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人疱疹病毒增强谷氨酸神经毒性作用[1]

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Enhancing effects of *Herpesvirus hominis* on sodium glutamate neurotoxicity[1]

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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 Ca^{2+} and intracellular free Ca^{2+} ($[Ca^{2+}]_i$) by the Tb^{3+} fluorescent probe and Ca^{2-} 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 Tb^{3+} relative fluorescent intensity increased from 20 ± 3 and 20 ± 1 to 28 ± 5 and 34 ± 6, ie, the mitochondrial protein bound Ca^{2+} reduced significantly. MSG elevated the $[Ca^{2+}]_i$ levels from 0.11 ± 0.03 to 0.69 ± 0.19 μmol·L^{-1} by not only stimulating the Ca^{2+} influx but also releasing the Ca^{2+} from intracellular stores. These findings suggested that MSG neurotoxicity was probably related to the overloading of neuroplasmic Ca^{2+}, the destruction of neuronal abilities to deplete or sequester the intracellular Ca^{2+}, as well as the irreversible neuronal injury and death.

KEY WORDS sodium glutamate; *Herpesvirus*

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hominis; blood - brain barrier; Fura - 2; calcium; neurochemistry

摘要 人疱疹病毒 (HVH, 0.2 ml/mouse ip) 加重谷氨酸单钠 (MSG, 2.5 g·kg^{-1} sc) 对下丘脑弓状核的损毁，同时，下丘脑和脊髓的线粒体膜结合钙明显降低. Fura-2 单细胞检测表明，MSG 既可引起胞外 Ca^{2+} 内流，又可促进胞内钙库释放 Ca^{2+}，使胞内 Ca^{2+} 超载，最终导致细胞死亡. 结果提示，MSG 可引起胞内 Ca^{2+} 超载而破坏神经元排空或封存 Ca^{2+} 的能力，从而损伤细胞. 而 HVH 可增强上述损伤效应.

关键词 谷氨酸钠；人疱疹病毒；血脑屏障；Fura-2；钙；神经化学

谷氨酸单钠(MSG)可损毁未成熟动物不受血脑屏障保护的脑区[1,2]，影响动物成年后的学习记忆能力[3]. 近年来认为某些神经退化病如早老性痴呆、肌萎缩性侧索硬化症等可能与兴奋性氨基酸的神经毒性作用相关[4]，但对谷氨酸能否透过成年人和哺乳动物血脑屏障产生神经毒性的问题仍存在争议. 鉴于亲神经性人疱疹病毒(*Herpesvirus hominis*, HVH)能影响屏障效应[5]，且可促进脑内谷氨酸释放[6]，本文利用 HVH 观察该病毒能否增强 MSG 的神经毒性效应，即是否能诱导谷氨酸透过成年动物血脑屏障，或直接损伤成年或幼年动物脑内神经元.

MATERIALS AND METHODS

给药方式 昆明系小鼠100只，♀♂各半，10日龄60只 (体重 7.0 ± s 0.2 g)，18日龄40只 (体重 16.5 ± s 0.5 g)，均随机分为对照组和实验组. 实验鼠分别用 MSG (2.5 g·kg^{-1}) 或/和 HVH (0.2 ml/mouse) 处理. HVH 一次性 ip，其组织培养感染剂量(tissue cultured infection dose, TCID) 10^{-2}. MSG 于 ip HVH 后立即 sc，以后隔天 sc 同剂量 MSG，共3次.

光镜样本 最后一次给药后24 h，用10 %福尔马林溶液灌流心脏. 取脑置10 %福尔马林溶液中固定1 wk，石蜡包埋，冠状面连续切片6 μm. 焦油紫染色. 选取垂体柄基部前200 μm 为下丘脑弓状核代表切面，光镜检查.

线粒体制备和线粒体—Tb^{3+}荧光测定 小鼠经最后一次药物和 HVH 处理后 24 h 拉断颈椎处死，分离出下丘脑和脊髓，置 Teflon 棒玻璃匀浆器中以蔗糖 0.32 mol·L^{-1}制成匀浆，再按差速离心法[7]提取粗制线粒体并测定蛋白浓度[8]. 每管取线粒体蛋白40 μg，加生理盐水至 2.2 ml，再加六次甲基四胺缓冲液 0.6 ml (0.5 mol·L^{-1}, pH 6.2). 最后加 0.2 ml $TbCl_3$ (0.2 mol·L^{-1})，37 ℃平衡1 h，用 Hitachi RF-540型荧光分光光度仪测定 Tb^{3+}荧光强度. λ_{em} = 545 nm，λ_{ex} = 295 nm.

