

石杉碱甲促进蟾蜍椎旁神经节的胆碱能传递¹张根葆, 汪萌芽¹, 郑建全 (皖南医学院细胞电生理研究室, 芜湖 241001, 中国)唐希灿² (中国科学院上海药物研究所, 上海 200031, 中国)Facilitation of cholinergic transmission by huperzine A in toad paravertebral ganglia *in vitro*¹ZHANG Gen-Bao, WANG Meng-Ya¹, ZHENG Jian-Quan (Cell Electrophysiology Laboratory, Wannan Medical College, Wuhu 241001, China)

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ABSTRACT Using the intracellular recording techniques of toad paravertebral ganglia (PVG), the effects of cholinesterase inhibitor huperzine A (Hup-A), an alkaloid first isolated from *Huperzia serrata* (Thunb) Trev in China, on the synaptic transmission were studied. In 30 PVG cells tested, no remarkable changes in membrane potential and input resistance were observed during superfusion of Hup-A 0.3 or 1 $\mu\text{mol} \cdot \text{L}^{-1}$ for 15 min. The rate of orthodromic action potential evoked by preganglionic stimulation was increased by Hup-A 0.3 or 1 $\mu\text{mol} \cdot \text{L}^{-1}$ ($n=12$, $P < 0.05$), and much faster, stronger, and longer in action at 50 or 100 $\mu\text{mol} \cdot \text{L}^{-1}$ ($n=11$). The amplitude and duration of exogenous acetylcholine-, but not carbachol-, induced depolarization were increased ($P < 0.05$). It is concluded that Hup-A is a selective and potent cholinesterase inhibitor, by which action it facilitates the cholinergic transmission of PVG neurons.

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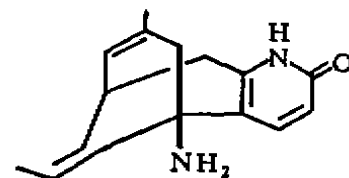
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KEY WORDS huperzine A; cholinesterase inhibitors; acetylcholine; carbachol; sympathetic ganglia; electrophysiology

A 摘要 应用离体蟾蜍椎旁神经节(PVG)细胞内记录技术,研究了石杉碱甲(Hup-A)对胆碱能突触传递的影响。Hup-A 0.3 或 1 $\mu\text{mol} \cdot \text{L}^{-1}$ 升高节前神经刺激诱发的顺行动作电位发放率并增强外源性 ACh 电位的幅度,延长时程,但不影响卡巴胆碱电位。结果显示 Hup-A 是一种高度选择性的 ACh-E 抑制剂,由此促进 PVG 的胆碱能传递。

关键词 石杉碱甲; 胆碱酯酶抑制剂; 乙酰胆碱; 卡巴胆碱; 交感神经节; 电生理学

石杉碱甲(huperzine A, Hup-A)是由蛇足石杉 *Huperzia serrata* (Thunb) Trev 中分离的生物碱¹, 其结构式和化学名如下:



Huperzine A

Hup-A 是一种可逆性胆碱酯酶抑制剂², 增强骨骼肌收缩, 改善外周肌无力³, 促进大鼠学习, 记忆功能⁴, 用于治疗重症肌无力和老年记忆功能减退⁵。为观察 Hup-A 对交感神经系统的作用, 本文应用离体交感神经节细胞内记录技术进行了研究。

METHODS

取蟾蜍 36 只, 体重 85 ± 20 g, ♀♂ 兼用。制备椎旁神经节(PVG)标本^[6,7], 固定在浴槽(0.5 ml)内, 以 Ringer 溶液^[6] $0.5-1 \text{ ml} \cdot \text{min}^{-1}$ 灌流。另将第 7 或第 8 脊神经中枢端及以上的交感链穿入双极吸引电极用于节前神经刺激。

以内充 $\text{KCl } 3 \text{ mol} \cdot \text{L}^{-1}$ 玻璃微电极(尖端电阻 $20-50 \text{ M}\Omega$), 经微操纵仪穿刺 PVG 细胞作细胞内记录, 电信号经微电极前置放大器(8100, Dagan)放大后, 在记忆示波器(Vc-10, Nihon Kohden)监视, 同时由二道生理记录仪(LMS-2A, 成都仪器厂)连续记录, 间断采样的快信号经示波器数字化记忆后描出。

氯化乙酰胆碱(acetylcholine chloride, ACh, 上海试剂三厂)和氯化卡巴胆碱(carbachol, Sigma)以 $0.1 \text{ mol} \cdot \text{L}^{-1}$ 浓度, 经压力注射仪(BH-2 型)由微管(尖端约 $5 \mu\text{m}$)向细胞旁进行微量压力注射(200 kPa , $20-100 \text{ ms}$)。Hup-A 游离碱粉剂由中国科学院上海药物研究所植物化学研究室刘加森提供, 以稀 HCl 助溶, 配成 $1 \text{ mmol} \cdot \text{L}^{-1}$ 的贮存液, 临用前以 Ringer 溶液稀释灌流。结果以 $\bar{x} \pm s$ 表示, 用药前后的差异进行配对 *t* 检验, 不同剂量组作用强度对比用组间 *t* 检验。

