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### Electrophysiological effects of *m*-nisoldipine and nisoldipine on pacemaker cells in sinoatrial node of rabbits

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**ABSTRACT** The effects of *m*-nisoldipine (*m*-Nis) and nisoldipine (Nis) on the electrical activity of pacemaker cells in sinoatrial node (SAN) of rabbit were studied using intracellular microelectrodes. The results: APA and  $V_{max}$  in SAN pacemaker cells were markedly reduced by *m*-Nis and Nis. The inhibitory effects of Nis on APA and  $V_{max}$  were greater than those of the *m*-Nis. Elevation of  $Ca^{2+}$  concentration in the perfusate partially antagonized the inhibitory actions of *m*-Nis and Nis. VDD of SAN pacemaker cells were reduced by *m*-Nis. The change of RPF was not consistent with that of VDD. CSRT was prolonged by *m*-Nis and Nis in a dose-dependent manner. The inhibitory effects of Nis on SAN pacemaker cells were greater than that of the *m*-Nis. Both MDP and  $APD_{50}$  were not affected by *m*-Nis and Nis.

**KEY WORDS** *m*-nisoldipine; nisoldipine; micro-electrodes; sinoatrial node; action potentials;

electrophysiology

*m*-Nis, a new dihydropyridine calcium channel blocker<sup>(1)</sup>, remarkably affected action potentials (AP) in normal and partially depolarized papillary muscles of guinea pig, through its blocking action on voltage-dependent  $Ca^{2+}$  channels<sup>(2)</sup>. *m*-Nis also showed inhibitory effects on ischemic arrhythmias in rats<sup>(3)</sup>. However, the electro-physiological effects of *m*-Nis on pacemaker cells in sinoatrial node (SAN) have not yet been studied. In this article, the effects of *m*-Nis and Nis on transmembrane potentials of pacemaker cells and corrected SA recovery time (CSRT) in SAN of rabbits were studied with intracellular microelectrodes.

#### MATERIALS AND METHODS

Rabbits weighing  $2.2 \pm SD 0.4$  kg (both sexes) were stunned by heavy blow on the

head. The SAN preparation was excised from the right atrium<sup>(4)</sup>, and fixed to the silicon rubber placed on the bottom of perfusing chamber by stainless steel needles. The preparation was perfused with modified Krebs-Henseleit solution (K-H solution) at a flow rate of  $4 \text{ ml} \cdot \text{min}^{-1}$ . The perfusate maintained at  $35^\circ\text{C}$  was continuously equilibrated with  $95\% \text{O}_2 + 5\% \text{CO}_2$ .

The experiment began after the SAN preparation had been equilibrated for at least 30 min. APs were recorded from dominant pacemaker cells in SAN. The criteria to identify dominant pacemaker cells had been described by Brown<sup>(4)</sup>. After recording 3 control APs, the preparation was perfused with K-H solution or high  $\text{Ca}^{2+}$  ( $5 \text{ mmol} \cdot \text{L}^{-1}$ ) K-H solution containing  $0.2 - 1 \mu\text{mol} \cdot \text{L}^{-1}$  *m*-Nis and Nis. The solvent and resources of *m*-Nis and Nis were previously described<sup>(1)</sup>. The time required to change the perfusate was within 1 min. The AP was recorded at 5, 10, 20 min after application of *m*-Nis or Nis. The preparation was washed with K-H solution to observe the recovery of AP.

The glass microelectrode was inserted into the SAN pacemaker cells to record the electrical signal intracellularly. The signal after amplifier (MEZ-8201) was monitored with a memory oscilloscope (VC-11). The micro-computer (APPLE-II) was used to process the amplified signals.

Maximal diastolic potential (MDP), amplitude of action potentials (APA), duration of 50% repolarization ( $\text{APD}_{50}$ ), maximal rate of depolarization ( $V_{\text{max}}$ ), rate of pacemaker firing (RPF), and velocity of diastolic (phase 4) depolarization (VDD) were calculated by the computer. Parameters of AP were stored into diskette.

CSRT was defined by the computer using a program designed by our department. AP signals of SAN pacemaker cells amplified by oscilloscope were fed to the A/D convertor

and RPF was calculated. The overdriving stimuli for SAN preparation (frequency 120% RPF, duration 2 ms, intensity 1.2 times the threshold) were provided by the stimulator (SEN-3201) controlled by computer. The stimulation lasted for 1 min. Sampling began as soon as the stimulation ended. SAN recovery time (SRT) and CSRT were calculated and printed<sup>(5)</sup>.

The protocol includes 3 parts of experiment: (1) Effects of *m*-Nis and Nis on SAN pacemaker cells. The animals were divided into 5 groups: control, *m*-Nis  $0.2 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $0.5 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $1 \mu\text{mol} \cdot \text{L}^{-1}$  and Nis  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ; (2) The effects of high  $\text{Ca}^{2+}$  on inhibitory actions of *m*-Nis and Nis; (3) The effects of *m*-Nis and Nis on CSRT.

