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Inhibitory effects of vinpocetine on sodium current in rat cardiomyocytes[1]

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KEY WORDS vinpocetine; sodium channels; tetrodotoxin; myocardium; patch-clamp techniques; *Vinca* alkaloids

AIM: To study the effects of vinpocetine (Vin) on the sodium current (I_{Na}) in cardiomyocytes. **METHODS**: The sodium current in adult rat ventricular myocytes was measured by whole cell patch-clamp technique. **RESULTS**: The I_{Na} in cardiomyocytes was blocked reversibly by Vin, in concentration-dependent and voltage-dependent manner, but not rate- or use-dependent. The I_{Na} was attenuated by 13 % - 75 % when the Vin concentration was raised from 10 to 80 μmol · L^{-1}. The IC_{50} (95 % confidence limits) was 36.4 (28.1 - 47.1) μmol · L^{-1}. When the membrane potential depolarized over the range of -90 mV to +40 mV in 10-mV step, inhibitory effect of Vin on the I_{Na} was 39 % at first, then maintained at a higher level, about 52 % ± 5 %. The maximal depression (57 %) reached at about 0 mV. Vin influenced both the activation and inactivation processes of sodium channel, and resulted in attenuation of the window currents (the slowly inactivating sodium currents). **CONCLUSION**: Vin inhibited sodium currents in rat ventricular myocytes.

Vinpocetine (Vin), (ethyl apovincamin-22-oate), has been used in the treatment of cerebrovascular diseases: dementias, convulsion, etc. Vin was first described as cerebral vasodilate because it increased global cerebral blood flow under normal and hypoxic conditions in animals[1]. Then it was found that Vin reduced the loss of hippocampal neurones in rat cerebral ischemia[2], and potentiated the neuroprotective effect of adenosine against hypoxic damage[3]. Recently, it was reported that Vin improved the deformability of erythrocytes[4] and Vin had a block effect as potent as phenytoin on the voltage-gated Na^+ channel in rat primary cultured cerebral neurones[5]. They may be the main role of Vin in ischemia. The Na^+ channel in cardiomyocytes was also dealt with the damage of ischemia[6]. The fast sodium current was responsible for the upstroke phase of action potentials, which influenced excitability and conduction of impulses in heart tissues[7]. The slowly inactivating or late sodium current which precise information was obtained from single channel recording would have a special meaning from the clinical point of view, *eg*, in ischemia[8,9]. To clarify the role of Vin to cardiovascular diseases, we studied the action of Vin on voltage-gated Na^+ channel in rat ventricular cells.

MATERIALS AND METHODS

Isolation of cardiomyocytes Adult Sprague-Dawley rats, ♂, weighing 232 ± *s* 19 g, (supplied by Animal Department of Shanghai Brain Research Institute, Chinese Academy of Sciences, conventional animals, Certificate № 005) were stunned. Langendorff hearts were perfused at 37 ℃ with a nominally zero calcium (existed a little Ca^{2+} in distilled water) solution for 8 min, then perfused with enzymatic solution, *ie*, zero calcium solution plus type I collagenase (Sigma) 0.26 - 0.31 g · L^{-1} and albumin (fatty-acid-free, V Boehringer) 1 g · L^{-1} for 5 - 15 min. When the heart was soft, the ventricle was chopped and gently stirred in KB solution. Cardiomyocytes appeared in the supernatant were filtered, stored in fresh KB solution at 18 - 22 ℃, and used within 10 h.

Whole cell patch-clamp recording Cells were examined with an inversed microscope (Nikon, Japan). After attached to the bottom of the bath, the cells were perfused by the extracellular solution 6 - 7 mL · min^{-1}. The solution in the bath was replaced completely within 1 - 2 min. The glass microelectrodes were pulled in two steps by microelectrode puller (Narrishage, Japan), resistance 0.8 - 2.8 MΩ after filled with intracellular solution. An Ag-AgCl electrode was used as the reference electrode. Membrane currents were recorded with an Axopatch 200A amplifier (Axon Instrument, USA). The

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pCLAMP 6.0 software (Axon Instrument, USA) was used to produce signals, collect and process data. Capacitive transients and series resistance were compensated and linear leakage currents were subtracted using the p/4 protocol (leakage current was calculated based on 4 small and short hyperpolarising pulses before conditioning and test-pulses. See user manual of pCLAMP 6.0). The temperature in bath was kept at 18 - 22 ℃.

