

- 3 Wilhelmsen L, Sweden G. Coronary heart disease: Epidemiology of smoking and intervention studies of smoking. *Am Heart J* 1988; 115 : 242
- 4 Du FC, Wang JL, Ma WZ. Matched investigation on the relationship between heavy smoking and coronary heart disease. *Chin J Cardiol* 1982; 10 : 81
- 5 Kannel WB. Update on the role of cigarette smoking in coronary artery disease. *Am Heart J* 1981; 101 : 319
- 6 Bekheit S, Fletcher E. The effects of smoking on myocardial conduction on the human heart. *Ibid* 1976; 91 : 712
- 7 Deng QG, Chen PY, Mu LH. Effect of smoking on left ventricular performance. *Chin J Cardiol* 1985; 13 : 197
- 8 Burn JH, Rand MJ. Action of nicotine on the heart. *Br Med J* 1985; 18 : 137
- 9 Dong LM, Sperelakis N, Wahler GM. Development of physiological responses to acetylcholine during organ culture of young embryonic chick hearts. *Dev Physiol J* 1986; 8 : 307

* * * * *

中国药理学报 *Acta Pharmacologica Sinica* 1989 Jul; 10 (4) : 328-331**甲基莲心碱对兔窦房结和培养的乳鼠心肌细胞跨膜电位的影响**李贵荣、李孝光¹、吕富华(同济医科大学药理教研室, 武汉 430033; ¹西安医科大学电生理研究室, 西安 710033, 中国)**Effects of neferine on transmembrane potential in rabbit sinoatrial nodes and clusters of cultured myocardial cells from neonatal rats**LI Gui-Rong, LI Xiao-Guang¹, LU Fu-Hua(Department of Pharmacology, Tongji Medical University, Wuhan 430033; ¹Research Laboratory of Electrophysiology, Xi-an Medical University, Xi-an 710033, China)

ABSTRACT Neferine (Nef), a bis-benzyl-isoquinoline alkaloid first isolated from the seed embryo of *Nelumbo nucifera* G in China, possesses an anti-arrhythmic action. The effects of Nef on the transmembrane potential were studied in rabbit sinoatrial nodes and the clusters of cultured cardiac myocytes from neonatal rats.

Nef 30 $\mu\text{mol/L}$ suppressed the amplitude of action potential (APA) from 57 ± 5 to 42 ± 4 mV, and the maximal upstroke velocity (\dot{V}_{max}) from 1.7 ± 0.3 to 0.9 ± 0.4 V/s ($n=5$, $P<0.01$) in rabbit sinoatrial nodes. Nef ($40 \mu\text{mol/L}$) decreased the APA from 92 ± 8 mV of control to 80 ± 4

mV, and \dot{V}_{max} from 20 ± 5 to 12 ± 4 V/s ($P<0.01$) in the clusters of cultured cardiac myocytes from neonatal rats. The effects on APA and \dot{V}_{max} were concentration-dependent. The results indicate that Nef has an inhibitory effect on the slow transmembrane Na^+ and/or Ca^{2+} current of myocardium.

KEY WORDS neferine; sinoatrial node; cultured myocardial cells; action potentials

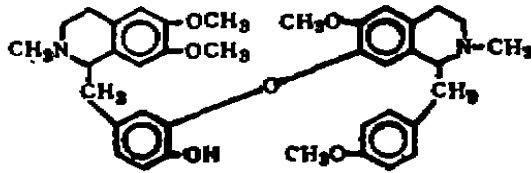
摘要 甲基莲心碱 (Nef) $30 \mu\text{mol/L}$ 给药 30 min 时, 兔窦房结跨膜电位 APA 及 \dot{V}_{max} 从给药前的 57 ± 5 mV 和 1.7 ± 0.3 V/s 抑制到 42 ± 4 mV 和 0.9 ± 0.4 V/s. $40 \mu\text{mol/L}$ 亦可使培养的乳鼠心肌细胞跨膜电位 APA, \dot{V}_{max} 及 MDP 从对照的 92 ± 8 mV, 20 ± 5 V/s 和 -66 ± 8 mV 抑制到 80 ± 4 mV, 12 ± 4 V/s 和

Received 1988 Feb 10 Accepted 1989 Jan 23

-59 ± 7 mV. 对 \dot{V}_{max} 和 MDP 为浓度依赖性抑制, 结果表明 Nef 对心肌跨膜慢 Na^+ 和/或 Ca^{2+} 内流具有抑制作用。