RESULTS

在 38 个 PVG 进行细胞内记录, 选择静息电位负于 -40 mV , 动作电位(AP)大于 50 mV 并能稳定记录 30 min 以上的细胞进行下述实验。

Hup-A 对膜电性质和逆行 AP 的作用
 节前神经刺激(0.2 Hz , 0.5 ms , $1-20 \text{ V}$)可在 PVG 神经元诱发快兴奋性突触后电位(f-EPSP)或逆行 AP (Fig 1)。若调整刺激强度使逆行 AP 间断发放后, 再用 Hup-A 0.3 或 $1 \mu\text{mol} \cdot \text{L}^{-1}$ 灌流 PVG 15 min, AP 发放率显著增加, 冲洗 30 min 作用达峰值, 冲洗 120 min 后基本恢复至对照水平(Fig 1, Tab 1)。作用峰期测得 AP 发放率升高值, $0.3 \mu\text{mol} \cdot \text{L}^{-1}$ 组为 $28 \pm 17\%$ ($n=7$, $P<0.01$), 而 $1 \mu\text{mol} \cdot \text{L}^{-1}$ 组为 $62 \pm 39\%$ ($n=5$, $P<0.05$), 两剂量组间发放率升高值差异有显著性意义($P<$

0.05), 且逆行 AP 的潜伏期也轻度缩短(Fig 1)。但在受试细胞($n=30$)未见静息电位和膜电阻的可测性变化。

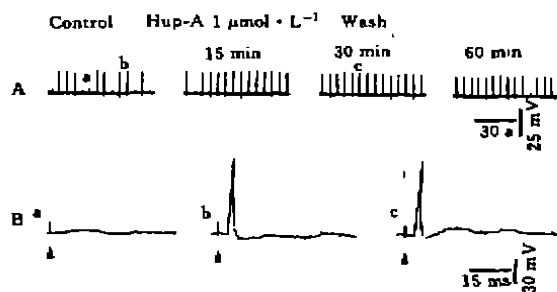


Fig 1. Facilitatory action of huperzine A on cholinergic synaptic transmission evoked by preganglionic stimulation (triangle arrows). A) Continuous chart recording with some intervals (upward deflections representing excitatory postsynaptic potential or orthodromic action potential; amplitudes of action potential were attenuated by limited frequency response of pen recorder). B) Digitized oscilloscopic recordings sampled at times marked by a, b, and c on A. The resting potential was -50 mV .

Tab 1. Effects of huperzine A $1 \mu\text{mol} \cdot \text{L}^{-1}$ on evoked rate of orthodromic action potential by preganglionic stimulation, ACh-potential, and carbachol-potential in paravertebral ganglion neurons. $\bar{x} \pm s$, paired *t* test. ^a $P>0.05$, ^b $P<0.05$, ^c $P<0.01$ vs control.

	n	Control	Hup-A 15 min	Wash 30 min
Evoked rate of action potential (%)	5	48 ± 20	71 ± 29^b	77 ± 33^b
ACh-potential	5	19 ± 10	29 ± 7^b	12 ± 11^c
Amplitude/ mV				
Duration/ s		22 ± 17	53 ± 32^b	90 ± 11^b
Carbachol-potential	4	18 ± 5	19 ± 4^c	18 ± 3^c
Amplitude/ mV				
Duration/ s		26 ± 11	29 ± 16^c	26 ± 11^c

以 Hup-A 50 或 $100 \mu\text{mol} \cdot \text{L}^{-1}$ 灌流 PVG $5-10 \text{ min}$ ($n=11$), 由节前刺激产生的逆行 AP 发放率增加, 其作用开始时间提前, 在药物灌

流期间,就可使发放率达100%;并且作用持久,冲洗200 min尚不能恢复至对照水平. 在全部受试细胞均未观察到 Hup-A 对逆行 AP 和直接 AP (细胞内注射去极化脉冲引起)的抑制现象.

Hup-A 对 ACh 电位及 carbachol 电位的作用 向记录的 PVG 细胞旁压力注射 ACh $0.1 \text{ mol} \cdot \text{L}^{-1}$ 或 carbachol $0.1 \text{ mol} \cdot \text{L}^{-1}$, 可获得稳定的去极化反应, 称 ACh 电位或 carbachol 电位 ($n=9$). Hup-A $1 \mu\text{mol} \cdot \text{L}^{-1}$ 灌流 PVG 标本 15 min, 在5个神经元使 ACh 电位的幅度增大, 约为对照水平2—4倍, 时程延长 ($P < 0.01$, Fig 2A, Tab 1). 且作用的峰期及恢复时间与 Hup-A 对逆行 AP 的作用相似. 但 Hup-A 对 carbachol 电位无明显影响 ($n=4$, $P > 0.05$, Fig 2B, Tab 1).