Solutions and drugs Solutions: zero calcium solution contained: NaCl 120, KCl 10, KH_2PO_4 1.2, $MgCl_2$ 1.2, taurine 10, pyruvate 5, glucose 20 ($mmol \cdot L^{-1}$), and its pH was adjusted to 7.2 with NaOH. KB solution contained: KOH 70, KCl 40, KH_2PO_4 20, $MgCl_2$ 3, *L*-glutamic acid 50, taurine 20, egtazic acid 0.5, HEPES 10, glucose 10 ($mmol \cdot L^{-1}$), and its pH was adjusted to 7.4 with KOH. Intracellular (pipette) solution contained: CsCl 130, NaF 13, $MgCl_2$ 2, egtazic acid 2, HEPES 10 ($mmol \cdot L^{-1}$), and its pH was adjusted to 7.2 with CsOH. Extracellular solution contained: NaCl 75, CsCl 75, $MgCl_2$ 1, KCl 5, $CoCl_2$ 2.5, TEA 10, glucose 5, HEPES 10 ($mmol \cdot L^{-1}$), and pH was adjusted to 7.4 with CsOH. Drugs: vinpocetine (Takeda Chemical Inc Ltd, Japan) was first dissolved in HCl 1 $mol \cdot L^{-1}$ at a concentration of 20 $mmol \cdot L^{-1}$, and then diluted with extracellular solution. The pH was adjusted to 7.3 with CsOH. Tetrodotoxin (TTX) was purchased from Fisheries Research Institute, Hebei Province, China.

Data analysis and curve fitting Data are presented as $\bar{x} \pm s$, and analyzed by paired or group t test. The steady-state inactivation or activation curves were fitted with Boltzman equation: $I_{Na} = I_{Max}/[1 + \exp(V - V_{0.5})/K]$[10], (or $I_{Na} = I_{Max}/[1 - \exp(V - V_{0.5})/K]$). The I_{Na} is the sodium current; I_{Max} is the maximal amplitude of sodium current; V is the voltage of the conditioning pulse; $V_{0.5}$ is the voltage for half inactivation; and K is a slope factor. The concentration-response curve was fitted according to the equation: $I_{Na} = I_{Max}/(IC_{50}/C + 1)$, where the I_{Na} and I_{Max} are the same as above. The C is the concentration of Vin and IC_{50} is the concentration of inducing half-inhibition.

RESULTS

Effects of Vin on sodium current

Quiescent, single, small-sized ventricular cells with clear striations were selected for recording sodium currents. When the cell was evoked by a 50-ms step depolarization to - 50 mV from a holding potential of - 90 mV, an inward current with amplitude of 4.2 ± 1.6 nA (n = 24) appeared. It was blocked partially by adding TTX 30 $\mu mol \cdot L^{-1}$ (Fig 1) or completely by 100 $\mu mol \cdot L^{-1}$. After 5 - 8 min of equilibration, the sodium currents were recorded.

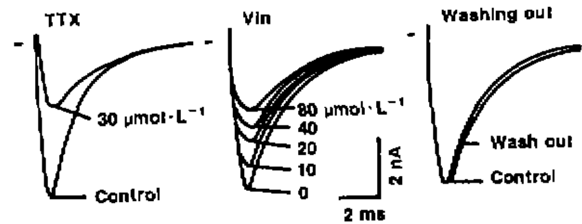

Fig 1. Inhibition of myocardial sodium current by TTX or Vin.

One cell was used for only one concentration. The inhibitory effect appeared within 2 min after adding Vin, and increased with raising concentrations. As the Vin concentrations were 10, 20, 40, and 80 $\mu mol \cdot L^{-1}$, the amplitudes of the currents were attenuated by 13 % ± 2 % ($n = 6$, $P < 0.05$), 29 % ± 6 % ($n = 6$, $P < 0.01$), 53 % ± 6 % ($n = 6$, $P < 0.01$), and 75 % ± 6 % ($n = 6$, $P < 0.01$), respectively (Fig 1). There was a correlation (r = 0.99684, $P < 0.01$) between the log concentration (X) and the % inhibition (Y) (the regression equation: $\hat{Y} = -0.5876 + 0.6976X$). The IC_{50} (95 % confidence limited) was 36.4 (28.1 - 47.1) $\mu mol \cdot L^{-1}$. The I_{Na} recovered completely after washing out of Vin from the extracellular solution (Fig 1).

