BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; **13** (4) : 341-344

Effects of *m*-nisoldipine on transmembrane currents of guinea pig papillary muscles

AN Rui-Hai, FAN Zhen-Zhong, HE Rui-Rong (*Department of Physiology, Institute of Basic Medicine, Hebei Medical College, Shijiazhuang 050017, China*)

ABSTRACT Effects of *m*-Nis on the transmembrane currents were studied using single sucrose gap voltage clamp technique. The amplitude of slow inward current (I_{si}) was 10.6 ± 4.1 μA. Maximal inward current was induced at a membrane potential range between −20 to −25 mV. The amplitude of I_{si} were significantly decreased by *m*-Nis (0.2 μmol · L^{-1}) with a reduction of 47.3%. The transient inward current (I_{ti}) induced by ouabain was also greatly depressed or prevented by *m*-Nis, resulting in the inhibition of DAD.

KEY WORDS *m*-nisoldipine; papillary muscles; electrophysiology; membrane potentials

In our previous papers, the effects of *m*-nisoldipine (*m*-Nis) on the action potentials and delayed afterdepolarization (DAD) were observed in guinea pig papillary muscle, rabbit sinoatrial node and canine Purkinje's fiber[1-3]. In the present paper, the effects of *m*-Nis on the slow inward current (I_{si}) and transient inward current (I_{ti}) of guinea pig papillary muscle were studied to analyze the cellular mechanism by means of single sucrose gap voltage clamp technique.

MATERIALS AND METHODS

Tissue preparation Guinea pigs (either sex) weighing 0.25 ± *s* 0.1 kg were stunned by heavy blow on the head. The papillary muscles with diameter of 0.8 mm or less were carefully picked. The papillary muscle bundle was pulled through the tightly fitting holes in 2 rubber membranes which formed the S chamber between T and K chambers (*vide infra*). Less than 0.5 mm of the papillary muscles was exposed to Tyrode solution in order to ensure a spatially uniform clamp. The 3 compartments were first perfused with Tyrode solution equilibrated continuously with O_2 and maintained at 35 ± 0.5 ℃, allowing the preparation to recover from injury. After the action potential with normal configuration was recorded, the S and K chambers were then perfused with isotonic sucrose solution and high K^+ solution, respectively. The potential difference between T and K chambers (gap potential, E_g) was monitored with microelectrode amplifier (MEZ-8201). Voltage clamp experiment was made after the E_g reached −60 to −80 mV.

Voltage clamp circuit Membrane potential (E_m) was measured by microelectrode amplifier between an intracellular microelectrode inserted into the papillary muscle in the T chamber and a virtual electrode in the T chamber. E_m and the command signal (E_c) provided by the step pulse generator (SET-1100) were fed to the voltage clamp amplifier (CEZ-1200) for comparison. The controlling current from the CEZ-1200 was conducted to the K chamber to clamp the membrane potential to the command level (Fig 1). The

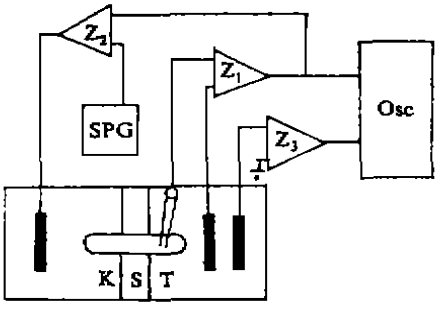

Fig 1. Perfusing bath and circuit used in single sucrose gap voltage clamp experiment on guinea pig papillary muscles. Z_1: microelectrode amplifier; Z_2: voltage clamp amplifier; Z_3: current-voltage converter; SPG: step pulse generator; OSC: storage oscilloscope; K: potassium chamber; S: sucrose chamber; T: test chamber.

membrane current (I_m) was measured by a current-voltage convertor in CEZ-1200. I_m and

Received 1991 Nov 28 Accepted 1992 May 4

E_m were monitored with a memory oscilloscope (VC-11) and fed to the microcomputer (Apple-Ⅱ) through the A/D convertor to be analyzed. The amplitude and duration of I_m and E_m were measured by the microcomputer automatically.

The inward currents were activated by a depolarizing step from a conditioning clamp (the holding potential, E_h). The E_h was set to −40 mV for inactivating the fast inward current (sodium current, I_{Na}), allowing to observe the I_{si} without interference from I_{Na}. The command signal was a deploarizing pulse (E_c −15 mV) with a duration of 500 ms. Stepwise pulses (from −35 to +10 mV) provided by step pulse generator were used as command signals to define the threshold potential and current-voltage relationship.