关键词 甲基莲心碱; 窦房结; 培养心肌细胞; 动作电位

甲基莲心碱 (neferine, Nef) 是从睡莲科植物莲 *Nelumbo nucifera* G 的成熟种子的胚芽中提出的一种双苄基异喹啉类生物碱。Nef 具有较广泛的抗心律失常作用^(1,2)。抗心律失常作用可能与其降低心肌自律性、兴奋性及延长不应期有关⁽³⁾。电生理研究证明其作用可能与非特异性抑制跨膜 Na^+ , K^+ , Ca^{2+} 的转运有关⁽⁴⁾。本文在离体兔窦房结和培养的新生乳鼠心肌细胞, 观察 Nef 对跨膜电位的影响, 以探讨 Nef 抗心律失常机理。



Neferine

METHODS

兔窦房结 兔体重 $2.0 \pm \text{SD } 0.4$ kg, 击后脑致昏, 取心脏, 浸入氧饱和的 Tyrode 液 (含 Tris 5 mmol/L) 中, 分离窦房结组织^(5,6), 将组织块内膜朝上, 用不锈钢针将其固定在 3 ml 浴槽底部硅橡胶上, 标本用循环的 Tyrode 液 ($36 \pm 0.5^\circ\text{C}$) 灌液, 通 100% O_2 , 用玻璃微电极 (充 KCl 3 mmol/L, 电阻 20-30 M Ω) 记录窦房结优势细胞跨膜电位。

培养乳鼠心肌细胞 新生 2-3 d Wistar 乳鼠, 常规消毒皮肤, 在超净台中剪取心室, 置于 Eagle 培养基中, 洗去积血, 剪碎, 用 0.06% 胰蛋白酶溶液 12 ml, 在磁力搅拌器上 37°C 水浴中消化 5 min 后, 自然沉淀, 弃去上清液, 再用胰蛋白酶溶液消化 10 min, 沉淀, 弃去上清液后, 加冷 Eagle 培养基 10 ml, 再沉淀, 弃去上清液, 加入含 15% 小牛血清的 Eagle 培

养基后, 混匀, 将其接种在含盖玻片的培养瓶中, 在 37°C 培养箱中密闭培养, 2-3 d 后, 根据细胞搏动情况更换一次培养基, 培养 7-10 d, 取有搏动良好的细胞簇的盖玻片置于开放的浴槽中进行电生理实验观察⁽⁷⁾。标本放入浴槽中, 加入原培养液 4 ml, 上面盖一层液体石蜡⁽⁸⁾。浴槽温度 $36 \pm 0.5^\circ\text{C}$, 标本槽置于倒置显微镜 (英国产 Vickers-M-55) 上。玻璃微电极 (充 KCl 3 mmol/L, 电阻 >30 M Ω) 固定在微电极操纵仪上, 以 45° 角度对准欲穿刺的细胞簇, 穿刺并记录跨膜电位及其零相上升最大速率 (\dot{V}_{max}), 最大舒张电位 (MDP) 直接从微电极放大器 (日本产 MEZ-8201) 读出, 跨膜电位经摄影记录。

Nef 由中国预防医学科学院卫生研究所提供, 用 HCl 配制溶液, pH 5.8。

RESULTS

对兔窦房结跨膜电位的影响 Nef 30 $\mu\text{mol/L}$ 对 5 例兔窦房结跨膜电位的影响如 Tab 1 所示: Nef 能显著抑制兔窦房结细胞跨膜电位幅度 (APA), \dot{V}_{max} 和 4 相除极速度 (SP_4), 延长动作电位复极 50% 的时程 (APD_{50}) 和窦性周期 (SCL), Fig 1 示其中一例。

Tab 1. Effects of neferine 30 $\mu\text{mol/L}$ on transmembrane potential of rabbit sinoatrial nodes. $n=5$, $\bar{x} \pm \text{SD}$. ** $P < 0.05$, *** $P < 0.01$.

	Control	10 min	20 min	30 min
APA (mV)	57 ± 5	$53 \pm 4^{**}$	$47 \pm 5^{***}$	$42 \pm 4^{***}$
\dot{V}_{max} (V/s)	1.7 ± 0.3	$1.5 \pm 0.3^{**}$	$1.2 \pm 0.3^{***}$	$0.9 \pm 0.4^{***}$
APD_{50} (ms)	129 ± 30	$140 \pm 27^{***}$	$152 \pm 25^{***}$	$168 \pm 28^{***}$
SP_4 (mV/s)	50 ± 17	$36 \pm 6^{***}$	$31 \pm 7^{***}$	$28 \pm 8^{***}$
SCL (ms)	692 ± 47	$779 \pm 80^{**}$	$825 \pm 92^{***}$	$860 \pm 93^{***}$