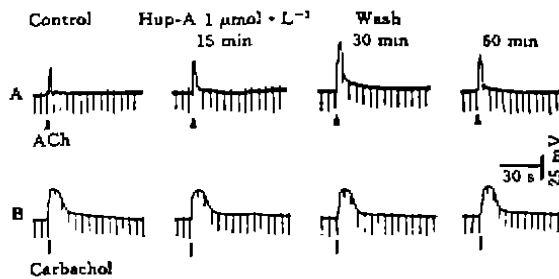


Fig 2. Effects of Hup-A on exogenous ACh-potential and carbachol-potential in PVG neurons of toad. A) ACh-potential induced by pressure ejection (triangle arrow, $0.1 \text{ mol} \cdot \text{L}^{-1}$, 20 ms, 200 kPa) was enhanced by Hup-A. B) Carbachol-induced depolarization was not much affected by Hup-A. Downward deflexions represent electrotonic potentials by injecting hyperpolarizing current pulses (0.2 nA, 40 ms). The resting potentials of Cell A and B were -50 and -45 mV , respectively.

DISCUSSION

本文首次在蛙类 PVG 单神经元细胞内生物电研究中,证明 Hup-A 通过其胆碱酯酶抑制作用促进 PVG 的胆碱能传递过程^[6], 证据

有三:首先是同时增强节前神经刺激诱发的 f-EPSP 或逆行 AP 发放率和外源性 ACh 电位, 且其作用强度与时间过程基本一致, 表明 Hup-A 的促进作用没有突触前机制, 这与 Hup-A 对 ACh 合成速率, 释放过程的阴性结果^[8,9]是一致的. 再之, Hup-A 对 PVG 神经元的膜电位及膜电阻无影响, 且不增大 carbachol 电位, 表明 Hup-A 不改变突触后膜的电学性质, 通道功能及受体敏感性, 其作用也非突触后机制. 最后, Hup-A 的作用表现缓慢持久的过程, 冲洗后可逆, 且对胆碱酯酶不敏感的 carbachol 电位无效, 作用性质与其它胆碱酯酶抑制剂作用^[10,11]相似, 均证明与其胆碱酯酶的抑制作用^[4]有关. 另外, Hup-A 对突触后膜电性质和 carbachol 电位无作用, 以及增强 AP 发放率作用的浓度依赖性质, 均证明非特异性作用的可能性可以排除.

由于 Hup-A 对交感神经节胆碱能传递有促进作用存在, 除对 Hup-A 在临床应用中的副作用, 毒性反应的机制及预防有一定意义外, 提示有可能推广应用于某些与交感系统功能低下有关的疾病治疗. 此外, 高浓度的 Hup-A (50 及 $100 \mu\text{mol} \cdot \text{L}^{-1}$) 对胆碱能突触传递仍表现出增强作用, 与其他胆碱酯酶抑制剂在高浓度抑制胆碱能传递^[10,11]显然不同, 表明 Hup-A 是一种具有高度选择性的可逆性胆碱酯酶抑制剂. 也有临床应用前景.

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人疱疹病毒增强谷氨酸神经毒性作用¹

R965.2

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Enhancing effects of *Herpesvirus hominis* on sodium glutamate neurotoxicity¹

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ABSTRACT The neurotoxic effects of sodium glutamate (MSG, 2.5 g · kg⁻¹ sc) and the enhancing effects of neurotropic *Herpesvirus hominis* (HVH, 0.2 ml/mouse ip) on MSG toxicity were studied through histomorphological observations, together with detection of the concentration of both mitochondrial protein bound Ca²⁺ and intracellular free Ca²⁺ ([Ca²⁺]_i) by the Tb³⁺ fluorescent probe and Ca²⁺ indicator Fura-2/AM, respectively. It was found that in 10-d-old mice the neurons in

arcuate hypothalamic nucleus degenerated severely after treatment with HVH + MSG, showing swollen edematous cytoplasm, dark pyknotic nuclei as well as a decrease in the amount of the neurons. The hypothalamic and spinal cord mitochondrial Tb³⁺ relative fluorescent intensity increased from 20 ± 3 and 20 ± 1 to 28 ± 5 and 34 ± 6, ie, the mitochondrial protein bound Ca²⁺ reduced significantly. MSG elevated the [Ca²⁺]_i levels from 0.11 ± 0.03 to 0.69 ± 0.19 μmol · L⁻¹ by not only stimulating the Ca²⁺ influx but also releasing the Ca²⁺ from intracellular stores. These findings suggested that MSG neurotoxicity was probably related to the overloading of neuroplasmic Ca²⁺, the destruction of neuronal abilities to deplete or sequester the intracellular Ca²⁺, as well as the irreversible neuronal injury and death.

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KEY WORDS sodium glutamate; *Herpesvirus*