.

Basic cycle length

The results showed that there was a significant dose-dependent increase ($P < 0.001$) in the basic cycle length for quinidine, procainamide and N-acetylprocainamide. Dicentrine exerted no significant effect on this parameter.

Atrial function

Quinidine and procainamide both caused a significant degree of lengthening ($P < 0.01$) of the intra-atrial conduction time (Figure 2). At the highest dose tested, the latter caused a significantly higher degree of prolongation of the SA interval (29%) than the former (13%) ($P < 0.01$). On the other hand, dicentrine and N-acetylprocainamide exerted no significant effect on this parameter.

All 4 drugs produced a significant lengthening of the atrial effective refractory period ($P < 0.01$) (Figure 2). At the highest dose tested, the lengthening of the atrial effective refractory period for procainamide (54%) and quinidine (43%) was much more than that for N-acetylprocainamide (26%) and dicentrine (15%).

Figure 3 Comparison of the effect of dicentrine (○), quinidine (●), N-acetylprocainamide (△) and procainamide (▲) on rabbit AV nodal function. * $P < 0.001$.

AV nodal function

Quinidine and procainamide both caused a significant degree of lengthening ($P < 0.01$) of the AV nodal conduction time (Figure 3). At the highest dose tested, the former exerted more effect (21%) than the latter (18%) ($P = 0.01$). Dicentrine and N-acetylprocainamide exerted no significant effect on the AV nodal conduction.

All 4 drugs caused a significant lengthening of the Wenckebach periodicity ($P < 0.001$) (Figure 3). Significant lengthening of the Wenckebach periodicity over the control values was observed at all doses of quinidine and procainamide, at medium and high doses of N-acetylprocainamide and at high dose dicentrine only.

The reason for the missing data in the AV nodal effective refractory period measurement was that, in some experiments, the atrial tissue became refractory to premature atrial stimulation prior to the AV nodal tissue becoming refractory, thus the AV nodal effective refractory period could not be measured directly. In those experiments in which this parameter could be measured, all drugs caused a significant lengthening of the AV nodal effective refractory period ($P < 0.001$) (Figure 3). Significant lengthening of the AV nodal effective refractory period was seen at all doses of quinidine and at the 'high' dose of dicentrine, procainamide and N-acetylprocainamide only. At the highest dose tested, the degree of lengthening of the AV nodal effective refractory period was significantly more with quinidine (59%) than with N-acetylprocainamide (38%), dicentrine (36%) or procainamide (33%).

His-Purkinje function

Dicentrine, quinidine and procainamide all exerted a significant lengthening effect on the His-Purkinje conduction time in the highest doses tested ($P < 0.01$) (Figure 4). At these doses the degree of lengthening of this parameter with procainamide (33%) was more than that with dicentrine (13%) or quinidine (15%). N-acetylprocainamide exerted no effect on the His-Purkinje conduction time.

Figure 4 Comparison of the effect of dicentrine (○), quinidine (●), N-acetylprocainamide (Δ) and procainamide (▲) on rabbit His-Purkinje function. * $P < 0.001$.

All 4 drugs caused a significant dose-dependent increase in the His-Purkinje effective refractory period ($P = 0.001$) (Figure 4). At the highest dose tested the degree of lengthening of this parameter with procainamide (36%) was more

Figure 5 Comparison of the effect of dicentrine (○), quinidine (●), N-acetylprocainamide (Δ) and procainamide (▲) on rabbit ventricular function. * $P < 0.001$.

Figure 6 Effect of dicentrine on action potential of the rabbit atrial (a) and ventricular (b) cells. The cells were bathed in Ca^{2+} -Tyrode solution and stimulated at a frequency of 0.2 Hz. Superimposed action potentials obtained before and 4 min after treatment (●) with $9 \mu\text{M}$ dicentrine are shown. Note the atrial cells became inexcitable after treatment with $9 \mu\text{M}$ dicentrine. Therefore, the stimulation current should be increased to elicit the action potential of the atrial cell.

than that with N-acetylprocainamide (33%), quinidine (29%) or dicentrine (20%).

Ventricular function

All 4 drugs produced a significant dose-dependent increase in the corrected QT interval and in the ventricular effective refractory period ($P = 0.001$) (Figure 5). The degree of lengthening of the QTc interval with all 4 drugs was similar and their dose-response curves were parallel. At the highest dose tested the degree of prolongation of the ventricular effective refractory period with procainamide (31%) was more than that with quinidine (27%), dicentrine (22%) or N-acetylprocainamide (18%).

