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Whole-cell patch clamp measurements and berberine inhibition of hyperpolarization-activated inward current in rabbit sinoatrial node cells[1]

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ABSTRACT Single rabbit sinoatrial node (SAN) cells were isolated by means of an enzymatic dispersion procedure and used for the whole-cell patch clamp experiment. At a holding potential of −40 mV, a time- and voltage-dependent inward current, I_f, was activated at different hyperpolarization potentials from −60 mV to −110 mV. The current-voltage relation curve showed that I_f was activated at potential more negative than −40 mV. Five min after treatment by CsCl 2 mmol·L^{-1}-containing Tyrode solution, I_f was almost completely blocked. At a hyperpolarization potential of −110 mV, I_f was reduced from 1.7±0.2 nA of the control to 1.2±0.4 nA after superfusing with Tyrode solution containing berberine (Ber) 1 μmol·L^{-1} for 5 min. And it was difficult to wash out this action. Ber also had inhibitory effects on other currents to a certain extent. The results indicate that I_f is a Cs^+-sensitive current and that the negative chronotropic effect of Ber may be due to the inhibition of I_f that functions as an important pacemaking modulator for the spontaneous depolarization of SAN tissue.

KEY WORDS electrophysiology; berberine; sinoatrial node; patch clamp; ion channels

Normally the heart rate is originated from the sinoatrial node (SAN) tissue in which a spontaneous electric activity is enabled to occur[1]. The hyperpolarization-activated inward current, I_f, as a modulator of heart rate, plays an important role in the spontaneous depolarization of maximum diastolic potential (MDP) in SAN cells[2]. It would be predictable that most of anti-arrhythmic agents against fast supraventricular arrhythmia will bring about their negative chronotropic effect on myocardium through affecting the I_f of SAN cells. Effects of berberine (Ber) on the cardiovascular system in the human and experimental animals have been characterized by increasing the contractile force and slowing the heart rate[3,4]. Under the voltage clamp condition, Ber had a dose-dependent biphase effect on the slow inward ionic current (I_{si}) in guinea pig papillary muscle[5]. However, there are no more electrophysiological data to show the influence of Ber on the pacemaking activity and the ionic currents of sino-atrial node cells. In order to search for the mechanism of the negative chronotropic effect of Ber, in this study, we established the method of determining I_f under patch-clamp condition and investigated the basic effects of Ber on I_f.

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MATERIALS AND METHODS

Isolation of single SAN cells Rabbits (♂ or ♀), weighing 1.5±0.3 kg, were anesthetized with iv pentobarbital sodium 100 mg·kg^{-1}. The SAN was dissected out in oxygenated Tyrode solution at room temperature 20℃. The single rabbit SAN cells were isolated according to the enzymatic procedures[7] with some modifications. In brief, the SAN tissue strips were incubated in a Ca^{2+}-free Tyrode solution containing collagenase (151 U·mg^{-1}, Worthington Biochemical Co) 3 g·L^{-1} and elastase (Type IV, Sigma) 0.25 g·L^{-1} at 37℃ for 1 h. After the enzymatic dispersion, these strips were kept in a "KB" (Kraftbrühe, power soup)[8] recovery solution which was refrigerated at 0—4℃ for at least 1 h. before use.

The SAN cells suspending in KB solution were allowed to settle down at the bottom of the recording

chamber mounted on the stage of an inverted microscope (CK2, Olympus) for 5—10 min. and then superfused with Tyrode solution at 37℃ until drugs were given. Those cells nearest to the central area of SAN were used for the whole-cell patch clamp experiment.

Superfusion solutions and drugs Tyrode solution was composed of NaCl 143, KCl 4, $MgCl_2 \cdot 6H_2O$ 0.5, $CaCl_2$ 1.8, HEPES 5.0, NaH_2PO_4 0.3, glucose 5.5 $mmol \cdot L^{-1}$. The pH was adjusted to 7.4 by NaOH 1 $mol \cdot L^{-1}$. Calcium-free solution contained NaCl 143, KCl 5.4, $MgCl_2$ 1.8, HEPES 5.0, NaH_2PO_4 0.33 and glucose 5.5 $mmol \cdot L^{-1}$. The pH was adjusted to 7.4 by NaOH 1 $mol \cdot L^{-1}$. KB recovery solution contained glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, HEPES 10, $MgCl_2 \cdot 6H_2O$ 0.5, EGTA 0.5, and glucose 11 $mmol \cdot L^{-1}$. The pH was adjusted to 7.3—7.4 by KOH 1 $mol \cdot L^{-1}$. Internal solution contained KCl 130; HEPES 5, $MgCl_2 \cdot 6H_2O$ 1, ATP·K_2 5, CPK 5, and EGTA 1 $mmol \cdot L^{-1}$. The pH was adjusted to 7.2 by KOH 1 $mol \cdot L^{-1}$. CsCl was purchased from Sigma Co and berberine hydrochloride (Ber) was purchased from the Dong Bei Pharmaceutical Co.

