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胆碱能激动剂和阻断剂对大鼠背侧海马 CA1 区单位放电的影响

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Effects of cholinergic agonists and antagonists on unit discharge of dorsal hippocampal CA1 area in rat

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ABSTRACT The effects of icv cholinergic agonists (acetylcholine 6 μg, physostigmine 10 μg, and nicotine 20 μg) and cholinergic antagonists (scopolamine 40 μg and hexamethonium 250 μg) on 174 cells unit discharges of the dorsal hippocampal CA1 area in 42 rats were studied. The frequencies of the unit discharges were increased in 41 cells over 84 cells by icv M or N receptor cholinergic agonists, but reduced in 22 cells over 42 by M or N receptor cholinergic antagonists. After the electrolytic lesion of the septal region, the effects of acetylcholine and nicotine on unit discharges disappeared. The results indicated that the cholinergic agents changed the electric activity of cholinergic sensitive neurons via the septum-hippocampal cholinergic pathway.

KEY WORDS parasympatholytics; parasympathomimetics; hippocampus; septal nuclei; electrophysiology

提要 icv 胆碱能 M 或 N 型受体激动剂 acetylcholine, physostigmine 或 nicotine 后, 大鼠背侧海马 CA1 区单位放电以增频反应为主. 而 M 或 N 型受体阻断剂 scopolamine 或 hexamethonium, 却以减频反应为主; 损毁膈区后, icv acetylcholine 或

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nicotine 对海马单位放电的影响大多消失. 提示胆碱能药物可能经隔–海马通路, 影响海马胆碱能敏感神经元的电活动.

关键词 拟副交感神经药; 抗副交感神经药; 海马; 隔核; 电生理

边缘系统中的海马含有胆碱能神经元, 并接受起自隔区的胆碱能传入纤维[1,2], 向海马内注入胆碱能受体激动剂能增强动物的学习记忆能力, 注入胆碱能受体阻断剂则与之相反[3–5]. 这类实验研究也多以动物行为为指标, 且甚少涉及其机制. 为此本研究的目的在于观察侧脑室注射胆碱能 M 型和 N 型受体激动剂和阻断剂, 对大鼠背侧海马 CA1 区单位放电的影响, 以及隔区在其中所起的作用, 并试图探讨背侧海马 CA1 区胆碱能神经元参与学习记忆与其电活动的关系.

MATERIALS AND METHODS

Wistar ♂ 和 ♀ 大鼠 42 只, 体重 260 ± *s* 20 g. 用 20% urethane 和 1% α–chloralose 混合液 8 ml · kg^{-1} 作腹腔麻醉, 气管插管, 将大鼠固定于脑立体定位仪后, 用 0.1% tubocurarine (1 mg · kg^{-1} ip)制动. 按 Bures 大鼠脑图谱, 于侧脑室(P 0.6; L 1.6; H 3.2)埋置不锈钢套管, 以自动抽注机, 驱动微量注射器向脑室内注入 10 μg 下列胆碱能药物: acetylcholine (ACh, 上海化学试剂二厂) 6 μg, Physostigmine (Phy, 瑞士 Fluka 产品) 10 μg, nicotine (Nic, 德国 Merck 产品) 20 μg, scopolamine (Sco, 上海化学试剂二厂) 40 μg, hexamethonium (Hex, 上海化学试剂二厂) 250 μg. 分别观察它们对背侧海马 CA1 区单位放电的影响. 每次给药历时 3 min, 注药后立即开始观察, 约 1–1.5 min 时单位放电开始出现变化, 2 min 时开始作磁带记录, 药物作用持续 8–10 min. 单位放电便恢复至对照水平, 为此于注药后 40 min, 再另寻新的放电单位, 重复上述实验, 每只大鼠重复给药 3–5 次. 凡放电频率净增 30%以上者, 称为增频单位或兴奋性单位, 净减 30%以上者, 称为减频单位或抑制性单位, 未达 30%的称无关单位或无变化单位.