Current-voltage relationship The membrane potential was held at - 80 mV and the inward current was evoked in every 10-mV step depolarization to various levels. The maximal activation potential appeared at - 40 mV for control and - 30 mV for Vin 40 $\mu mol \cdot L^{-1}$. Sodium currents were decreased by Vin at membrane potentials from - 50 mV to + 10 mV. The extent of % inhibition was from 33 % to 60 % ($P < 0.05$). The mean reversal potential did not change and was at about + 29 mV in both control and Vin group (Fig 2).

Steady-state sodium current inactivation

Various membrane potentials between - 120 mV and - 20 mV were held for 5 s and then depolarized by test pulses to - 30 mV. Vin 40 $\mu mol \cdot L^{-1}$ had an inhibitory effect to inactivation course of sodium channel. These currents were not leak-subtracted. The curve was fitted with Boltzman equation. The half-inactivation voltage and slope factor were - 72.8 mV and 7.9 for control, and - 82.4 mV and 9.7 for Vin ($P < 0.05$), respectively (Fig 3).

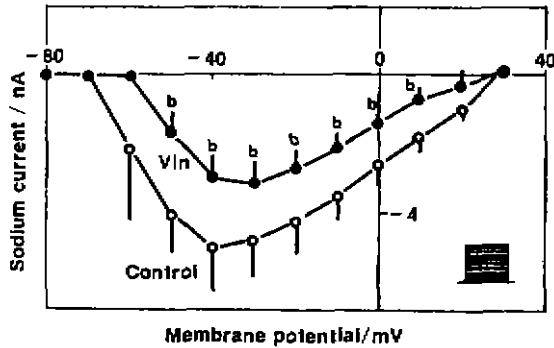

Fig 2. Current-voltage relationship of sodium current for control and Vin 40 μmol·L^{-1}. $n = 5$ cells, $\bar{x} \pm s$. [b]$P < 0.05$.

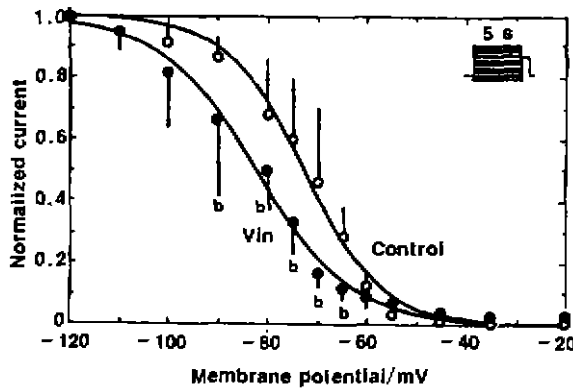

Fig 3. Steady-state inactivation curve of sodium current. The inactivation course was inhibited by Vin 40 μmol·L^{-1}. $n = 5$ cells, $\bar{x} \pm s$. [b]$P < 0.05$.

Effect of Vin on activation and inactivation

The Vin 40 μmol·L^{-1} inhibited both activation and inactivation of sodium channel, and attenuated the window current (Fig 4).

Voltage-dependent and use-dependent blocking The membrane potentials were held from −90 mV to +40 mV by condition pulses, then depolarized to −30 mV by a test pulse. The results (I_{test}/ $I_{condition}$) indicated that the blocking effects of Vin 40 μmol·L^{-1} were produced below the threshold (−60 mV) of sodium current and stronger when the potentials were increased. There was a correlation ($r = 0.5464$, $P < 0.05$) between the % inhibition (Y) and the log membrane potential (X) (the regression equation: $\hat{Y} = 0.1634 + 0.2021X$). The maximal blocking effect was about at 0 mV. Afterwards, the effect was not increased obviously (Fig 5).

