have been ascertained.

REFERENCES

1 Yin HQ. Neuropeptide Y. *Prog Physiol Sci* 1986; **17** : 270–2.
2 Edvinsson L, Ha˚kanson R, Wahlestedt C, Uddman R. Effects of neuropeptide Y on the cardiovascular system. *Trends Pharmacol Sci* 1987; **8** : 231–5.
3 de Quidt ME, Emson PC. Distribution of neuropeptide Y–like immunoreactivity in the rat central nervous system–II: immunohistochemical analysis. *Neuroscience* 1986; **18** : 545–618.
4 Giardino L, Calza L, Zanni M, Parchi P, Battistini N, Marrama P. Iodinated–NPY binding sites: autoradiographic study in the rat brain. *Neuropeptides* 1989; **13** : 23–8.
5 Ruit KG, Neafsey EJ. Cardiovascular and respiratory responses to electrical and chemical stimulation of the hippocampus in anesthetized and awake rats. *Brain Res* 1988; **457** : 310–21.
6 Miyazawa TM, Gelsema AJ, Calaresu FR. Septal neurons respond to activation of baro– and chemoreceptors in the rat. *Am J Physiol* 1988; **254** : R331–7.
7 Gelsema AJ, Calaresu FR. Chemical microstimulation of the septal area lowers arterial pressure in the rat. *Am J Physiol* 1987; **252** : R760–7.
8 Calaresu FR, Mogenson GJ. Cardiovascular responses to electrical stimulation of the septum in the rat. *Am J Physiol* 1972; **223** : 777–82.
9 Li HL, Wang Q, Wang Q, Gu YH. Functional relationship between pressor effect of substantia nigra and depressor effect of nucleus arcuatus hypothalami. *Acta Physiol Sin* 1988; **40** : 28–35.

大鼠脑内注射神经肽 Y 的心血管效应

杨绍年、杨 蔚、唐毓环、王 绍 (白求恩医科大学生理教研室，长春 130021，中国)

提要 麻醉大鼠海马 CA3 区(CA3)内微量注射神经肽 Y(NPY)引起剂量依赖性血压下降和心率减慢. 外侧隔核(LSN)内微量注射 NPY 引起血压升高和心率加快，其中血压升高是剂量依赖性的，但心率加快则没有剂量依赖性. 黑质(SN)内微量注射 NPY 引起剂量依赖性血压下降，但心率在统计学上无明显变化. 结果表明，外源性 NPY 在 CA3，LSN 和 SN 有明显的心血管效应.

关键词 神经肽 Y; 血压; 心率; 海马; 隔核; 黑质

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Mar; **13** (2) : 118–122

Rate– and voltage–dependent effects of *m*–nisoldipine on action potential of partially depolarized guinea pig papillary muscle

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

ABSTRACT The rate– and voltage–dependent effects of *m*–Nis were studied using standard microelectrode technique and real–time microcomputer analyzing system. The onset rate for rate–dependent inhibition (RDI) on action potentials of partially depolarized papillary muscle of guinea pig was accelerated as the concentration of *m*–Nis was increased from 0.5 to 2 $\mu mol \cdot L^{-1}$ or the driving frequency decreased from 0.8 to 0.2 Hz. The steady–state values of V_{max} and APA were markedly decreased by elevating the concentration of *m*–Nis or increasing the driving frequency. The recovery time constants of V_{max}, APA, and latency period from RDI were all increased by *m*–Nis (1 $\mu mol \cdot L^{-1}$). The inhibitory

Received 1990 Nov 7 Accepted 1991 Dec 12

effects of *m*–Nis on V_{max} and APA were more pronounced as the resting membrane potential was decreased by elevating K^+ concentration in the perfusate.

KEY WORDS *m*–nisoldipine; papillary muscles; action potentials; microelectrodes

m–Nisoldipine (*m*–Nis) is a new dihydropyridine calcium channel blocker[1]. Our previous paper reported that *m*–Nis exerted significant inhibitory effects on action potentials (AP) in normal and partially depolarized papillary muscle[2]. This study was undertaken to further elucidate the characteristics of its inhibitory effects.

MATERIALS AND METHODS

Guinea pigs weighing 0.41 ± *s* 0.11 kg (either sex) were used. The methods of preparation and perfusion of papillary muscle were described previously[2]. The preparation was allowed to be equilibrated in the modified K–H solution[3] containing KCl 18 mmol · L^{-1} for at least 30 min before the experiment.