Induction of delayed afterdepolarization and I_{ti} To induce stable DAD and I_{ti}, the preparation in T chamber was perfused with Tyrode solution containing ouabain 0.8 μmol · L^{-1} for 30–40 min. After stable DAD had been induced, the voltage clamp experiment was started to evaluate the effects of *m*-Nis on I_{ti} which was responsible for the occurrence of DAD.

Solutions and drugs Modified Tyrode solution was prepared just before the experiment by mixing the stock solutions[4]. The composition of solvent and the sources of *m*-Nis were described previously[5]. *m*-Nis solution was prepared before each experiment from stock solution and kept away from intense light.

Results were expressed as $\bar{x} \pm s$. The changes in parameters after drug administration were analyzed using *t* test.

RESULTS

Identification of I_{si} While a depolarizing clamp was delivered (E_h −75 mV, E_c −25 mV), 2 inward currents were seen (Fig 2). The first inward current with amplitude of 60–80 μA, fast activation and inactivation appeared just after the depolarizing capacity current. This inward current was sensitive to change in Na^+ concentration and inactivated by tetrodotoxin (TTX, 10 μmol · L^{-1}) or by elevating the E_h to above −55 mV, and insensitive to *m*-Nis. These characteristics verified that this current was the inward sodium current (I_{Na}).

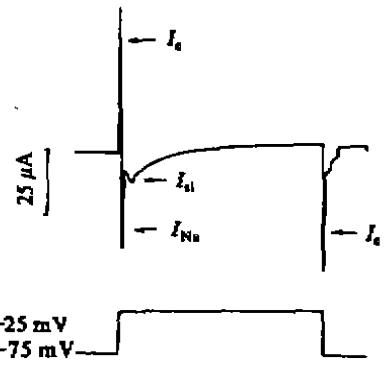

Fig 2. Currents during a depolarizing step in papillary muscles. I_c: capacity current; I_{si}: slow inward current; I_{Na}: sodium current.

The I_{Na} was followed by another inward current with low amplitude, slow activation, and inactivation (Fig 2). It was insensitive to TTX or depolarization (E_m up to −40 mV), but sensitive to $CoCl_2$ (2 mmol · L^{-1}) and to changes in Ca^{2+} concentration. These results indicated that the inward current is I_{si}.

Effects of *m*-Nis on I_{si} The I_{Na} was fully inactivated by a conditioning deploarization to −40 mV. Only I_{si} was induced under this depolarization level. In the 8 preparations of the control group, the threshold potential of I_{si} was −32.4 ± 5.1 mV measured by clamping the membrane to a train of stepwise pulses (pulse step was 1 mV). The amplitude of I_{si} was initially increased then gradually declined as the E_c was increased positively. The duration from the start of a depolarizing clamp to the peak of I_{si} was also gradually reduced as the depolarizing step increased (Fig 3). So long as the E_m increased to about 0 mV, the inward current reversed to the outward current. Maximal imward current was initiated at a E_c range between −20 and −25 mV (Fig 3).

After treatment with *m*-Nis (0.2 μmol · L^{-1}), the amplitude of I_{si} began to reduce in 10 min. This change reached its nadir at 20 min of perfusion with *m*-Nis, with a reduc-

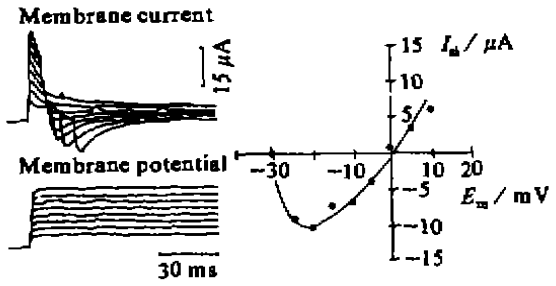

Fig 3. Current–voltage relationship of I_{si} in papillary muscles ($n=8$). Left: Multiple superimposed records of I_{si} with stepwise depolarizations from holding potential ($E_h=-40$ mV) to −30, −25, −20, −15, −10, −5, 0, +5 mV.

tion of 47.3% (from 10.6± 4.1 to 6.1± 4.1 μA, $n=8$, $P<0.05$). The duration of I_{si} was shortened and threshold potential was elevated to −28± 7 mV. The reduction in I_{si} induced by *m*–Nis was reversed by washing with normal Tyrode solution (Fig 3).

