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二氢埃托啡耐受后小鼠脑内 cAMP 含量的减少及氨基酸含量的增加[1]

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Decrease of cAMP and increase of amino acids contents in mouse brain after dihydroetorphine tolerance[1]

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KEY WORDS dihydroetorphine; drug tolerance; cyclic AMP; glutamic acid; aspartic acid; glutamine; GABA

AIM: To study the mechanism of dihydroetorphine (DHE) tolerance. **METHODS**: DHE tolerance was produced by repeated sc injections in progressively increased doses to mice for 8 d. The concentrations of amino acids and cAMP were detected by RP-HPLC/fluorescence assay and radioimmunoassay, respectively. **RESULTS**: The basal contents of glutamic acid (Glu), aspartic acid (Asp), and GABA in whole brain (cerebellum removed) were increased respectively from 14.1 ± 2.1, 3.0 ± 0.4, and 1.8 ± 0.8 μmol/g tissue in control mice to 17.2 ± 2.2, 4.1 ± 0.6, and 3.2 ± 1.0 μmol/g tissue in tolerant mice, and the rates of increase were 22.0 % ($P < 0.05$), 36.7 % ($P < 0.01$), and 77.8 % ($P < 0.05$ *vs* control), respectively. There was no significant difference in the basal contents of Gl_n (5.1 ± 1.0 *vs* 4.5 ± 1.7 μmol/g tissue of control). The basal contents of cAMP in hypothalamus and striatum were decreased respectively from 271 ± 38 and 189 ± 31 nmol/g tissue in control mice to 96 ± 15 and 65 ± 21 nmol/g tissue in tolerant mice ($P < 0.01$), and the rates of decrease were 64.6 % and 65.6 %, respectively. There was no significant difference of cAMP in cerebral cortex (72 ± 20 *vs* 55 ± 15 nmol/g tissue of control). **CONCLUSION**: The increases of Glu, Asp, and GABA in brain and the decrease of cAMP in hypothalamus and striatum were involved in DHE tolerance.

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关键词 二氢埃托啡; 药物耐受性; 环腺苷一磷

酸；谷氨酸；门冬氨酸；谷氨酰氨；GABA

目的：探讨二氢埃托啡(DHE)耐受的机制. **方法**：放免法及反相高效液相色谱荧光检测法测定环腺苷一磷酸(cAMP)及谷氨酸(Glu)、门冬氨酸(Asp)、谷氨酰氨(Gln)、GABA的含量. **结果**：DHE反复sc 8 d后产生耐受. 小鼠去小脑全脑Glu、Asp、GABA的基础含量分别由对照组的14.1±2.1、3.0±0.4、1.8±0.8升高至耐受组的17.2±2.2、4.1±0.6、3.2±1.0 μmol/g组织，Gln的含量无明显改变. 下丘脑及纹状体的cAMP基础含量分别由对照组的271±38、189±31降至耐受组的96±15、65±21 nmol/g组织，大脑皮层cAMP的含量无明显改变. **结论**：DHE耐受与脑内Glu、Asp、GABA基础含量的升高及下丘脑、纹状体内cAMP的基础含量的降低有关.

二氢埃托啡(dihydroetorphine, DHE)是一种强效镇痛药，选择作用于μ阿片受体[1]，脑内高密度的特异结合区有纹状体、大脑皮层等[2]，与其它阿片类一样，反复应用会产生耐受[1]. NMDA受体拮抗剂可阻止吗啡耐受的发生[3]，GABA能取消福尔马林所致的吗啡耐受的延迟[4]，提示氨基酸类递质可能参与吗啡耐受，但耐受是否改变脑内氨基酸类递质的含量尚不清楚. 慢性给吗啡后，吗啡抑制腺苷酸环化酶(AC)活性及cAMP产生的作用减弱、丧失也被认为是阿片类耐受的机制之一[5]. 因此，本实验观察DHE耐受后小鼠脑内氨基酸及纹状体、大脑皮层、下丘脑内cAMP含量的变化，以探讨DHE耐受的部分机制.

MATERIALS AND METHODS

DHE耐受模型 用小鼠热板法(55±0.5 ℃)测痛，以舔后足的潜伏期(s)为痛阈指标. 取基础痛阈在10－20 s的小鼠(♀，18±1 g，本校实验动物部，医动2-22-1)，每日两次(8:00, 16:00)皮下注射(sc) DHE(本校合成药化教研室合成)，按以下剂量逐日递增：4.8, 9.6, 14.5, 21.8, 29.0, 36.3, 43.5, 48.4 nmol·kg^{-1}，共注射8 d，末次sc后18 h(d 9)给DHE (4.0 nmol·kg^{-1}, 10 mL·kg^{-1}, sc)后测痛. 对照组sc生理盐水(NS, 10 mL·kg^{-1}) 8 d, d 9小鼠分成两组：对照组sc NS后测痛，阳性对照组sc DHE 4.0 nmol·kg^{-1}后测痛.

cAMP的测定 耐受及对照小鼠于末次sc后18 h断头取脑，在冰台上取下丘脑、纹状体及大脑皮层，置干冰中，用cAMP放免药盒(上海第二医科大学)测定cAMP的量.

氨基酸的测定 耐受及对照组小鼠于末次sc后18 h取(去小脑)全脑，用反相高效液相色谱荧光检测法[6]测定Glu、Asp、GABA及Gln的含量.

