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甲啡肽,纳络酮对红藻氨酸诱发大鼠癫痫活动的影响

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提 要 目的:探索甲啡肽(ME)、纳络酮(NLX)对红藻氨酸(KA)诱发大鼠癫痫发作的行为和组织学效应。方法:大鼠经戊巴比妥钠腹腔麻醉后固定于SN-I型立体定位仪上,按大鼠海马定位图谱把直径为0.5 mm的金属导管植于海马双侧CA1区:用微量注射器将ME、NLX于10 min内注入,随即腹腔内注射KA 10 mg/kg或12 mg/kg海马组织石蜡切片、HE染色,显微镜观测。结果:海马内微量注入ME可易化癫痫的发生,加重海马组织损伤。NLX可拮抗ME的作用,减轻海马组织损伤。结论:内源性阿片肽在KA诱发大鼠癫痫发作中,对海马的兴奋性的调节起重要作用。

关键词 甲啡肽;纳络酮;红藻氨酸;癫痫;大鼠;海马
中图法分类号 R742.1

癫痫是以反复发作的阵发性神经性行为表现为特征的脑功能失调。海马存在结构明确的阿片肽能神经通路。阿片肽与癫痫的发生密切相关,其在各种癫痫模型中的作用十分复杂,常因模型不同或给药途径不同出现不一致甚至相互矛盾的结果[1,2]。红藻氨酸(Kainic acid, KA)对哺乳动物中枢神经系统有很强的兴奋作用。本实验利用可靠的KA腹腔内注射(ip)所致大鼠癫痫模型[3],用行为与组织学研究相结合的方法,观察了海马内注射(ih)甲啡肽(Metenkephalin, ME)或阿片受体阻断剂纳络酮(Naloxone, NLX)对癫痫发生的影响。

1 材料与方法

61只体重200~300 g的健康Wistar大鼠,雌雄不拘,以1%戊巴比妥钠(3ml/kg ip)麻醉后,固定在SN-I型脑立体定位仪上,按Pellegrino大鼠脑立体定位图谱[3]确定海马座标为P 3.0, L 3.0, H 3.0将8号注射针头制成的注射引导管植入双侧背海马并以牙托粉固定于颅骨上,以4号半注射针头做注射管,其下端突出引导管0.5 mm,上端经硅胶管与自动微量注射器联接。术后2 d将动物随机分组,先用自动推挽微量注射器于10 min内匀速向双则海马内注射ME或NLX;随即腹腔内注射致痫剂KA 10 mg/kg(致痫阈下剂量)或12 mg/kg(致痫阈剂量),实验分组及给药情况如下:

I组: saline 5μl ih+ saline 20 ml/kg ip
II组: saline 5μl ih+ KA 12 mg/kg ip
III组: saline 5μl ih+ KA 10 mg/kg ip
IV组: ME 25μg (5μl) ih+ KA 10 mg/kg ip
V组: ME 25μg (5μl) ih+ KA 10 mg/kg ip+ NLX 100μg (5μl) ih
VI组: NLX 100μg (5μl) ih+ KA 12 mg/kg ip

(注:海马内所给ME及NLX的剂量在正常动物未引起行为和海马组织的异常,V组中NLX先于ME 5min注射。)

观察各组大鼠给药后24 h内的行为变化,尔后1%戊巴比妥钠再次麻醉动物,经引导管注入微量旁胺天蓝(Pontamin sky blue)标定海马内给药位置,开胸经左心室灌注10%福尔马林液至剪破的右心室流出液清亮为止。开颅取脑,10%福尔马林液固定2周。石蜡切片、HE染色,光镜下观察海马组织的改变。

实验药品ME; NLX; KA均为Sigma产品。

实验结果行相关组间比较并做χ^2检验。

2 结果

2.1 KA引起的痫样行为及ME、NLX对其致痫作用的影响,见表1

表1 海马内注射ME, NLX对KA致痫作用的影响

Tab 1 Effects of ME and NLX applied via ih on the development of seizure induced by KA in rats