The changes in parameters of AP expressed as mean  $\pm$  SD were analyzed using *t* test (one way). Differences among groups were tested using *F* test.

## RESULTS

**Effects of *m*-Nis on automaticity of SAN** RPF of control group was  $126 \pm 12$  bpm. The changes in RPF induced by *m*-Nis were varied with the concentrations used. RPF was increased from 126 to 135 bpm by *m*-Nis at low concentration ( $0.2 \mu\text{mol} \cdot \text{L}^{-1}$ ), but was markedly decreased from 126 to 91 bpm at high concentration ( $1 \mu\text{mol} \cdot \text{L}^{-1}$ ) and insignificantly changed at moderate concentration ( $0.5 \mu\text{mol} \cdot \text{L}^{-1}$ ). VDD of control group was  $52 \pm 6 \text{ mV} \cdot \text{s}^{-1}$ .

At the 10th min of perfusion with K-H solution containing *m*-Nis, VDD began to decrease. Fifteen min after medication, VDD of any group perfused with *m*-Nis-containing K-H solution was greatly depressed. The values of VDD at concentrations of 0.2, 0.5 and  $1 \mu\text{mol} \cdot \text{L}^{-1}$  were 34, 38 and  $26 \text{ mV} \cdot \text{s}^{-1}$ , respectively. The changes in VDD were not parallel to that of RPF. Elevation of  $\text{Ca}^{2+}$  concentration in perfusate partially antagonized the inhibitory action of *m*-Nis and

**Tab 1. Effects of *m*-nisoldipine (*m*-Nis) and nisoldipine (Nis) on transmembrane potentials of pacemaker cells and CSRT in SAN of rabbit. *n* = 10,  $\bar{x} \pm \text{SD}$ . \**P* > 0.05, \*\**P* < 0.05, \*\*\**P* < 0.01 vs control. †*P* > 0.05, ††*P* < 0.05, †††*P* < 0.01 vs *m*-Nis at equal dose.**

Group	Dose ( $\mu\text{mol} \cdot \text{L}^{-1}$ )	MDP (mV)	APA (mV)	$V_{\text{max}}$ ( $\text{V} \cdot \text{s}^{-1}$ )	VDD ( $\text{mV} \cdot \text{s}^{-1}$ )	RPF (bpm)	APD <sub>50</sub> (ms)	CSRT (ms)
Control		-50 ± 6	61 ± 6	10.1 ± 3	52 ± 6	126 ± 12	121 ± 16	117 ± 21
<i>m</i> -Nis	0.2	-51 ± 5*	57 ± 5*	6.5 ± 3*	34 ± 8***	135 ± 26*	131 ± 21*	136 ± 19***
	0.5	-49 ± 7*	51 ± 4**	6.1 ± 4**	38 ± 6*	129 ± 15*	121 ± 19*	143 ± 17**
	1.0	-54 ± 7*	47 ± 13***	6.0 ± 4**	26 ± 7**	91 ± 15***	120 ± 17*	161 ± 21***
Nis	0.5	-46 ± 5**†	47 ± 3***†	4.0 ± 2***†	36 ± 6***†	108 ± 21***†	128 ± 17*†	162 ± 27***†
<i>m</i> -Nis (High Ca <sup>2+</sup> )	0.5	-50 ± 5*	57 ± 4*	8.1 ± 3*	42 ± 5*	131 ± 17*	118 ± 16*	120 ± 13*
Nis (High Ca <sup>2+</sup> )	0.5	-52 ± 5*	55 ± 8*	7.4 ± 3*	43 ± 4*	120 ± 15*	121 ± 19*	129 ± 16*

Nis (Tab 1). The inhibitory effects of Nis on RPF was greater than that of *m*-Nis at 0.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , but there was no significant difference in the effects on VDD.

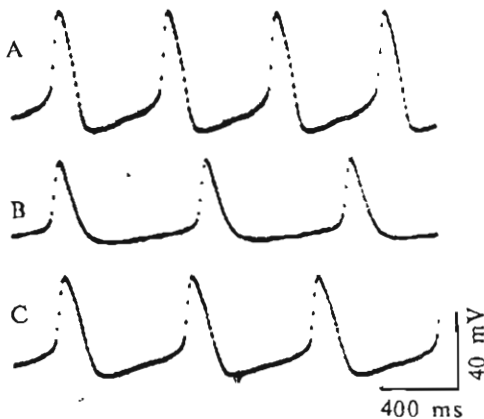
**Effects of *m*-Nis on transmembrane potentials of pacemaker cells in SAN** *m*-Nis showed no effect on APA and  $V_{\text{max}}$  at 0.2  $\mu\text{mol} \cdot \text{L}^{-1}$ . However, the depolarization process was obviously depressed by *m*-Nis at 0.5 and 1  $\mu\text{mol} \cdot \text{L}^{-1}$ . Both the rate and amplitude of depolarization were decreased in a dose dependent manner: APA was reduced by 19%, and  $V_{\text{max}}$  by 40%. Elevation of Ca<sup>2+</sup> concentration in perfusate partially

antagonized the inhibitory action (Fig 1). *m*-Nis at any concentration used had no effect on repolarization of the cells, as evidenced by no detectable change in APD<sub>50</sub> (Tab 1). Similar to *m*-Nis, Nis exerted no effect on MDP and APD<sub>50</sub>.