以充灌 KCl 3 mol · L^{-1} 溶液的玻璃微电极引导背侧海马 CA1 区 (P 1.7–1.9; R 0.4–0.6; H 3.6–3.8)单位放电活动, 用示波器显示, 磁带记录, 微机处理单位放电并绘制序列密度直方图. 实验结束后, 分别经套管和微电极向侧脑室和海马内注入 2% pontamine sky blue, 标记套管和微电极尖端所在部位.

用尖端裸露的绝缘电极, 通以 1 mA, 30 s 阳极电流, 损毁隔区(A 2.0; LR 0; H 5.0). 损毁 30 min 后继续实验. 实验后, 用 1% potassium ferrocyanide 和 10% formalin 灌流脑, 固定后行脑切片, 隔区被损毁的范围约为直径 1 mm.

Tab 1. Effects of icv acetylcholine, physostigmine, nicotine, scopolamine or hexamethonium on spontaneous discharges of hippocampal neurons. Unit number (% total unit number). $^{**}P<0.05$, $^{***}P<0.01$ *vs* inhibitory units.

Drug	Rats	Excitation	Inhibition	Unchange	Total
Acetylcholine	9	20 (48.8%)***	8 (19.5%)	13 (31.7%)	41 (100%)
Physostigmine	5	10 (50.0%)***	2 (10.0%)	8 (40.0%)	20 (100%)
Nicotine	5	11 (47.8%)**	4 (17.4%)	8 (34.8%)	23 (100%)
Scopolamine	4	2 (10.5%)**	9 (47.4%)	8 (42.1%)	19 (100%)
Hexamethonium	6	4 (17.4%)***	13 (56.5%)	6 (26.1%)	23 (100%)

RESULTS

侧脑室注射胆碱能药物对海马单位放电的影响

1 胆碱能受体激动剂的作用　icv ACh、Phy 或 Nic 后，各有近半数单位发生增频反应，发生减频反应和无变化的单位为数均较少，增频与减频单位数相比，差异显著($P<0.01$、$P<0.01$、$P<0.05$)，见 Tab 1 和 Fig 1.

2 胆碱能受体阻断剂的作用　icv Sco 或 Hex 后，发生减频反应的单位数各为 47.4% 和 56.5%，与发生增频反应的单位数各为 10.5%和 17.4%的比较，差异显著($P<0.05$、$P<0.01$)，见 Tab 1 和 Fig 1.

损毁隔区后侧脑室注射胆碱能受体激动剂对海马单位放电的影响　损毁隔区后，icv ACh 或 Nic 海马单位放电大多消失，代之以放电频率无变化的居多数，它们分别为 70.8%和 66.7%，与其各自增频、减频的单位数之和的%比较，差异显著($P<0.01$、$P<0.05$)．比较损毁隔区前后 icv ACh 和 Nic 无变化单位数的%，其差异也显著($P<0.01$、$P<0.05$)．在发生反应的单位中，发生增频反应的单位数仍明显多于减频反应的单位数，见 Tab 2.