Effects of dicentrine on action potential of rabbit cardiac myocytes

In rabbit atrial cells driven at 0.2 Hz, the action potential duration measured at 50% and 90% repolarization was 9.7 ± 1.6 ms and 69.3 ± 7.8 ms ($n = 7$), respectively. After treatment with $9 \mu\text{M}$ dicentrine, these values increased to 66.0 ± 5.1 ms and 137.2 ± 9.8 ms, respectively (Figure 6). In rabbit ventricular cells exposed to $9 \mu\text{M}$ dicentrine, the APD_{50} was unchanged but the APD_{90} was lengthened by

$13.2 \pm 1.4\%$ ($n = 6$). It is noteworthy that the spontaneous shortening of APD_{50} and APD_{90} due to the 'rundown' of L-type Ca^{2+} current with time (Belles *et al.*, 1988) was observed in rabbit ventricular cells in the absence of dicentrine. In our control study, APD_{50} and APD_{90} measured at 8 min after disruption of the membrane patch was shortened to $72.3 \pm 2.7\%$ (from 315.0 ± 6.1 ms to 227.5 ± 8.5 ms) and $78.4 \pm 5.2\%$ (from 337.5 ± 6.0 ms to 265.0 ± 17.5 ms) of that measured at 4 min after disruption of the membrane patch. When spontaneous shortening of APD was corrected, APD_{50} and APD_{90} after exposure to $9 \mu\text{M}$ dicentrine were lengthened by $14.1 \pm 1.8\%$ and $16.8 \pm 1.8\%$, respectively.

In the absence of drugs, the effective refractory period obtained at 8 min after disruption of the membrane patch was shortened to 82.5% of (from 385 to 318 ms) that measured at 4 min after disruption of the membrane patch. When the spontaneous shortening of ERP was corrected, ERP after exposure to $4.5 \mu\text{M}$ was lengthened by $45.7 \pm 2.3\%$ ($n = 6$, Figure 7). In cells treated with the same concentration of quinidine, ERP was lengthened by $97.9 \pm 4.8\%$ ($n = 6$, Figure 7).

Effect of dicentrine on sodium current and potassium current of rabbit cardiac myocytes

Figure 8a shows the transient potassium outward current (I_{to}) elicited by depolarization from a holding potential of

Figure 7 Effects of dicentrine and quinidine on recovery of membrane excitability of rabbit ventricular cells. Following a conditioning stimulation (TS, 4 ms, 1 nA), the premature action potential was elicited by test stimulation (TS, 4 ms, 1 nA) at different time interval. Each twin stimulation was applied at 0.2 Hz. (a) Typical action potentials in the absence of drug. (b) Action potentials obtained from the cell after exposure to $4.5 \mu\text{M}$ dicentrine. (c) Action potentials obtained from the cell treated with $4.5 \mu\text{M}$ quinidine.

Figure 8 Effect of dicentrine on potassium outward current of the rabbit atrial cell and ventricular cell. (a) Effect of $9 \mu\text{M}$ dicentrine on transient outward current of the rabbit atrial cell. The cell was bathed in Tyrode solution containing $0.5 \mu\text{M}$ nifedipine. Transient outward currents were elicited by ms depolarization of membrane potentials to -40 , -20 , 0 , 20 , 40 and 60 mV. Transient outward current was defined as the peak outward current measured at 5 to 10 ms after the start of depolarizing step. Insets are typical current obtained before and after exposure to dicentrine. (b) Effect of $9 \mu\text{M}$ dicentrine on potassium current of rabbit ventricular cells. The cells were bathed in Tyrode solution containing $0.5 \mu\text{M}$ nifedipine. Insets are typical inwardly rectifying current obtained before and after exposure to $9 \mu\text{M}$ dicentrine.

– 80 mV to different potentials between – 40 to + 60 mV in atrial and ventricular cells. Four minutes treatment with 9 μM dicentrine reduced the amplitude of I_{to} by 67.7%. If the integral of potassium outward current from the start of depolarization to the end of the depolarization step was measured, 86.6% reduction of potassium outward current was obtained. I_{to} is less prominent in rabbit ventricular cells than in rabbit atrial cells (Giles & Imaizumi, 1988) but the magnitude of inward rectifying current is larger in ventricular cells than in atrial cells. In Figure 8b, the relative amplitude of outward current through K_1 channels was decreased to nearly 40% after 4 min exposure to 9 μM dicentrine but a 62.5% inward current through K_1 -channels remained. Figure 9a shows the effects of dicentrine on the current voltage relationship for I_{Na} . I_{Na} was elicited by 15 ms depolarization from – 80 mV to various potentials ranging between – 60 and 0 mV. Under control conditions (in the presence of 0.5 μM nifedipine), I_{Na} was activated at a threshold potential of around – 60 mV and attained a maximum value at – 30 to – 20 mV. Dicentrine (9 μM) blocked I_{Na} completely. If the holding potential was changed from – 80 mV to – 90 mV, a partial recovery of I_{Na} was obtained.