**Effects of *m*-Nis on CSRT** For control group, CSRT was 117 ± 21 ms. At the concentrations of 0.2, 0.5 and 1.0  $\mu\text{mol} \cdot \text{L}^{-1}$  of *m*-Nis, CSRT was increased dose-dependently by 17%, 23% and 38%. Elevation of calcium concentration in perfusate attenuated the changes induced by *m*-Nis and Nis (Tab 1).

**DISCUSSION**

*m*-Nis and Nis greatly reduced VDD, which depended on the calcium influx in SAN pacemaker cells<sup>(6)</sup>. Elevation of calcium concentration in perfusate partially antagonized the inhibitory effects of *m*-Nis on VDD. The inhibitory effects of *m*-Nis and Nis on VDD might be attributed to blocking action on calcium influx. The inhibitory effect of *m*-Nis on RPF depended on the doses used. This characteristic of action was described elsewhere<sup>(7)</sup>. It is well known that the RPF is determined by VDD, level of threshold and MDP. Based on our result that MDP remained unchanged under the action of *m*-Nis, the change in RPF might be due to variations



**Fig 1 Effects of *m*-nisoldipine (*m*-Nis) and elevation of calcium concentration in perfusate on transmembrane potentials of SAN pacemaker cells of rabbit. A) control, B) *m*-Nis 0.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , C) CaCl<sub>2</sub> 5  $\mu\text{mol} \cdot \text{L}^{-1}$ .**

of VDD or / and threshold potential.

APA and  $V_{\max}$  in SAN pacemaker cells were greatly reduced by  $m$ -Nis. In some preparations, APs were abolished by  $m$ -Nis. The results showed that depolarization process was markedly affected by  $m$ -Nis. Elevation of calcium concentration in perfusate increased APA and  $V_{\max}$ . Therefore, the inhibitory effects on APA and  $V_{\max}$  in SAN might be resulted from the blockade of calcium influx responsible for depolarization.

APD<sub>50</sub> in SAN pacemaker cells was not changed by  $m$ -Nis and Nis, thereby suggesting that repolarization process of SAN cells were not sensitive to  $m$ -Nis and Nis. It probably related to the fact that only limited calcium influx involved in the repolarization phase of SAN pacemaker cells, in contrast to that of papillary muscles. These results also confirm that  $m$ -Nis and Nis selectively act on calcium current and have no effect on potassium current<sup>(8)</sup>.

CSRT was often determined to reflect the function of pacemaker in SAN<sup>(9)</sup>, and it was defined as the time required for the SAN to recover its automaticity after overdriving<sup>(5)</sup>. The pacemaker function of SAN was significantly depressed by  $m$ -Nis and Nis, so that the prolonged CSRT was observed. The depression of automaticity may be due to  $m$ -Nis-induced changes in intracellular calcium concentration.

The characteristics of electrophysiological effects of Nis on SAN pacemaking cells were essentially similar to that of  $m$ -Nis. But the inhibitory effects of Nis on APA, RPF and CSRT were stronger than that of  $m$ -Nis at equal dose.

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## 间尼索地平 and 尼索地平对兔窦房结起搏细胞的电生理效应

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**提要** 利用细胞内微电极技术, 观察间尼索地平 ( $m$ -Nis) 和尼索地平 (Nis) 对兔 SAN 起搏细胞电活动的影响, 结果:  $m$ -Nis 和 Nis 可显著抑制 SAN 起搏细胞 APA 和  $V_{\max}$ , 提高灌流液中  $Ca^{2+}$  浓度可对抗其抑制作用;  $m$ -Nis 和 Nis 使 SAN 起搏细胞 VDD 降低, RPF 的变化与 VDD 改变不一致;  $m$ -Nis 和 Nis 显著延长 CSRT, 此效应有明显的剂量依赖性, Nis 对 SAN 起搏细胞的抑制作用强于等剂量  $m$ -Nis;  $m$ -Nis 和 Nis 对 MDP 和 APD<sub>50</sub> 无影响。

**关键词** 间尼索地平; 尼索地平; 微电极; 窦房结; 动作电位; 电生理学