Whole cell patch clamp procedures Membrane potentials and currents of single SAN cells were elicited by whole-cell patch clamp technique[8]. Electrode tip was allowed to be about 1 μm in diameter with resistances of 5—10 MΩ and positioned near to the surface of the cell by controlling a 3-dimentional hydraulic microdrive manipulator (MO-303, Narishige). After a GΩ seal was formed by a gentle suction using an 1-ml syringe, all the junction potentials were compensated and the electrode current became zero. As the membrane patch within the electrode tip was ruptured by a further suction step, the whole cell recording mode was formed.

A stimulator (SEN-7103, Nihon Kohden Co) was used to provide the command pulses for the single SAN cell through a patch clamp amplifier (CEZ-2200, Nihon Kohden Co). The currents were filtered at 1 kHz and recorded through a data processor together with potential signals in a FM tape recorder (RMG-5304, Nihon Kohden Co) for later analysis. Voltage and current records were simultaneously monitored using a digital storage oscilloscope (VC-10A, Nihon Kohden Co). All data were transferred through an AD/DA converter to an IBM personal computer (PC/AT) and analyzed using a software (pClamp Ver 5.5, Axon Instruments Inc). Voltage and current tracings were played back and displayed on the storage oscilloscope and photographed with a continuous recording camera (RLG-6201, Nihon Kohden Co). The data were expressed as $\bar{x} \pm s$.

RESULTS

Spontaneous action potential Spontaneous action potential of single SAN cell was recorded in current clamp mode (I=0) immediately after the cell membrane was ruptured by a suction step. Fig 1 illustrated a typical record of spontaneous activity of a SAN cell that was most likely to be one adjacent to the central area of the SAN, since its MDP was about −55 mV, followed by a rapid spontaneous depolarization. Those cells near to the central region of the SAN had MDP of about −50 to −60 mV with a spontaneous beating rate of 200±69 beats·min^{-1} (n=5), but those in the periphery of the SAN had the MDP of about −65 to −75 mV with a slow beating rate of 185±70 beats·min^{-1} (n=4). The patterns of the action potentials of the central region cells were quite similar to those reported elsewhere[7,9-11].

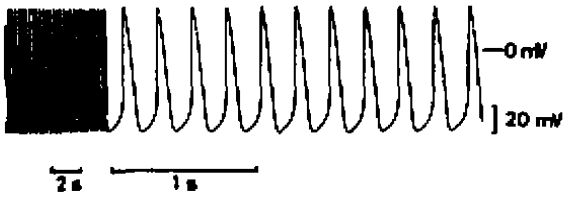

Fig 1. Spontaneous action potentials of an isolated sinoatrial node (SAN) cell of rabbit.

Hyperpolarization-activated current I_f I_f was elicited and determined under voltaged-clamp condition. Fig 2A showed typical current records of I_f from a SAN cell held at −40 mV and given successive 2-s voltage-clamp hyperpolarizations in 10 mV steps down to −110 mV. The threshold of I_f for activation was

about −60 mV, which corresponded to the MDP of this cell (Fig 2A). The amplitude of it increased in a voltage- and time-dependent manner. Fig 2B illustrated the current-voltage (I—V) relation of I_f, showing that the activation of this current required a more negative membrane potential than the holding potential. These features of I_f were identified with those of the voltage- and time-dependent I_f reported in the literatures[7,9,12-14].

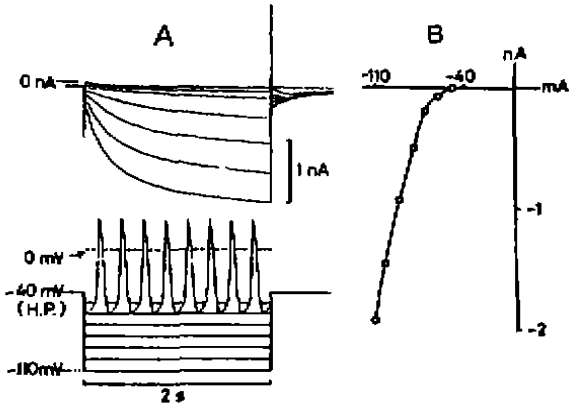

Fig 2. Activation of hyperpolarization-activated inward current, I_f(A) and the I—V curve of I_f(B).