Fura-2/AM 测定单细胞$[Ca^{2+}]_i$ 取1－2日龄小鼠，分离下丘脑，立即置冰冷 Hank 氏液 (NaCl 137.0, KCl 5.0, $CaCl_2$ 1.3, $MgSO_4$ 0.4, $MgCl_2$ 0.5, KH_2PO_4 0.4, Na_2HPO_4 0.6, $NaHCO_3$ 3.0、glucose 5.6 mmol·L^{-1}, pH 7.4) 中. 仔细剔除软脑膜和血管，用 Hank 氏液冲洗3－4次. 切碎后加入 0.125 %胰蛋白酶液，37 ℃ 水浴中用吸管轻轻吹吸，以进一步分离细胞. 然后用冰冷 DMEM 培养液 (含10 %小牛血清)终止消化. 过200目筛网，滤液以300×*g* 离心5 min，弃上清. 沉淀再以 Hank 氏液洗一次，将细胞悬浮于 HEPES 缓冲的 Hank 氏液中. 将分离的神经细胞与 Fura－2/AM (终浓度2 μmol·L^{-1}) 在37 ℃温育45 min. 细胞悬液以300 ×*g* 离心，弃上清. 用 HEPES-Hank 氏液洗涤2次，再将细胞用 HEPES-Hank 氏液悬浮[9]. 采用 AR-CM-MIC 阳离子测定系统检测细胞的荧光强度. λ_{ex} = 340 nm 和 380 nm; λ_{em} = 505 nm，观察不同实验条件下荧光强度的变化. 根据荧光比值，用 DM－3000软件计算$[Ca^{2+}]_i$[10].

药品 MSG，上海海普药厂产针剂，浓度287.5 mg·ml^{-1}；HVH，由南京大学医学院微生物教研室瞿涤老师提供，效价10^{-8}；Fura-2/AM，Sigma 产品，用前以 Me_2SO 配成0.25 mmol·L^{-1}贮备液，分装后置－20 ℃ 避光保存；HEPES (N-2-羟乙基哌嗪-N'-乙烷磺酸)，上海生化试剂商店进口分装.

RESULTS

形态学观察 10日龄小鼠20只，分成4组、小鼠 sc 生理盐水后下丘脑弓状核的形态结构

没有影响，神经元结构完整，分布均匀．sc MSG 或 ip HVH 后，弓状核神经元受到轻度损伤，部分神经元发生核固缩． HVH + MSG 组的下丘脑弓状核神经元受损严重，核团萎缩变小，坏死神经元已被吞噬，神经元数显著减少（Fig 1，Plate 4）.

18日龄小鼠 sc MSG 或 ip HVH 后，未见弓状核神经元有明显损伤，且 HVH + MSG 组的受损程度远比10日龄组小鼠轻.

MSG和HVH对线粒体膜结合钙的影响 MSG 处理后，10日龄组小鼠的下丘脑和脊髓线粒体膜结合 Tb^{3+} 荧光强度均显著增高，亦即线粒体膜结合钙均明显减少(Tab 1)． 加 HVH 后，该脑区的线粒体膜结合钙进一步降低． 而 HVH 处理组脊髓的线粒体膜结合钙亦显著减少.

在18日龄组中，单用 MSG 或 HVH 处理，对下丘脑和脊髓的线粒体膜结合钙均无明显影响，但 HVH + MSG 后，两者的 Tb^{3+} 荧光强度发生显著变化，其结果与10日龄组相似.

Tab 1. Mitochondrial protein bound Tb^{3+} fluorescent intensities in hypothalamus and spinal cord of 10-d-old mice treated with sodium glutamate (MSG，2.5 g·kg^{-1}，sc) and *Herpesvirus hominis* (HVH，TCID，10^{-3}，0.2 ml/mouse，ip)．$n = 5-6$ experiments，$\bar{x} \pm s$． $^aP > 0.05$ *vs* Hypothalamus，$^bP < 0.05$，$^cP < 0.01$ *vs* control．$^eP < 0.05$，$^fP < 0.01$ *vs* MSG group.

	Control	MSG	HVH+MSG
Hypothalamus	20±3	24 ± 1^b	28 ± 5^{ce}
Spinal cord	20 ± 1^a	25 ± 2^{ac}	34 ± 6^{acf}

MSG 对胞内$[Ca^{2+}]_i$的影响 用 AR-CM-MIC 阳离子测定系统检测，同时在 Diapho-TMD 型荧光倒置显微镜下观察同步发生的神经细胞形态变化． 正常神经元的$[Ca^{2+}]_i$为 0.11± 0.03 μmol·L^{-1}($n = 27$，$\bar{x} \pm s$)，在 150 μl 细胞悬液中加入10 μl MSG (2.875 mg)后，$[Ca^{2+}]_i$升至0.69 ± 0.19 μmol·L^{-1}，增高5倍多． Fig 2 和 Fig 3为其中一个神经元$[Ca^{2+}]_i$的动态变化过程，加入 MSG 后，该细胞的$[Ca^{2+}]_i$增至静息状态时的7.5倍． 这时神经元肿胀，300 s 后神经元破裂死亡.