The papillary muscle preparations were driven by pulses (duration 1 ms, 1.5 times the threshold intensity) provided by electronic stimulator (SEN–3201) through a bipolar electrode. The transmembrane potential was fed to the microelectrode amplifier (MEZ–8201) and monitored with a memory oscilloscope (VC–11). AP signal was collected from VC–11 synchronously by microcomputer (APPLE–II) at a rate of 12 bits / 60 μs. Using a program designed in our department[4], parameters such as amplitude of action potential (APA) and slow component of maximal rate of depolarization in phase 0 (V_{max}) were calculated automatically. Parameters of AP were conveyed to a statistics program to be fitted to different curves, and a best function was selected.

Onset dynamics of rate–dependent effects The papillary muscle was kept in resting state for at least 2 min before each test, then it was provided a 32–pulse train with the stimulator controlled by the microcomputer. The stimulation frequencies were 0.2, 0.5, and 0.8 Hz, respectively. AP trains were collected and analyzed by the microcomputer. V_{max} and APA were decreased in a single exponential curve as the AP were repeatedly induced. The onset rate was calculated by fitting the standard values of V_{max} and APA with the number of the AP repeated[5].

Offset dynamics from the rate–dependent inhibition To test the offset course from rate–dependent inhibition, the preparation was driven by a 20–pulse train (cycle length 1800 ms) after 2 min of rest. A beat–to–beat decrease in V_{max} and APA were produced and the steady state reached. Then, a test stimulus was applied at various coupling intervals following a stimulation train. The first AP signal of the train and the premature AP induced by test stimulus were collected and analyzed by microcomputer. The recovery time constants of V_{max}, APA and latency period of the AP were calculated by fitting the standard values with the coupling interval to a single exponential function[5].

Voltage–dependent effects of *m*–Nis The preparations were stimulated with low–frequency pulse (cycle length 2 s) to eliminate the rate–dependent effects of *m*–Nis. The control perfusate was replaced by K–H solution containing different concentrations of K^+ (from 4.7 to 35 μmol · L^{-1}) after 30 min of equilibration. The resting membrane potential varied in the range of −30 to −70 mV. The AP signal was collected and analyzed by microcomputer after the preparation was equilibrated in the high K^+ perfusate for 15 min. The influence of resting potential level upon the inhibitory action of *m*–Nis on AP

was analyzed by linear regression.

Statistics Data were expressed as $\bar{x} \pm s$. Differences among groups were tested using F test. The time courses of onset and offset of rate-dependent effects were determined using a least-square exponential fitting routine.

RESULTS

The guinea pig papillary muscles were depolarized to -53 ± 4 mV after 30 min of perfusion with high K^+ K-H solution. APA was 87 ± 5 mV and V_{max} was 25 ± 2.3 V / s. These results are consistent with that of our previous paper[2].

Onset dynamics of rate-dependent effects of *m*-Nis V_{max} and APA in the control group were only slightly reduced as the preparation was activated repetitively by a train of stimuli at the rate of 0.5 and 0.8 Hz ($P > 0.05$, Fig 1). In the *m*-Nis (0.5, 1, and 2 μmol · L^{-1}) treated groups, V_{max} and APA of the first AP were reduced *vs* those of the control groups (−8.1%, −22%, and −36.2% for V_{max}; −8.6%, −18.1%, and −27.6% for APA). These reductions in V_{max} and APA were considered as the tonic inhibitory effects.

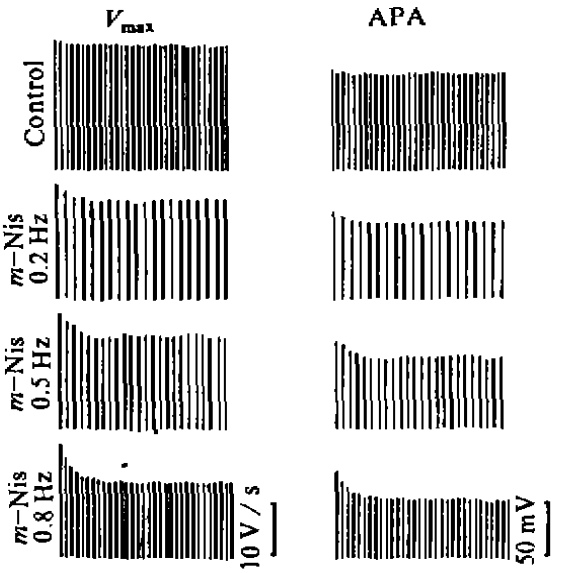

Fig 1. V_{max} **and APA induced by rate-dependent inhibition by *m*-nisoldipine 1 μmol · L^{-1} on partially depolarized guinea pig papillary muscle.**

Rate-dependent inhibition of *m*-Nis was seen while the preparation was driven by a train of stimuli at a rate of 0.2, 0.5, or 0.8 Hz. V_{max} and APA were gradually reduced and finally reached a steady state as the AP were induced repeatedly (Fig 1). The standard values of V_{max} and APA were fitted to the following function:

$$Y = B + A \cdot \exp(-n / \tau)$$

$1 / \tau$ was defined as the rate of onset. The results indicated that the rate of onset for rate-dependent inhibition was accelerated as the concentration of *m*-Nis was increased from 0.5 to 2 μmol · L^{-1} or the driving frequency decreased from 0.8 to 0.2 Hz. (Tab 1).