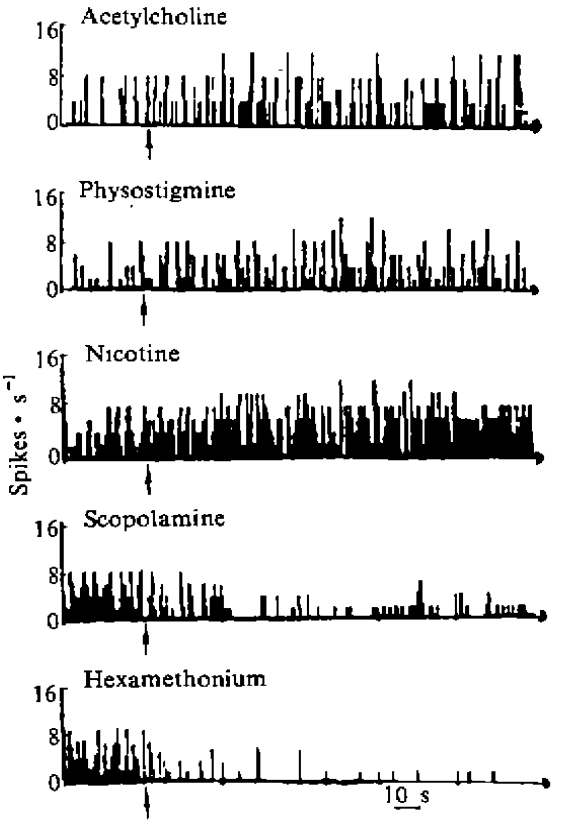

Fig 1. Density of single unit discharges of dorsal hippocampal CA1 area before and 2 min after icv drugs (arrows).

DISCUSSION

以往研究胆碱能药物对学习记忆的影响多以行为为指标，研究胆碱能药物对海马神经元的影响常以膜电位、突触后电位等为指标，且多以海马脑片为观察对象[6]．此外，对海马神经元自发放电的影响，也仅限于对 ACh 作了观察[7]．本实验采用微电极技术，通过侧脑室

Tab 2. Effects of icv acetylcholine or nicotine on spontaneous discharges of hippocampal neurons before and after the septum-lesion in rats. Unit number (% total unit number). $^{*}P<0.05$, $^{**}P<0.01$ *vs* **change units (excitation + inhibition) of post-lesion.** $^{+}P<0.05$, $^{++}P<0.01$ *vs* **unchange units of prelesion.**

Drug	Rats	Excitation	Inhibition	Unchange	Total
Acetylcholine	9 pre-lesion	20 (48.8%)	8 (19.5%)	13 (31.7%)	41 (100%)
	7 post-lesion	5 (20.8%)	2 (8.4%)	17 (70.8%)$^{**++}$	24 (100%)
Nicotine	6 pre-lesion	11 (47.8%)	4 (17.4%)	8 (34.8%)	23 (100%)
	6 post-lesion	5 (20.8%)	3 (12.5%)	16 (66.7%)$^{**++}$	24 (100%)

内注射胆碱能 M 或 N 型受体激动剂 ACh、Phy 和 Nic 使海马大多数神经元自发放电明显增多，呈现兴奋性效应，而于侧脑室内注入 M 或 N 型受体阻断剂 Sco 和 Hex、则使海马大多数神经元自发放电显著减少、出现抑制性作用．从而清楚地表明大鼠背侧海马 CA1 区中存在着胆碱能敏感神经元、它们既有胆碱能 M 型受体，又有 N 型受体．本文结果与用化学方法所得结果[8]相符．

侧脑室内或海马内注射胆碱能受体激动剂能显著增进动物学习记忆的能力、注射胆碱能受体阻断剂则与之相反[5,9]．因此本实验结果提示胆碱能受体激动剂可能部分通过增强海马内胆碱能敏感神经元的电活动，阻断剂可能部分经由压抑其电活动，从而影响动物的学习记忆能力．表明，海马内胆碱能敏感神经元电活动的变化有可能是学习记忆增强或减弱的基础．从兔瞬膜条件反射不断巩固时，其海马神经元的自发放电亦随之逐渐增多，这一效应能为胆碱能阻断剂二羟苯乙酸奎宁酯(quinuclidinyl benzilate, QNB)所阻断的报道[10]来看，也说明海马胆碱能敏感神经元电活动与学习记忆的关系极为密切．

已知海马内大多数胆碱能神经元属隔-海马胆碱能神经元[11]．本实验在损毁隔区后，观察到侧脑室内注射胆碱能受体激动剂对海马神经元放电活动的影响大多消失．损毁隔区能导致动物学习记忆能力明显减退[12]．因此有理由认为胆碱能受体激动剂可部分经由隔区-海马通路，增强海马胆碱能神经元电活动和兴奋性，而起到增进学习记忆的作用．

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