Effects of Cs^+ on I_f Fig 3A showed the effect of CsCl on I_f after a 5—min superfusion with Tyrode solution containing CsCl 2 mmol·L^{-1}. CsCl inhibited most part of this current at different hyperpolarization potentials. Washing for 10 min easily reversed this inhibitory effect. This indicated I_f was a Cs^+-sensitive current. The current-voltage curve of I_f in the presence of CsCl 2 mmol·L^{-1} was shown in Fig 3B. Cs^+ 2 mmol·L^{-1} also decreased the slope of slow diastolic depolarization (SDD) resulting in a reduction of spontaneous electric activity of SAN cell. However, Cs^+ 2 mmol·L^{-1} did not impede the activity (Fig 3D).

Other ionic currents and effects of Cs^+ When the membrane potential was depolarized from a holding potential of −40 mV to +30 mV in 10 mV steps, an inward Ca^{2+} current (I_{Ca}), and an outward K^+ current (I_{Kout}) were recorded in different potential levels at the beginning and the endof each 2-s depolarization pusle (Fig 3C). A time-inactivated outward current, called "tail current" (I_{tail}) with a pattern of 2-exponential, was also observed after the depolarization step finished. CsCl slightly decreased I_{Kout} and I_{tail}, but had no effect on I_{Ca} after a 5-min superfusion with a Tyrode solution containing CsCl 2 mmol·L^{-1}.

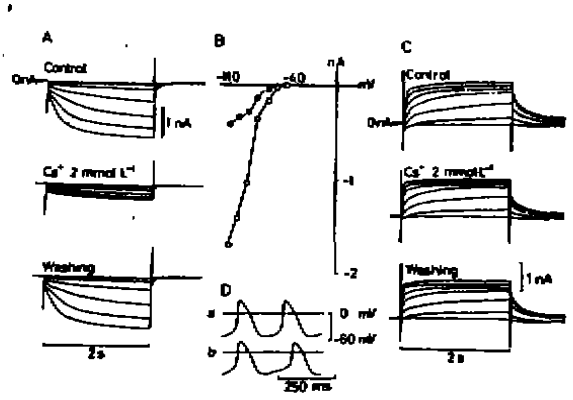

Fig 3. Effects of CsCl 2 mmol·L^{-1} on I_f, other ionic currents, and spontaneous action potentials. A) Holding potential: −40 mV; Hyperpolarization protocol: from −40 mV to −110 mV in 10 mV steps. B) I—V curve of I_f in the absence (○) and presence (●) of CsCl 2 mmol·L^{-1}. C) Holding potential: −40 mV; Depolarization protocol: from −40 mV to +30 mV in 10 mV steps. D) a: Control; b: 5 min after CsCl 2 mmol·L^{-1}.

Effects of Ber on action potential, I_f and other ionic currents Under current clamping condition, Ber 1 μmol·L^{-1} significantly decreased the firing rate of spontaneous action potential through reducing the slope of SDD. Fig 4 is a typical record of this action, showing that the firing rate was reduced by approximately 50% of the control (230 beats·min^{-1}) in the presence of Ber 1 μmol·L^{-1}. It was also observed that amplitude of action

potential (APA) was diminished after a 5-min superfusion with Tyrode solution containing Ber 1 μmol·L^{-1}(Fig 4).

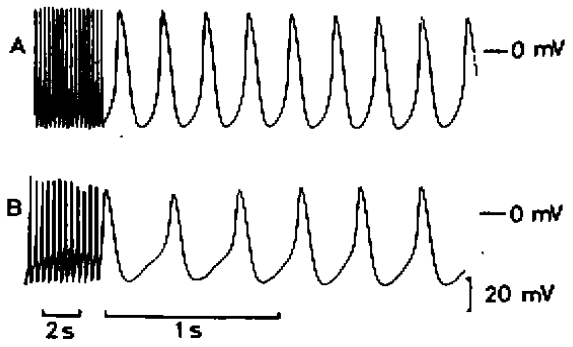

Fig 4. Effect of berberine (Ber) 1 μmol·L^{-1} on spontaneous action potential of a typical SAN cell. A. Control; B. After 5 min.