Effects of *m*–Nis on I_{ti} I_{ti}, as well as DAD, were not seen in any preparation perfused with Tyrode solution. After the preparation had been perfused with Tyrode solution containing ouabain for 30–40 min and stable DAD had been induced, I_{ti} was elicited upon repolarizing clamp from depolarization (Fig 4). The amplitude of I_{ti} was 4.8

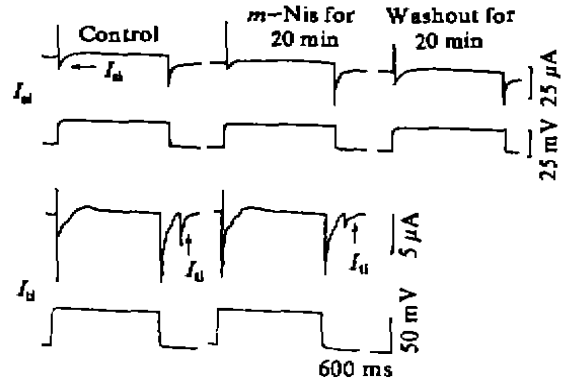

Fig 4. Effects of *m*–Nis 0.2 μmol · L^{-1} on I_{si} and I_{ti} in papillary muscles. Upper tracing: membrane current; Lower tracing: membrane potential.

± 2.1 μA with a duration of 412± 101 ms. The current was dependent on the Ca^{2+} concentration and was depressed by $CoCl_2$. After treatment with *m*–Nis (0.2 μmol · L^{-1}), the amplitude of I_{ti} was significantly reduced (from 4.8± 2.1 to 1.2± 1.1 μA, $n=12$, $P<0.01$) and disappeared in 2 preparations. Meanwhile, DAD was also markedly inhibited or prevented.

DISCUSSION

Single sucrose gap voltage clamp technique makes it possible to control the membrane potential and to measure the transmembrane currents at the same time, and it was suitable to observe the slow response I_{si}[6]. Two inward currents (I_{si} and I_{Na}) observed in our experiment were identified by applying specific channel blocker, changing Ca^{2+} or Na^{+} concentration in the perfusate or altering the holding potential of the conditioning clamp. These precedures ensured an accurate measurment of I_{si} without the interference from I_{Na}. The parameters and properties of I_{si} recorded in our experiment were consistent with those reported by other workers[7].

The amplitude of I_{si} was significantly depressed by treatment with *m*–Nis at dose that affected the cardiac electrophysiological parameters[1,2]. The inhibition in I_{si} was essentially due to a reduction in calcium influx by specific blocking action on the potential–dependent calcium channels. The results confirmed our previous assumption that the inhibitory effects of *m*–Nis on the depolarizing process of action potential in partially depolarized papillary muscle and sinoatrial node might be resulted from a specific blocking action on calcium influx.

In our previous paper, *m*–Nis showed great prophylactic and inhibitory effects on the occurrence of DAD[3]. The occurrence of DAD has been attributed to I_{ti} elicited by an abnormal rise in cytoplasmic Ca^{2+} concen–

tration[8]. In the present work, I_{ti}, as well as DAD, was not induced in Tyrode solution. However, it was readily elicited by ouabain. The fact that I_{ti} was sensitive to Ca^{2+} concentration in the perfusate and could be depressed by $CoCl_2$ indicated the occurrence of I_{ti} was dependent on the calcium influx.

I_{ti} was significantly depressed or prevented by *m*-Nis, resulting in a decrease of the amplitude of DAD. The blocking effects of *m*-Nis on the calcium influx and the resultant alleviation of cytoplasmic calcium overload may be the main mechanism underlying the inhibition of I_{ti} and DAD.

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间尼索地平对豚鼠乳头状肌跨膜电流的影响

安瑞海, 范振中, 何瑞荣 (河北医学院基础医学研究所生理室, 石家庄 050017, 中国)

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提要 利用单蔗糖间隙电压箝技术观察了间尼索地平(*m*-Nis)对豚鼠乳头状肌跨膜电流的影响. 慢内向电流(I_{si})幅值为 10.6 ± 4.1 μA, 最大 I_{si} 出现在膜电位为 -20 到 -25 mV 之间. *m*-Nis (0.2 μmol · L^{-1})能明显减小 I_{si} 的幅值和持续时间. *m*-Nis 对哇巴因诱发的短暂内向电流(I_{ti})也有显著的抑制作用, 使迟后除极化减小.

关键词 间尼索地平; 乳头状肌; 电生理学; 膜电位