The effect of dicentrine on the steady state inactivation of I_{Na} was studied by using a double pulse protocol. The suppressive effect of dicentrine was accompanied by a shift of the I_{Na} availability (h_{∞} –V) curve. The h_{∞} –V curves were

obtained by normalizing the I_{Na} (induced by a test pulse to – 20 mV) by taking the maximum value as unity. The curves were fitted by the Boltzmann equation:

$$h_{\infty} = 1/[1 + \exp(E - V_h)/s]$$

Where E denotes the first conditioning potential, V_h the potential at which h_{∞} is equal to 0.5, and s, the slope factor. Dicentrine at 1.5, 4.5 and 15 μM altered V_h from – 81.7 mV to – 89 mV, – 99.4 mV and – 115.9 mV, respectively (Figure 9). The maximal I_{Na} induced by a test pulse to – 20 mV from a holding potential of – 140 mV was reduced from – 5.6 nA to – 5.0 nA, – 3.2 nA and – 1.3 nA, respectively. In rabbit ventricular cells, similar exposure to 3 μM dicentrine resulted in a change of V_h from – 82.6 $\pm$ 2.9 mV to – 87.1 $\pm$ 3.5 mV (Figure 9). The maximal I_{Na} induced by test pulse to – 20 mV from a holding potential of – 140 mV was reduced from – 7.9 $\pm$ 1.2 nA to – 5.6 $\pm$ 1.2 nA ($n = 5$). This result indicates that the sensitivity of rabbit ventricular cells to dicentrine is similar to that of atrial cells.

Discussion

Several new antiarrhythmic drugs, previously available only for investigative purposes have been approved for general use, such as tocainide, mexiletine, flecainide, encainide and

Figure 9 Effect of dicentrine on sodium inward current of rabbit atrial and ventricular cell. (a) Effect of 9 μM dicentrine on potential dependent activation of I_{Na} in the atrial cell. (b) Effect of 1.5, 4.5 and 15 μM dicentrine on voltage-dependent inactivation curves of I_{Na} in a rabbit atrial cell. (c) Effect of 9 μM dicentrine on voltage-dependent inactivation curves of I_{Na} in ventricular cells ($n = 6$).

amiodarone. However, prompted by the need for more effective and less toxic drugs, considerable interest in the search for new antiarrhythmic agents exists.

In 1969, Vaughan Williams proposed a classification for antiarrhythmic agents that has subsequently become widely accepted (Vaughan Williams, 1972; Singh *et al.*, 1972; Harrison, 1985; Hondeghem & Katzung, 1984). His classification, based on the effect of such agents on cardiac action potentials or intracardiac conduction intervals categorizes the drugs into 4 classes (Hoffman, 1985; Vaughan Williams, 1984; Weld *et al.*, 1982; Wettrel & Andersson, 1986).

In this study, dicentrine was found to inhibit action potential upstroke and prolong action potential duration. In a perfused heart model, dicentrine was found to inhibit H-V conduction but without significant inhibition of A-H conduction.

The inhibition of action potential upstroke and suppression of H-V conduction is correlated well with the inhibition of sodium current. Use-dependent inhibition of I_{Na} , retardation of the recovery of Na^+ channels from their inactivated state and negative shift of the voltage-dependent inactivation curve of I_{Na} are major mechanisms responsible for the action of most group I antiarrhythmic agents (Chen *et al.*, 1975; Lee *et al.*, 1981; Carmeliet & Saikawa, 1982; Sanchez-Chapula *et al.*, 1983; Clarkson & Hondeghem, 1985; Clarkson *et al.*, 1988; Su & Morad, 1987; Su *et al.*, 1990). Since the inhibition of I_{Na} by dicentrine is associated with a prominent leftward shift of the voltage-dependent inactivation curve of I_{Na} , the Na^+ channels available for activation may be markedly decreased after treatment with dicentrine.

Our voltage clamp study proved that the prolongation of rabbit atrial action potential duration by dicentrine is due to its inhibition of the transient outward current. In rabbit ventricular cells, the suppression of the transient outward current and the suppression of the potassium outward current through the inward rectifier both contributed to the prolongation of action potential duration. Our data from the

voltage clamp study correlated well with the data obtained from the perfused heart model.

Early afterdepolarizations (EADs) and associated triggered arrhythmias can be induced by interventions that either depress outward (repolarizing) current or augment inward (depolarizing) current. These include caesium and class III antiarrhythmic agents which block potassium channels (Isenberg 1976; Roden & Hoffman 1985; Quantz & Nattel 1986; Hondeghem & Snyder, 1990); the calcium channel activator Bay K 8644 (January *et al.*, 1988); and agents that interfere with sodium channel inactivation such as aconitine and anthopleurin-A (Craelius *et al.*, 1988). EADs and associated triggered activity occurring during APD prolongation can be blocked by sodium channel blockers such as tocainide or mexiletine (Valois & Sasyniuk 1987; Roden & Hoffman, 1987) or potassium channel openers such as cromakalim and pinacidil (Fish *et al.*, 1990).

In the present study, we found that dicentrine blocks both Na^+ and K^+ channels like procainamide, quinidine and amiodarone (Carmeliet & Saikawa, 1982; Colatsky, 1982; Sanchez-Chapula *et al.*, 1983; Clarkson & Hondeghem, 1985; Nattel 1986; Clarkson *et al.*, 1988), which are broadly used in treating supraventricular as well as ventricular arrhythmias. The I_{Na} blocking action can antagonize the generation of EADs which occur after inhibition of potassium outward currents. Furthermore, a lesser degree of L-type calcium current inhibition by dicentrine as compared to quinidine (Su *et al.*, 1994), along with its rapid onset in action in perfused heart preparations and isolated cardiac myocytes, will make dicentrine useful in treating acute cardiac arrhythmias.

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