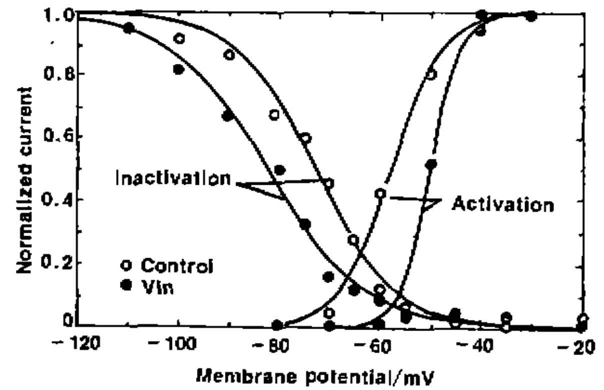

Fig 4. Activation and inactivation curves of sodium current for control and Vin 40 μmol·L^{-1}. $n = 5$ cells, $\bar{x} \pm s$.

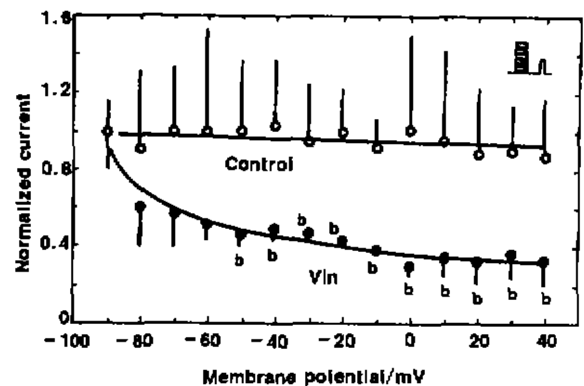

Fig 5. Voltage-dependent blockage of sodium current by Vin 40 μmol·L^{-1}. The normalized I_{test} (the peak amplitude of sodium current during the test pulse) was plotted as a function of the membrane potential during the conditioning pulse. $n = 5$ cells, $\bar{x} \pm s$. [b]$P < 0.05$.

To test the use-dependent blocking of the sodium currents, a train of 20 depolarizing pulses (the stimulating frequencies were 1, 3, and 5 Hz) was applied to a holding potential of −100 mV, and the membrane potential was depolarized to −30 mV. When the frequencies were 1, 3, and 5 Hz, the I_{Na} did not decline for control, but decreased by 36 %, 34 %, and 24 % for Vin 40 μmol·L^{-1}. However, the relative current (amplitude of I_{Na} at each stimulation compared with that of the initial one) was not much decreased for the presence of Vin 40 μmol·L^{-1}. For example, the peak amplitude of I_{20th} compared with that of I_{1st}, the % decrease was 10 %,

7 %, and 6 % ($n = 6$, $P > 0.05$) respectively, no obvious differences were found between 1, 3, and 5 Hz. So, we did not find the use- or rate-dependent effect of Vin.

DISCUSSION

The present results indicated that vinpocetine inhibited sodium current in isolated rat ventricular cells. This effect is the same as that in neuron cells[5]. The inhibition was reversible and concentration-dependent.

In addition, Vin influenced on both activation and inactivation process of sodium channel in cardiomyocytes. Vin raised the threshold potential and changed the activation curve toward depolarization direction. These findings suggested that the activation process of sodium channels be inhibited by Vin. The inactivation curve was shifted to hyperpolarization. It indicated that Vin also blocked the inactivated state of the channel. These results suggested that the window currents were inhibited by Vin (See Fig 4). The window current is a component of sodium current in inactivating state[9], which can be depressed by TTX. Furthermore, the I_{Na} blocking of Vin is, in a voltage-dependent manner, and is more strong at potentials positive to the region where steady-state inactivation seems to have been completed.

There are increasingly evidences demonstrating that the drugs that block the sodium channel may exert a protective effect on neuron cells and cardiomyocytes as well[6,11,13]. An increase in intracellular free Na^+ concentration during the early stage of ischemia is very harmful because it may lead to Ca^{2+} overload. The voltage-gated sodium channel can indeed be a source of significant increases in $[Na]_i$ because the sodium current inactivation is often incompletely in a large voltage range in ischemic cardiac tissue[12]. So, our findings can offer further evidence that the inhibitory effect of Vin on slow inactivation component of sodium currents may exert direct cardioprotection when the heart suffers from ischemia.