对培养新生乳鼠心肌细胞跨膜电位的影响 在 4 块来自不同培养瓶的盖玻片上, 首先记录培养的新生乳鼠心肌细胞簇单个细胞跨膜电

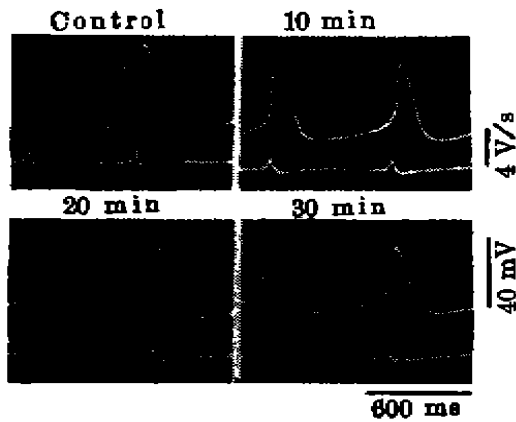


Fig 1. Effects of neferine 30 $\mu\text{mol/L}$ on the transmembrane potential (upper line) and \dot{V}_{max} (lower line) in one rabbit sinoatrial node.

Tab 2. Effects of neferine 40 $\mu\text{mol/L}$ on action potential of cultured neonatal rat myocardial cells. Number of cells in parentheses, $\bar{x} \pm \text{SD}$. ** $P < 0.05$, *** $P < 0.01$.

Parameter	Control (14)	Neferine (15)
APA(mV)	92 \pm 8	80 \pm 4***
\dot{V}_{max} (V/s)	20 \pm 5	12 \pm 4***
APD ₅₀ (ms)	56 \pm 8	72 \pm 15***
MDP(-mV)	66 \pm 8	59 \pm 7**
SCL(ms)	216 \pm 43	304 \pm 48***

Tab 3. Effects of neferine on action potential of cultured neonatal rat myocardial cells. Number of cells in parentheses, $\bar{x} \pm \text{SD}$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$.

	Neferine ($\mu\text{mol/L}$)				
	0 (29)	1 (16)	10 (12)	50 (19)	100 (13)
APA (mV)	90 \pm 6	83 \pm 6*	86 \pm 7*	77 \pm 8***	59 \pm 8**
\dot{V}_{max} (V/s)	18 \pm 4	12 \pm 2***	12 \pm 3***	9 \pm 4***	7 \pm 2***
APD ₅₀ (ms)	63 \pm 14	65 \pm 18*	50 \pm 13*	78 \pm 27*	72 \pm 8**
MDP (-mV)	66 \pm 8	58 \pm 5*	57 \pm 7***	51 \pm 5***	43 \pm 12***
SCL (ms)	248 \pm 31	251 \pm 56	248 \pm 56*	328 \pm 113***	311 \pm 94***

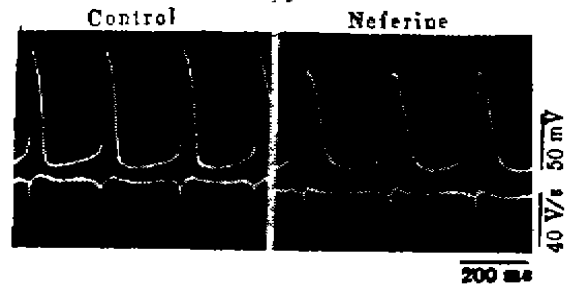


Fig 2. Effects of neferine 40 $\mu\text{mol/L}$ on the transmembrane potential and \dot{V}_{max} in one cultured neonatal rat myocardial cell.

位, 然后将 Nef 加入浴槽底部, 待自动扩散 10-20 min 后, 再记录给药后的跨膜电位。观察如 Tab 2 示: Nef 40 $\mu\text{mol/L}$ 使培养心肌细胞跨膜电位 APA, \dot{V}_{max} 和 MDP 显著抑制, APD₅₀ 和搏动周期(SCL)明显延长; Fig 2 示 Nef 40 $\mu\text{mol/L}$ 对同一培养心肌细胞跨膜电位的影响。

实验前 2 h 用 Nef 1, 10, 50, 100 $\mu\text{mol/L}$ 预处理培养标本, 然后记录培养心肌细胞跨膜电位。结果如 Tab 3: Nef 浓度依赖性地抑制培养心肌细胞跨膜电位 APA, \dot{V}_{max} 和 MDP, 浓度达 50 $\mu\text{mol/L}$ 时, 对 APD₅₀ 和 SCL 也有显著的延长作用。