Effects of *m*-Nis on steady-state level of V_{max} and APA The level of V_{max} and APA reached a steady state after about 10 AP while the preparation was driven by a train of stimuli. The steady-state V_{max} and APA were significantly decreased *vs* those of the first AP in the train, and decreased more prominently as the concentration of *m*-Nis or the rate of stimulation increased ($n = 10$, $P < 0.01$, Fig 2).

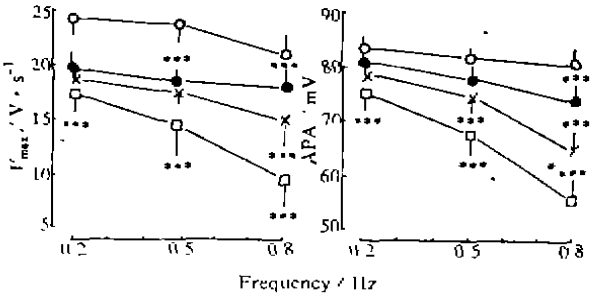

Fig 2. Effects of *m*-nisoldipine on steady-state V_{max} and APA of partially depolarized guinea pig papillary muscle. $n = 10$, $\bar{x} \pm s$ **Control (○), m-Nis 0.5 (●), 1 (×), and 2 μmol · L^{-1} (□)** $^{***}P < 0.01$ ***vs* control.**

Tab 1. Effects of *m*-nisoldipine on onset rate of rate-dependent inhibition for V_{max} and APA induced by stimuli of different frequency on partially depolarized guinea pig papillary muscle. $n = 10$, $\bar{x} \pm s$, $^{***}P < 0.01$ *vs* **0.5** μmol · L^{-1} **group,** $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* **0.2 Hz group.**

	Frequency / Hz	*m*-nisoldipine / μmol · L^{-1} 0.5	1.0	2.0
ORV_{max} / AP^{-1}	0.2	0.049 ± 0.011	$0.016 \pm 0.023^{***}$	$0.17 \pm 0.03^{***}$
	0.5	$0.021 \pm 0.011^{+++}$	$0.153 \pm 0.025^{***+}$	$0.160 \pm 0.03^{***++}$
	0.8	$0.020 \pm 0.015^{+++}$	$0.13 \pm 0.03^{***+++}$	$0.111 \pm 0.021^{***++}$
ORAPA / AP^{-1}	0.2	0.060 ± 0.021	$0.16 \pm 0.03^{***}$	$0.19 \pm 0.03^{***}$
	0.5	$0.016 \pm 0.017^{++}$	$0.13 \pm 0.03^{***+++}$	$0.15 \pm 0.035^{***++}$
	0.8	$0.010 \pm 0.014^{++}$	$0.13 \pm 0.04^{***+++}$	$0.14 \pm 0.03^{***+++}$

ORV_{max}: onset rate of rate-dependent inhibition of V_{max}
ORAPA: onset rate of rate-dependent inhibition of APA

Offset dynamics of rate-dependent effects of *m*-Nis The recovery of solvent control group from rate-dependent inhibition was very fast, with time constants of 513, 473, and 476 ms for V_{max}, APA, and latency period, respectively. *m*-Nis 1 μmol : L^{-1} greatly depressed the recovery time course from rate-dependent inhibition. Under the action of *m*-Nis (1 μmol · L^{-1}), the time constants of recovery of V_{max}, APA, and latency period were prolonged to 33.86, 13.72, and 13.19 s, respectively ($P < 0.01$),

Voltage-dependent inhibitory effects of *m*-Nis on AP While the resting membrane potential of papillary muscle was lowered from −70 to −30 mV by elevating the KCl concentration in the perfusate, the inhibitory effects of *m*-Nis (1 μmol · L^{-1}) on V_{maxs} and APA were progressively enhanced (from −16% to −57% for V_{maxs}, from −7.8% to −15.7% for APA). A good correlation existed between V_{maxs} and resting potential as well as between APA and RP ($r = -0.93$, $P < 0.01$ for V_{maxs}; $r = -0.89$, $P < 0.01$ for APA).