In conclusion, Vin inhibited sodium current in isolated rat ventricular cells in a reversible manner. This effect was concentration-dependent and voltage-dependent, but not rate- or use-dependent. Furthermore, Vin blockage of both inactivation and activation course of sodium channel resulted in the attenuation of window current.

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长春西汀对大鼠心肌细胞钠电流的抑制作用[1]

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关键词 长春西汀；钠通道；河鲀毒素；心肌；膜片箝技术；长春花生物碱类

目的：研究长春西汀对心肌细胞钠电流的作用．方法：用全细胞膜片箝技术记录大鼠心肌细胞钠电流．结果：长春西汀可逆性抑制心肌细胞钠电流的作用为剂量依赖性和电压依赖性，但未发现频率或使用依赖性．长春西汀 10－80 μmol·L^{-1}，对钠电流的抑制作用为 13 %±2 %至 75 %±6 %．半数抑制浓度 IC_{50}值(95 %可信限)为 36.4 (28.1－47.1) μmol·L^{-1}．在膜电位以 10 mV 的间隔从－90 mV 阶梯状去极化至＋40 mV 时，抑制作用呈逐渐增加的趋势，约在 0 mV 左右达到最大抑制．长春西汀对钠通道的稳态激活和失活过程的影响，可使钠窗电流(缓慢失活的钠电流)减少．结论：长春西汀抑制大鼠心肌细胞的钠电流．

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Effects of moxonidine injected into rostral ventrolateral medulla on blood pressure, heart rate, and renal sympathetic nerve activity in rats

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KEY WORDS moxonidine; medulla oblongata; sympathetic nervous system; blood pressure; heart rate; pressoreceptors

AIM: To examine the effects of moxonidine (Mox) injected into the rostral ventrolateral medulla (RVLM) on blood pressure (BP), heart rate (HR), and the renal sympathetic nerve activity (RSNA) in anesthetized normotensive rats. **METHODS**: BP, HR, and RSNA were simultaneously recorded after 1 μL Mox 1, 10, and 100 μmol·L^{-1} was injected into RVLM. **RESULTS**: Mox 1, 10, and 100 μmol·L^{-1} reduced BP from 13.9 ± 1.0 kPa to 13.0 ± 1.7 kPa ($P<0.05$), 13.8 ± 1.8 kPa to 11.4 ± 1.5 kPa ($P<0.01$), and 13.9 ± 1.9 kPa to 9.4 ± 1.7 kPa ($P<0.01$), respectively. Mox did not influence HR. RSNA varied with the doses: Mox 1 μmol·L^{-1} increased RSNA by 50 % ($P<0.05$), 10 μmol·L^{-1} insignificantly influenced RSNA ($P>0.05$), and 100 μmol·L^{-1} reduced RSNA by 23 % ($P<0.05$). In sinoaortic barodenervated rats, Mox 10 μmol·L^{-1} inhibited RSNA by 50 % ($P<0.05$), which substantially differed from that in buffer nerve intact rats ($P<0.01$). **CONCLUSION**: Mox injected into RVLM decreased BP, but did not influence HR. The changes of RSNA did not parallel with the depressor effect of Mox.

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Moxonidine (Mox), an imidazoline, represents a new generation of clonidine-like centrally acting antihypertensive drugs, which acts via I_1-imidazoline receptor[1]. Mox injected into the rostral ventrolateral medulla (RVLM) decreased blood pressure (BP) and heart rate (HR) in conscious or anesthetized hypertensive rats[2-4]. Neurons in RVLM send excitatory input directly to the spinal sympathetic preganglionic neurons. It is assumed that Mox produces its depressor effect by decreasing peripheral sympathetic tone. But there was no report about the effect of Mox directly applied into the RVLM on sympathetic nerve outflow. The renal sympathetic nerve activity (RSNA) is mostly used to evaluate peripheral sympathetic tone. Intravenous injection of Mox in the doses inducing hypotension inhibited RSNA in anesthetized cats[5] and in conscious rabbits[6], but it was thought that the decrease of RSNA induced by iv Mox was due to its central action as well as peripheral presynaptic inhibition. The aim of the present study was to investigate the effects of injection of Mox into RVLM on BP, HR, and RSNA in anesthetized