Under voltage clamping condition, Ber 1 μmol·L^{-1} brought about a partial inhibitory effect on I_f(Fig 5A). At the end of a 2-s hyperpolarization pulse of −110 mv from a holding-potential of −40 mv, the amplitude of I_f was reduced from 1.7±0.2 nA of control to 1.2±0.4 nA (n=4) after exposing SAN cells to Tyrode solution containing Ber 1 nmol·L^{-1} for 5 min. The inhibitory effect of Ber on I_f was recognized clearly in the I—V curve (Fig 5B).

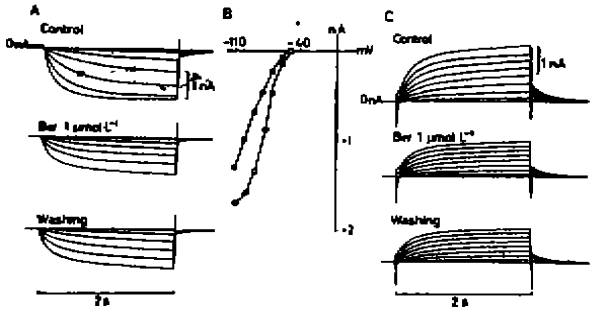

Fig 5. Effect of berberine (Ber) on I_f and other ionic currents. A) Holding potential: −40 mV; Hyperpolarization protocol: from −40 mV to −110 mV in 10 mV steps. B) I—V curve of I_f in the absence (○) and presence (●) of Ber 1 μmol·L^{-1}. C) Holding potential: −40 mV; Depolarization protocol: from −40 mV to +50 mV in 10 mV steps.

Fig 5C showed a typical record the effects of Ber on I_{Kout}, I_{tail}, and I_{Ca}. After SAN cells were exposed to Tyrode solution containig Ber 1 μmol·L^{-1}, I_{Kout}, I_{tail}, and I_{Ca} were reduced to 71.9±6.7% (n=4), 65.0±2.0% (n=3), and 99.7±13.7% (n=4) of control, respectively. Ber at this concentration inhibited I_{Kout} and I_{tail} partially, but did not significantly affect I_{Ca}.

DISCUSSION

In the present study, the cells isolated from SAN tissue were of different features. Those cells with high beating rate and MDP of −50 to −60 mV were selected for our use. This kind of cells were adjacent to, but not in the central region of SAN[7]. The leading pacemaker cells in the center of SAN were quite small and rapidly rounded up as soon as patch pipette touched them. And these cells sometimes seemed intolerant to calcium ions in Tyrode solution. Therefore, they were not used for experiment. Other cells without spontaneous activity or with low beating rate and more negative MDP were atrial cells or peripherial cells, so they were also not used.

Cumulative evidences[2,7,9,12,13] strongly indicate that I_f in SAN cells was a Cs^+-sensitive current with a activation threshold of −55 to −60 mV and play a very important role in the SAN pacemaker. The basic electric properties of I_f we observed in the persent experiment were correspondent with those described in the literatures[7,9-16].

In this study, we found that the partial depression of I_f by Ber is parallel to the decrease of the slope of SDD and the reduction of firing rate of SAN cell. This indicates that the negative chronotropic effect of Ber was performed probably mainly through the inhibi-

tion of I_f. In addition, the partial depressions of Ber on I_{Kout} and I_{tail} were also observed, indicating Ber was likely to perform a blocking effect on K^+-channels in SAN cells. The influence of this agent on I_{Ca} seemed to be more complicated since Ber 1 μmol·L^{-1} did not result in an obvious alternation of I_{Ca} while APA in current clamp mode was reduced markedly, and we had not found a biphase effect of Ber 1 μmol·L^{-1} on I_{Ca} in single SAN cells.

In conclusion, the I_f we obtained in the present experiment is a Cs^+-sensitive current activated within the range of MDP. Ber 1 μmol·L^{-1} partially inhibited I_f as well as I_{Kout} and I_{tail}.

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兔窦房结细胞超极化激活内向电流的全细胞膜片钳测定及小檗碱的抑制作用

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摘要 用酶分离的家兔窦房结细胞进行了全细胞膜片钳制实验，在保持膜电位—40 mV 条件下，用—60 mV 至—110 mV 的超极化电位，引出 I_f，该电流大部分被 CsCl 2 mmol·L^{-1}所抑制。在上述超极化电位范围内，小檗碱(Ber) 1 μmol·L^{-1}使 I_f 部分受到抑制。结果表明：本实验引出的 I_f 为 CsCl 敏感电流，Ber 抑制调节窦房结组织自发除极的 I_f 可能是该药负性频率作用机制之一。

关键词 电生理；小檗碱；窦房结；膜片钳；离子通道