

大鼠海马内强啡肽₁₋₈和亮啡肽在癫痫发作及电针时的变化

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Alteration of dynorphin₁₋₈ and leu-enkephalin in rat hippocampus during seizure and electroacupuncture

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ABSTRACT Immunocytochemical techniques were used to evaluate the influence of penicillin-induced seizure and electroacupuncture treatment on dynorphin₁₋₈ and leu-enkephalin immunoreactivity in hippocampus. It was found that 3 h after beginning of seizure there started a dramatic decrease in dynorphin₁₋₈ in hilus, mossy fiber of hippocampus but an increase in hilus, mossy fiber of hippocampus but an increase in leu-enkephalin in subiculum, CA1 area of hippocampus and some other limbic structures. Electroacupuncture treatment decreased the leu-enkephalin immunoreactivity in the nuclei mentioned above and increased dynorphin₁₋₈ immunoreactivity in hippocampus. The results show that epileptiform activity and electroacupuncture inhibitory effect on seizure may be related to the alteration of dynorphin₁₋₈ and leu-enkephalin in the brain.

KEY WORDS dynorphin; leucine enkephalin; seizures; hippocampus; immunohistochemistry; electroacupuncture

摘要 用免疫组织化学方法观察大鼠青霉素致痫及加电针后脑内强啡肽₁₋₈及亮啡肽的变化。

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致痫时, 在海马门区及苔样纤维上强啡肽₁₋₈含量明显减少, 在海马下脚、CA₁区等结构内亮啡肽含量明显增加, 电针后海马中强啡肽₁₋₈含量增加, 亮啡肽含量在上述各区显著减少。提示: 癫痫及电针抗痫可能与脑内强啡肽₁₋₈和亮啡肽的变化有关。

关键词 强啡肽; 亮啡肽; 发作; 海马; 免疫组织化学; 电针

免疫组化

在海马, 脑啡肽^[1,2]主要分布于CA₁区、下脚、齿状回及苔样纤维, 强啡肽则主要分布于CA₃及齿状回, 前者起兴奋作用, 后者则起抑制作用。在苔样纤维上脑啡肽的免疫染色远少于强啡肽的, 而perforant pathway上又只有脑啡肽的免疫染色^[3,4]。癫痫时海马匀浆及灌流液中强啡肽含量下降, 而脑啡肽含量上升^[5,6], 形态研究^[7,8]也获类似结果。电针抑制癫痫可能部分通过增加强啡肽释放和降低脑啡肽释放而起作用^[9,10], 但电针抑制癫痫时是否对强啡肽、脑啡肽的合成起调节作用? 为此用免疫组织化学方法观察了正常、致痫及致痫加电针时强啡肽、脑啡肽的变化。

MATERIALS AND METHODS

Wistar大鼠18只, 体重210±15 g, ♀♂不拘, 分成5组: A组是手术对照组, 4鼠; B、C组均是青霉素致痫组, 其中B致痫后3 h主动脉灌流, C组致痫后21 h灌流, B、C组各4鼠; D组单纯电针组, 3鼠。电针穴位分别在枕骨大孔及第九、十胸椎棘突间, 电流强度6 mA, 频率100 Hz, 持续45 min; E组是青霉素致痫加电针组, 3鼠, 其电针穴位、强度、频率、持续时间与D组同。

大鼠一侧海马埋入套管, 青霉素致痫方法详

见^[10]。脑固定及后固定方法:4%戊巴比妥钠40mg·kg⁻¹ ip麻醉鼠,先后分别用0.9% NaCl及4%多聚甲醛主动脉灌注。取脑,移入含20%蔗糖的4%多聚甲醛中后固定2h,再移入30%蔗糖液中过夜。A组套管内微量注入2μl人工脑脊液,D组不予手术,电针后2h 15min灌注固定脑,E组致痫后即予电针,痫后3h固定脑,均做冰冻冠状切片,片厚35μm,脑片用avidin-biotin peroxidase complex (ABC)法进行强啡肽_{1-8}}和亮啡肽的免疫组织化学定位,简述如下:脑薄片依次移入:10%山羊血清,21℃温育30min;强啡肽_{1-8}}抗体300μl (1:660)或亮啡肽抗体300μl (1:250),均37℃温育6h,再4℃温育36h;生物素化羊抗兔IgG 1:100,37℃温育1h,ABC液1:100,37℃温育1h,50% 3,3'-二氨基联苯胺 + 3% H₂O₂染色15min左右。

ABC药箱购自Vector Laboratories Inc;强啡肽_{1-8}}抗体购自Peninsula Laboratories Inc;亮啡肽抗体购自第二军医大学神经生物学教研室。