DISCUSSION

Rate- and voltage-dependency are common characteristics of the electrophysiological effects of calcium channel blockers[6]. Both verapamil and diltiazem are rate-dependent[7], while dihydropyridines less rate-dependent[8].

In the *m*-Nis treated partially depolarized papillary muscle, the steady- state values of V_{max} and APA were more intensely depressed, while the rate of stimulation and the concentration of *m*-Nis increased. The results indicated that the inhibitory effects of *m*-Nis on the AP of partially depolarized papillary muscle were to a certain extent dependent on the stimulation frequency. The results of onset dynamics analysis revealed that the rate of onset was accelerated as stimulation frequency was decreased or the concentration of *m*-Nis increased. It had been reported that the affinity between calcium channel blocker and calcium channel was modulated by the state of calcium channel and / or the membrane potential[9]. Dihydropyridines have a higher affinity for the receptor of an activated or inactivated calcium channel than for that of a resting channel[10]. This property could result in an increased binding of *m*-Nis with the activated and inactivated

calcium channel while the preparation was activated repetitively. The high concentration of *m*-Nis and high rate of stimulation could greatly enhance the binding, thus resulting in a slower reactivation or partial reactivation.

The present results also indicated the voltage-dependent inhibitory effects of *m*-Nis on AP of papillary muscle. This was consistent with the concept that the affinity of the calcium channel blockers with the channel was modulated by the resting potential[8]. Moreover, the slow recovery of calcium channel from previous activation at a lower resting potential was also in accordance with the voltage-dependent property.

Based on the characteristics of rate-dependency, *m*-Nis might have a stronger inhibition on tachyarrhythmias. Furthermore, by the voltage-dependent inhibition, *m*-Nis might act selectively on the partially depolarized myocardial cells caused by ischemia.

REFERENCES

1 An RH, Fu SX, Li YS. Effects of *m*-nisoldipine on ischemic arrhythmia in conscious rats. *Acta Pharmacol Sin* 1989; **10** : 151-6.

2 An RH, He RR. Electrophysiological effects of *m*-nisoldipine and nisoldipine on papillary muscles of guinea pig. *Acta Pharmacol Sin* 1990; **11** : 310-4.

3 Neely JR, Roverto MJ. Techniques for perfusing isolated rat hearts. *Methods Enzymol* 1975; **39** : 43-60.

4 Fan ZZ, An RH, He RR. System of sampling and processing cardiac transmembrane potential by microcomputer. *Chin J Phys Med* 1991; **13** : 39-42.

5 Kuang Y, Liu TP. Rate-dependent inhibitory effect of class I antiarrhythmic drugs. *Prog Physiol Sci* 1989; **20** : 307-12.

6 Sperelakis N. Electrophysiology of calcium antagonist. *J Mol Cell Cardiol* 1987; **19** Suppl 2 : 19-48.

7 Opie LH, Thandroyen FT, Muller CA, Hamm CW. Calcium channel antagonists as anti-arrhythmic agents: contrasting properties of verapamil and diltiazem versus nifedipine. In: Opie LH, editor. *Perspectives in cardiovascular research*; vol 9. NY: Raven Press 1984 : 303-11.

8 Molyvdas PA, Sperelakis N. Comparison of the effects of several calcium antagonistic drugs on the electrical activity of guinea pig purkinje fibers. *Eur J Pharmacol* 1983; **88** : 205-14.

9 Andersson K-E, Hogestatt ED. On the mechanism of action of calcium antagonists. *Acta Med Scand* 1984; Suppl 681 : 11-24.

10 Kanaya S, Arlock P, Katzung BG, Hondeghem LM. Diltiazem and verapamil preferentially block inactivated cardiac calcium channels. *J Mol Cell Cardiol* 1983; **15** : 145-8.

118-122

间尼索地平对部分除极化豚鼠乳头状肌动作电位的频率和电压依赖性抑制作用

安瑞海、何瑞荣 (河北医学院基础医学研究所生理室，石家庄 050017，中国) R965.1

提要 用微电极技术观察到随间尼索地平(*m*-Nis)浓度增高(0.5 至 2 μmol · L^{-1})和刺激频率减慢(0.8 至 0.2 Hz)频率依赖性抑制(RDI)的启动速率增大；APA 和 V_{max} 的稳态值随 *m*-Nis 浓度加大和刺激频率的加快而降低；*m*-Nis 使 V_{max}、APA 和 AP 潜伏期在 RDI 后的恢复时间常数明显增大；提高 K^+浓度使静息电位除极化到-30 mV，可明显增加 *m*-Nis 对 V_{max} 和 APA 的抑制作用。

关键词 间尼索地平；乳头状肌；动作电位；微电极