Group	n	Seizure behavior developed			
		n_1	%	n_2	%
I	4	0	0.00	4	100.00
II	13	12	92.31	1	7.96
III	11	2	18.18	9	81.82
IV	12	12	100.00	0	0.00
V	9	2	22.22	7	77.78
VI	12	1	8.33	11	91.67

n_1: No. of rats with epilepsy; n_2: No. of rats without epilepsy

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Ⅱ组给予致痫阈剂量的13只大鼠有12只出现癫痫样行为,动物于给药后2~5 min走动减少,出现洗面和凝视行为;20~30 min后出现湿狗样的颤动。在给药后1 h左右出现反复阵发性癫痫样发作,主要表现为头颈抽搐,前肢阵挛,眼球突出及多涎。每次发作时间为2~10 s,发作间期动物呈呆滞不动或燥动不安。反复发作3~4 h后动物症状开始减轻,7~8 h发作基本停止,此后动物呈衰竭状态而呆滞不动;14 h后基本恢复正常,但有5只大鼠又出现1~5次癫痫样发作。

2.2 海马组织学改变

Ⅰ组动物海马组织形态正常,Ⅱ和Ⅲ组未致痫大鼠海马CA1和CA3区锥体细胞核轻度固缩,细胞突起轻度肿胀,参考文献[4]将海马的这种病理改变称为癫痫发作无关的KA直接损坏,简称A型损坏,见图1A。Ⅱ和Ⅲ组诱发癫痫的大鼠海马组织明显水肿,可见小片状出血。CA1至CA3区锥体细胞及突起明显肿胀变形,细胞核固缩甚至溶解,细胞数目明显减少,海马的这种病理改变称为癫痫发作相关的组织损害,简称B型损害,见图1B。Ⅳ组所有大鼠的海马组织均呈B型损害,提示ME易化癫痫发作并加重了海马组织的损伤。Ⅴ和Ⅵ两组发生癫痫动物的海马组织学改变呈B型,未发生者呈A型,结合行为观察的结果提示:NLX能拮抗ME易化癫痫发生而加重海马组织损伤的作用,而单独将NLX注入海马则能防止癫痫发生,减轻海马组织损伤。

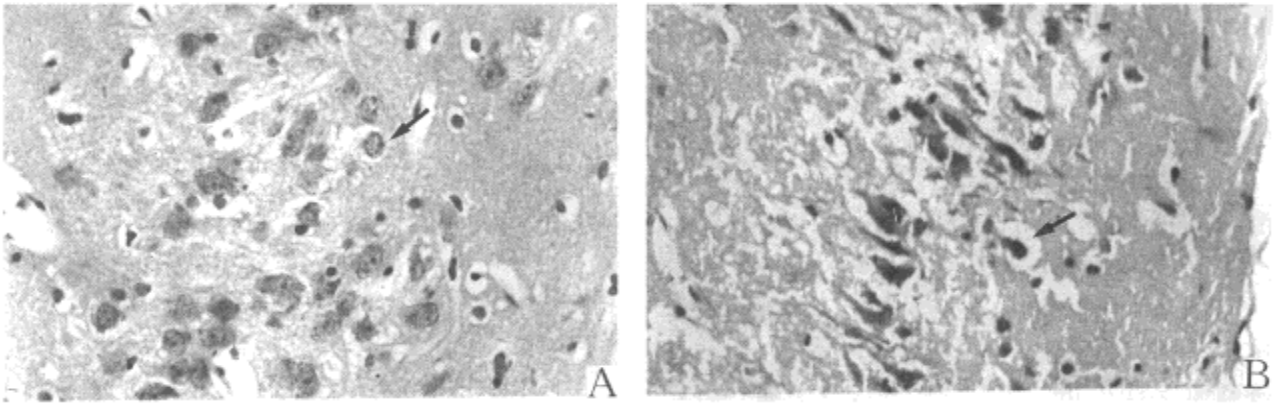


图1 大鼠海马内注射KA后,海马CA3区 (HE×400)