RESULTS

1 行为与脑电 B、C、E组在注入青霉素2—5min后出现高频、高幅的痫样放电,行为上大鼠出现翘尾、嘶叫、四肢抽搐、手术对侧前肢阵挛和“wet dog shakes”;C组在24h之后上述症状已消失;A组、D组脑电未见变化,行为上除电针引起的躯体轻微颤动外无明显变化;E组在加电针后痫波基本消失,行为上也转为安静。

2 免疫组织化学

2.1 强啡肽_{1-8}} A组在海马门区及苔样纤维上见到密集的细点状的神经纤维及散在的细胞体,CA₁区未见纤维或细胞染色, Fig 1 (Plate 1)。B组在海马门区及苔样纤维上基本未见到纤维、细胞的染色。D组结果与A组类似。E组在海马门区及苔样纤维上见到点状纤维,但不如A、D组多。

2.2 亮啡肽 A组:CA₁区、苔样纤维、门区、下脚、室床通路上见散在的较大的点状纤维(puncta)及短小的串珠样纤维染色,纤维交织

成网状,其密度远不如强啡肽_{1-8}}的纤维染色。在苔样纤维的细的轴突上很少见到亮啡肽免疫反应,这与Gall^[11]的报道一致,也见到散在的细胞染色, Fig 2 (Plate 2, 3)。B组在CA₁区、下脚、苔样纤维及门区均见到染色的纤维及细胞增加,在梨状皮层、内嗅皮层及室床通路上纤维染色亦加深、增多。在海马结构内纤维及细胞的增加以CA₁、下脚尤为明显,可见到密集的小圆形细胞及其伸向hippocampal fissure (hf)的树突,在门区及苔样纤维上以puncta增加为多,上述变化主要发生在注射青霉素侧海马,对侧海马未见明显变化。在致痫24h后在两侧海马CA₁区、下脚、苔样纤维、门区及室床通路上均见到脑啡肽的纤维、细胞染色数增加,在梨状皮层、内嗅皮层及杏仁核的亮啡肽染色也明显增加。D组与A组类似。E组:CA₁区及苔样纤维、门区、室床通路亮啡肽的胞体染色基本消失,纤维也显著减少, Fig 2 (Plate 2, 3)。

DISCUSSION

大鼠海马内强啡肽,亮啡肽在致痫时可能起着不同的作用,海马内注入亮啡肽能致痫样放电而强啡肽所作用的kappa受体的激动剂则有抗痫作用^[9,11]。在电惊厥及海马青霉素致痫模型上也曾观察到致痫时海马中亮啡肽释放增加、强啡肽释放减少,因此本实验中,致痫3h看到的海马内强啡肽_{1-8}}免疫染色减少、亮啡肽免疫染色增加可能是由于它们在海马细胞内合成变化引起的。本实验手术对照组中未见到强啡肽_{1-8}}及亮啡肽的变化,说明实验中致痫组及电针组强啡肽_{1-8}}及亮啡肽的变化与手术损伤无关。当一侧海马注入青霉素致痫时,在皮层、梨状皮层、内嗅皮层这些富含亮啡肽区域也染色增加,这表明在此种癫痫发生时除海马外还有其它边缘结构及皮层中亮啡肽系统的参与。White, Morris^[4,7,8,12,13]等以往也曾用免疫组织化学、放射免疫法及原位杂交技术在海马门毁损、海马点燃、杏仁核点燃、杏仁核海人

酸致痫及电惊厥等模型上看到致痫时海马内强啡肽 mRNA、强啡肽含量下降及脑啡肽 mRNA、脑啡肽含量增加, 本实验结果与上述文献结果基本相符, 但 Kanamatsu^[14]在海人酸注入纹状体致痫模型上用放射免疫方法测得海马匀浆中 met-脑啡肽及强啡肽分别下降 30% 和 63%, 到 24 h 才恢复到正常水平, 48 h 时, 脑啡肽增加到 270%, 本实验室未见到癫痫时的亮啡肽下降, 在致痫 3 h 即已见到亮啡肽的增加, 24 h 后亮啡肽增加由一侧海马波及另一侧海马。上述结果的不同, 可能是因为模型及脑啡肽类型的差异。

已往我们的工作中发现电针在抑制癫痫同时能阻止海马中强啡肽释放的减少及脑啡肽释放的增加。本实验中看到青霉素致痫时, 在海马门区及苔样纤维内强啡肽₁₋₈免疫染色明显减少, 亮啡肽在 CA₁、下托、门区、苔样纤维及梨状皮层、内嗅皮层、杏仁核明显增加, 电针处理后, 上述变化被拮抗或翻转。这提示: 癫痫及电针抑制癫痫可能与强啡肽₁₋₈及亮啡肽合成的变化有关。

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