数据处理 数据以 $\bar{x} \pm s$ 表示，单变量均数组间比较用 t 检验.

RESULTS

DHE的耐受 在sc NS 8 d的小鼠上，DHE (4.0 nmol·kg^{-1}, sc)有明显的镇痛作用，在15、30、45分钟时，小鼠的舔足潜伏期由对照组的18±6、16±3、16±4秒升高至阳性对照组的45±13 ($P<0.01$)、31±12 ($P<0.01$)、27±10 ($P<0.05$)秒，但对sc DHE 8 d的小鼠，同样剂量的DHE无镇痛作用，与对照组相比，$P>0.05$，说明小鼠对DHE已完全耐受(Fig 1).

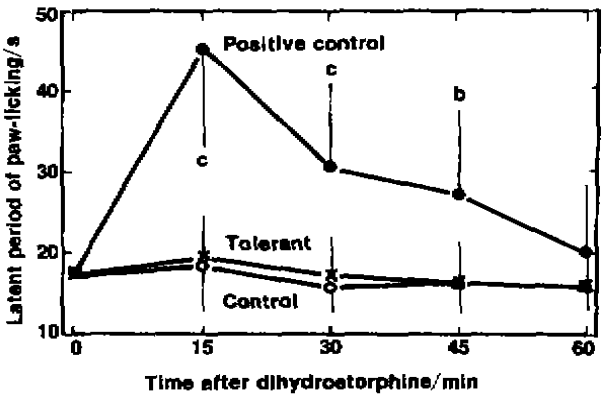

Fig 1. Tolerance of mice to DHE 4.0 nmol·kg^{-1}, sc. $n=10$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

DHE耐受对脑内氨基酸基础含量的影响 耐受小鼠去小脑全脑中Glu、Asp、GABA的基础含量明显高于对照组，分别升高22.0 % ($P<0.05$)、36.7 % ($P<0.01$)、77.8 % ($P<0.05$)；而Gln的变化不明显，与对照组相比，$P>0.05$ (Tab 1).

DHE耐受对脑内cAMP基础含量的影响 耐受小鼠下丘脑及纹状体内cAMP的基础含量明显低于对照组，分别减少64.6%、65.6 % ($P<0.01$)，大脑皮层cAMP的含量无明显改变，与对照组相比，$P>0.05$ (Tab 1).

DISCUSSION

Glouet等报道，吗啡耐受动物多数脑区的

Tab 1. Effects of dihydroetorphine tolerance on amino acids and cAMP in mouse brain. $n = 8$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control of amino acids. $^dP > 0.05$, $^fP < 0.01$ *vs* control of cAMP.

	Control	Tolerant
Amino acids, μmol/g tissue		
Glutamic acid	14.1 ± 2.1	17.2 ± 2.2[b]
Aspartic acid	3.0 ± 0.4	4.1 ± 0.6[c]
GABA	1.8 ± 0.8	3.2 ± 1.0[b]
Glutamine	5.1 ± 1.0	4.5 ± 1.7[a]
cAMP, nmol/g tissue		
Hypothalamus	271 ± 38	96 ± 15[f]
Striatum	189 ± 31	65 ± 21[f]
Cerebral cortex	72 ± 20	55 ± 15[d]

cAMP 含量无明显改变，纹状体及大脑皮层 cAMP 含量增加[7]，但他们是在末次给吗啡后 2 h 取脑测得的，此时脑内尚有较高的吗啡浓度，该浓度在非耐受动物上是足以产生镇痛作用的[8,9]。而我们的实验是在末次给药后 18 h 才取脑。根据 DHE 的药代动力学参数（$T_{\frac{1}{2}} = 27.7$ min，$T_{\frac{1}{2}\alpha} = 2.7$ min，单室模型）[10]计算，在我们取脑时小鼠血中 DHE 的浓度约为 0.1 nmol·L^{-1}，而 DHE 在血药浓度为 39 nmol·L^{-1}时已无镇痛作用，且主要分布于循环系统，因此可以认为我们对含量的测定是在脑内几乎无药物存在的条件下进行的，反映的是耐受所引起的 cAMP 基础含量的下降。

NMDA 受体拮抗剂能阻止吗啡耐受的发生[3]，说明兴奋性氨基酸递质系统活性的增强在吗啡耐受中起重要作用，但吗啡耐受后脑内 NMDA 受体下调[11]，这提示兴奋性氨基酸递质系统在吗啡耐受中的作用可能有赖于氨基酸含量的增加，我们的结果表明在 DHE 耐受后脑内 Glu 及 Asp 含量确实增加了，而单剂吗啡并不影响 Glu 及 Asp 的基础释放[12]，也说明这种增加是与耐受相关的。

GABA 可取消福尔马林所致的吗啡耐受的延迟[4]，提示 GABA 的增加能促进吗啡耐受，我们关于 DHE 耐受后脑内 GABA 含量升高的结果是与之相符的。

cAMP 依赖的蛋白激酶能增强 NMDA 在神经原上的反应[13]，那么在 DHE 耐受后 cAMP 含量降低的情况下，这种增强作用显然会减弱，这提示兴奋性氨基酸含量的增加也可能是对 cAMP 含量降低的一种代偿作用，而 GABA 的增加可能是由于 Glu、Asp 与 GABA 之间能相互促进释放[14]。

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