A:海马内注射KA 8 mg/kg+生理盐水5μl,大鼠未出现癫痫发作,神经细胞核及其突起无明显病变(↑);B:海马内注射KA 12 mg/kg生理盐水5μl,CA3区细胞明显水肿,锥体细胞及突起明显固缩(↑)

Fig 1 Hippocampal CA3 area after KA ip (HE×400)

A: Rats exposed to KA 8 mg/kg (ip) and saline 5μl ih. No signs of seizure. Only slight abnormalities are observed in the processes and nuclei of neurons(arrow); B: Rats exposed to KA 12 mg/kg (ip) and saline 5μl (ih) expressed seizures. Massive edema in all layers of the CA3 area can be seen and the pyramidal cells and their processes are agryrophilic (arrow).

3 讨论

本实验给大鼠腹腔注射KA 12mg/kg引起典型的颞叶癫痫样行为和海马组织学改变,与文献[4]报道一致,表明我们所复制的动物癫痫模型是成功的。

Ⅰ组大鼠行为正常,Ⅲ组有18.18% (2/11)的大鼠发生癫痫,而Ⅳ组大鼠全部(12/12)发生癫痫,两者差异非常显著,提示海马内注射ME易化了癫痫发生。Ⅴ组22.22% (2/9)大鼠致痫,与Ⅲ组比较差异不显著,提示NLX能拮抗ME协同KA致痫的作用。Ⅱ组致痫占92.31% (12/13),而Ⅵ组仅有8.3% (1/12)两者差异非常显著,表明双侧海马内注射NLX可防止癫痫发生。

实验观察到海马内微量注射ME可易化癫痫发生,加重海马组织损伤,其作用被NLX所拮抗,提示ME是特异的通过海马内阿片受体起上述作用的。ME在海马内影响癫痫发生的机理可能比较复杂,有报道脑啡肽可通过对抑制性中间神经元功能的削弱使海马锥体细胞处于脱抑制的高兴奋状态[5]。这无疑将有利于痫性电活动的同步化和扩散,从而易化癫痫发生;另外有报道阿片受体的激活可以显露NMDA受体,增强蛋白激酶的活性,从而加强兴奋性递质谷氨酸的作用[6]。ME或许通过这种途径易化癫痫的发生。至于本

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实验中 ME通过哪种阿片受体亚型产生效应尚需进一步研究。

值得注意的是,本实验向双侧海马内注射 NLX防止了 KA所致癫痫的发生,减轻了海马组织的损伤,是我们首次观察到这一现象。许多实验发现动物致痫过程中海马内有大量阿片类物质释放或其基因表达增强[7,8],结合本实验观察到的 ME的作用,我们推测这些阿片类物质可能易化癫痫的发生,而海马内注射 NLX后则拮抗了上述效应,从而防止了癫痫的发作。

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Effects of met-enkephalin and naloxone on kainic acid-induced epilepsy in rats

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Abstract **Objective**: To investigate the behavioral and histological effects of met-enkephalin (ME) and naloxone (NLX) on kainic acid-induced epilepsy in rats. **Methods**: After the rats were anesthetized with intraperitoneal injection of sodium pentobarbital (30 mg/kg), their heads were mounted onto a SN-1 stereotaxic apparatus (Narishige). Guiding cannula with a tip diameter of 0.5 mm was slowly implanted in CA1 area of the hippocampus (AP: 3.0; RL: 3.0; HO: 3.0) on both sides. ME and NAL were microinjected through the implanted cannula into the bilateral CA1 areas of awake rats within 10 min. Intraperitoneal injection of 10 mg/kg or 12 mg/kg was immediately conducted. The extracted hippocampal tissue was embedded with paraffin and sectioned. The sections were then stained with HE and observed with microscopy. **Results**: Intrahippocampal microinjection of ME augmented epileptogenic action and neurotoxic effects of kainic acid (KA) while NLX retarded them. **Conclusion**: The endogenous opioid peptides may play an important role in regulating hippocampal excitability in KA-induced epilepsy in rats.

Key words met-enkephalin; naloxone; kainic acid; epilepsy; hippocampus; rat

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