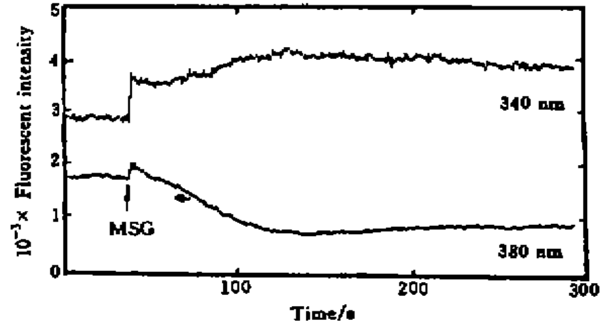

Fig 2. Fluorescent intensity of brain cell suspension in response to MSG106 mmol·L^{-1}.

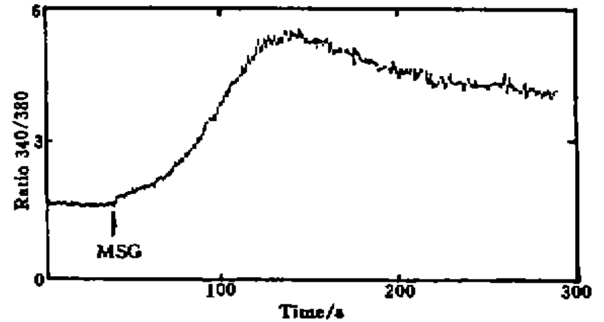

Fig 3. Increase of fluorescent intensity ratio (340/380) induced by MSG 106 mmol·L^{-1} to cell suspension.

在细胞悬液中加入过量 egtazic acid (EGTA，2.35 mmol·L^{-1}) 使细胞外 Ca^{2+} 螯合后，再加入 MSG 仍可引起神经元$[Ca^{2+}]_i$明显增加(Fig 4)，最后导致细胞坏死，说明 MSG 除促进胞外 Ca^{2+} 内流，还能引起胞内钙库释放 Ca^{2+}.

DISCUSSION

上述结果表明，HVH 可损伤幼年鼠下丘脑弓状核神经元，且明显加重 MSG 对神经元

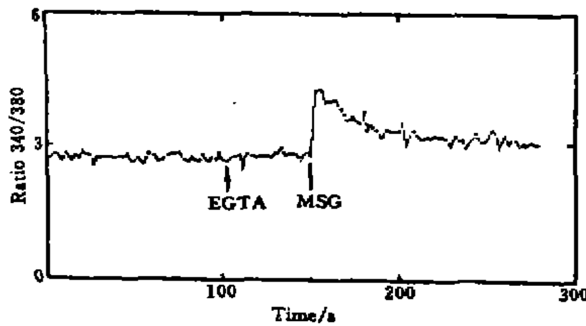

Fig 4. Fluorescent intensity ratio (340/380) after EGTA (2.35 mmol·L⁻¹) 50 s before MSG (106 mmol·L⁻¹) in cell suspension.

的不可逆损伤. 但在成年鼠实验中, 单独 MSG 处理对下丘脑弓状核神经元没有明显影响, HVH 预处理后, MSG 则引起下丘脑弓状核神经元轻度损伤, 出现 MSG 神经毒性的特征性病理学改变[1-3]及线粒体膜结合钙的相应变化, 提示 HVH 似能诱导谷氨酸透过成年动物血脑屏障, 增强 MSG 对成年动物的神经毒性作用. 从线粒体膜结合钙和单细胞$[Ca^{2+}]_i$的变化看, 谷氨酸不但引起胞外Ca^{2+}内流, 而且促进胞内钙库释放Ca^{2+}, 使胞内钙超载, 最终导致神经元死亡. 神经元钙库封存或排空Ca^{2+}功能的失调可能是 MSG 神经毒性作用的机制之一. 另一方面, 脊髓神经元线粒体膜结合钙亦发生明显变化, 这为谷氨酸可能损伤脊髓神经元提出了新的启示, 其形态学观察正在进行中.

谷氨酸 (味精的主要成分) 及其结构类似物, 如咖啡伴侣中的 palm 和 palm kernel (一种中性氨基酸毒素, 其毒性成分为 B-N-甲基氨基一丙氨酸, BMAA), 都是大众食品, 年耗量可观. 除亲神经性病毒外, 吗啡和镧系元素亦可增强谷氨酸的神经毒性效应[3,11]. 因此, 应重视兴奋性毒素的神经毒性作用, 其作用机制值得深入探讨.

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