DICUSSION

实验结果表明, Nef 30 $\mu\text{mol/L}$ 能显著抑制离体兔窦房结细胞及培养乳鼠心肌细胞的跨膜电位, 大于此浓度可能为其中毒浓度。

窦房结细胞动作电位除极相为 Ca^{2+} , Na^+ 经慢通道内流所致⁽⁸⁾; 窦房结起搏电流也主要与慢内向电流增加有关⁽¹⁰⁾, 在正常情况下, 慢内向电流的作用要比外向电流的作用更为重要⁽¹¹⁾。Nef 抑制兔窦房结跨膜电位 APA, \dot{V}_{max} 及 SP₁, 延长 SCL, 表明与阻滞慢内向电流有关。

培养心肌细胞跨膜电位对河豚毒素不敏感, 而对 Mn^{2+} 较敏感, 提示此期间动作电位的形成主要是缓慢 Ca^{2+} 内流引起⁽¹²⁾, 但也有认为与 Na^+ , Ca^{2+} 两种离子通道开放有关, 且

可将培养心肌细胞作为研究心室肌异位起搏细胞特性的模型^(13,14)。Nef 抑制培养心肌细胞跨膜电位 APA, \dot{V}_{max} 和 MDP, 延长 SCL, 表明是对心肌慢 Na^+ 或和 Ca^{2+} 内流的抑制, 也就是说 Nef 对室性早搏有抑制作用。

本实验在兔窦房结和培养的乳鼠心肌细胞进一步证实 Nef 对心肌慢离子流具有抑制作用, 此作用可能参与抗心律失常机理。

感谢 臧伟进、张社团、刘传浩同志参加部分工作。

REFERENCES

- 1 Xia GJ, Liu YF, Lü FH. Effects of methyl-lysine on experimental arrhythmias. *Acta Univ Med Tongji* 1986; 15 : 200
- 2 Li GR, Qian JQ, Lü FH. Comparison studies on the effect of neferine and quinidine on experimental arrhythmia. *Chin Tradit Herbal Drugs* 1988; 19 : 217
- 3 Li GR, Qian JQ, Lü FH. Effects of neferine on isolated rabbit myocardium. *Acta Pharmacol Sin* 1988; 9 : 139
- 4 Li GR, Li XG, Qian JQ, Lü FH. Effects of neferine on electrical and mechanical activity in isolated guinea pig myocardium. *Chin J Pharmacol Toxicol* 1987; 1 : 268
- 5 de Carvalho AP, de Mello WC, Hoffman BF. Electrophysiological evidence for specialized fiber types in rabbit atrium. *Am J Physiol* 1959; 196 : 483
- 6 Kreitnek D. Electrophysiological study of the two main pacemaker mechanism in the rabbit sinus node. *Cardiovasc Res* 1985; 19 : 304
- 7 Li XG, Yang L, Zhu MT, Zhao DZ. Determinations of electrical activity in clusters of cultured cardiac cells from neonatal rats. *J Xi-an Med Univ* 1986; 7 : 219
- 8 Elaine K. Nonlinear current-voltage relationship in cultured microphages. *J Cell Biol* 1980; 85 : 160
- 9 Irisawa H. Comparative physiology of the cardiac pacemaker mechanism. *Physiol Rev* 1978; 58 : 461
- 10 Vassalle M. Physiological basis of normal and abnormal automaticity. In: Rosenbaum MB, Elizari MV, eds. *Frontier of cardiac electrophysiology*. 1st ed. Boston: Martinus Nijhoff, 1983 : 120-3
- 11 Yanagihara K, Irisawa H. Potassium current during the pacemaker depolarization in rabbit sinoatrial node cell. *Pfluegers Arch* 1980; 388 : 255
- 12 Schanne OF, Ruiz-Ceretti E, Rivard C, Charlier D. Determinants of electrical activity in clusters of cultured cardiac cells from neonatal rats. *J Mol Cell Cardiol* 1977; 9 : 269
- 13 Lompre AM, Poggioli J, Vassort G. Maintenance of fast Na-channels during primary culture of embryonic of chick heart cells. *Ibid* 1979; 11 : 813
- 14 Schanne OF, Ruiz-Ceretti E, Payet MD, Deslauriers Y. Influence of varied $[\text{Ca}^{2+}]_i$ and $[\text{Na}^+]_i$ on electrical activity of clusters of cultured cardiac cells from neonatal rats. *Ibid* 1979; 11 : 477

First International Bi peptide Medical Sciences Symposium "Neuropeptide receptors and related antagonists"

1989 Sep 1-3 Shanghai

Please contact Professor W H Lee, c/o Department of Pharmacology, Faculty of Basic Medical Sciences, Shanghai Medical University, Shanghai 200032, China.