

与阿片受点结合比较牢固的四个阿片腺类化合物

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提要 NZO, HMZ 与大鼠脑 P₂ 膜制备共同保温后分别洗涤 4 或 10 次, 阿片受点可完全恢复与 [³H]Etor 的结合活性, 它们对 GPI 的作用亦可被洗掉或被 Nx 逆转. Scatchard 作图分析, 以 HMZ 或 HZO 处理后洗涤 6 次, 其高亲和力结合点活性仅恢复 10-18%, 而用 Mor 处理后洗涤 4 次即可恢复 70% 以上. 它们的镇痛持续时间约比 Mor 长 2 倍.

关键词 麻醉品; 腺类; 吗啡; 纳络酮; 内啡肽受体

Mor 的镇痛作用比较短暂是其缺点, 改变分子结构使其与受点结合稳定性增加有可能延长作用时间. 文献报道⁽¹⁻³⁾阿片胍或腺类可与阿片受点形成共价键, 是长效拮抗剂或镇痛剂. 但据我们的研究, 胍类化合物 NZI 及其 N-甲基衍生物 HZI 并非阿片受点不可逆配基, HZI 不是长效镇痛剂⁽⁴⁾. 本文对腺类衍生物 HZO, NZO 及尚未见文献报道的 HMZ 及 HDM 进行了研究.

材料及方法

化合物 HDM, HMZ, HZO, NZO 是上海第一医学院药理学系刘懋勤副教授合成. Mor 是东北第六制药厂出品, 用前经重结晶及氧化铝柱层析纯化. [³H]Etor (44 Ci/mmol) 是上海第一医学院药理学系标记.

阿片受点结合试验 [³H]Etor 0.2 ml (终浓度 1.2 nM) 加 0.2 ml Tris 缓冲液 (50 mM, pH 7.5, 简称 Tris 液; 总结合管) 或 Etor 溶液

(终浓度 5 μM, 非特异性结合管) 再加入 0.6 ml 大鼠脑(去小脑)P₂ 膜制备⁽⁴⁾, (含蛋白质 1 mg). 30°C 保温 20 min 后加入冷 Tris 液 5 ml, 用 GF/B 滤纸过滤, 以冷 Tris 液 5 ml 洗涤 1 次, 将滤纸放在计数杯底部, 加 10 ml 闪烁液⁽⁴⁾, 用液闪仪计数(效率 41%). 总结合管减非特异性结合管计数即 [³H]Etor 的特异性结合计数.

GPI 试验 方法见文献 4.

镇痛试验 以 150 W, 12 V 卤钨灯为光源, 将光线聚焦在小鼠(昆明种, ♂♀不限) (19.3±3.3 g) 尾端 1/3-1/2 处, 测定出现甩尾反应所需时间. 如给药后所需时间超过给药前数值加 2 倍 SD 则认为有镇痛作用.

结果

阿片腺类化合物与阿片受点的结合与洗脱 阿片腺类化合物对 [³H]Etor 与阿片受点结合的抑制作用见图 1, 其 IC₅₀ 值见表 1. 用可以产生最大抑制作用的浓度与 P₂ 保温(30°C, 15 min) 后反复洗涤, 再用 [³H]Etor 进行受体结合试验以测定阿片受点结合能力恢复情况. 用计算机绘出 Scatchard⁽⁵⁾ 图形, 且计算高、低亲和力结合点的结合量(图 2, 表 1). 由试验结果可以看到 NZO 与受点结合后洗涤 2 次仅低亲和力结合点恢复活性, 洗涤 4 次, 高、低亲和力结合点活性均已恢复, 其情况与 Mor 相似. HZO 与阿片受点结合较牢固, 洗涤 6 次, 低亲和力结合点活性虽可恢复 90%, 但高亲和力结合点仅恢复约 20%, 7、8 位有双键(HMZ)与阿片受点结合亦甚牢固, 洗涤 6 次, 高亲和力结合点活性约恢复 10%, 其二甲腺与受点结合牢固程度亦较高.

此外, 我们又测定了在上述实验中与阿片受点结合最牢的 HMZ 与受点结合后经过洗

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略写 Etor = 依托啡(etiophine); GPI = 豚鼠回肠(guinea pig ileum); HDM = 14-羟基吗啡二甲腺(14-hydroxydimethylmorphazone); HMZ = 14-羟基吗啡腺(14-hydroxymorphazone); HZI = 14-羟基双氢吗啡胍(14-hydroxydihydromorphazine); HZO = 14-羟基双氢吗啡腺(14-hydroxydihydromorphazone); Mor = 吗啡(morphine); Nx = 纳洛酮(naloxone); NZI = 纳洛胍(naloxazine); NZO = 纳洛腺(naloxazone)

Tab 1. Binding of hydrazone derivatives of opiates with opiate receptors and their analgesic effects. ($\bar{x} \pm SD$)

Compounds	%recovery of binding capacity of opiate receptors after washing for: ¹⁾ (n = 2)						Inhibition of [³ H]Etor binding IC ₅₀ (nM) ²⁾ (n = 4)	Analgesic effect (mice, sc) Duration min ³⁾ (dose mg/kg)	ED ₅₀ (mg/kg) (n = 2)
	2 times		4 times		6 times				
	H	L	H	L	H	L			
HDM	—	—	6	94	—	—	150 ± 5.0	117 ± 28(5.2)n = 3	1.51 ± 0.17
HMZ	—	—	13	82	10	92	63 ± 4.7	132 ± 27(2.5)n = 3	0.52 ± 0.04
HZO	13	60	—	—	18	90	71 ± 11	195 ± 24(1.25)n = 4	0.18 ± 0.014
Mor	16	100	77	100	—	—	140 ± 3.7	80 ± 5(5)n = 3	2.0 ± 0.07
NZO	7	100	100	100	—	—	4.8 ± 1.5	(Antagonistic effect)	

1) Binding capacity of high (H) and low (L) affinity binding sites were calculated from Scatchard plots by a PS-80 computer. 2) Inhibition curves are given in Fig 1. 3) Duration curves are given in Fig 5.

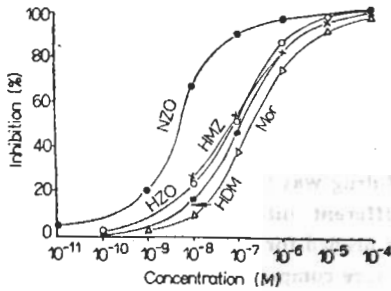


Fig 1. Inhibition of [³H]etorphine-opiate receptor binding by hydrazone derivatives of opiates. P₂ membrane preparation of rat brain (1 mg protein) was incubated with [³H]etorphine 1.2 nM in the presence (nonspecific binding, cpm_n) or absence (total binding, cpm_t) of Etor (5 μM) or drug solutions of different concentrations (binding in the presence of drugs, cpm_d). The % of inhibition of [³H] etorphine binding by drugs were given by (cpm_t - cpm_d)/(cpm_t - cpm_n). ED₅₀ found by probit-log plotting were given in Tab 1. n = 4.

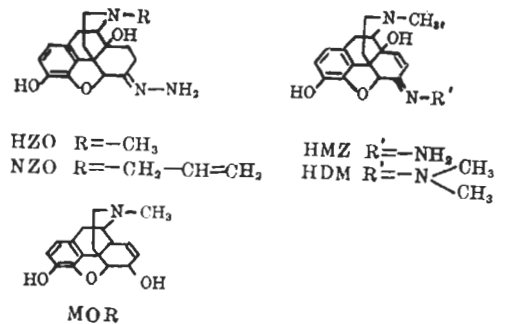
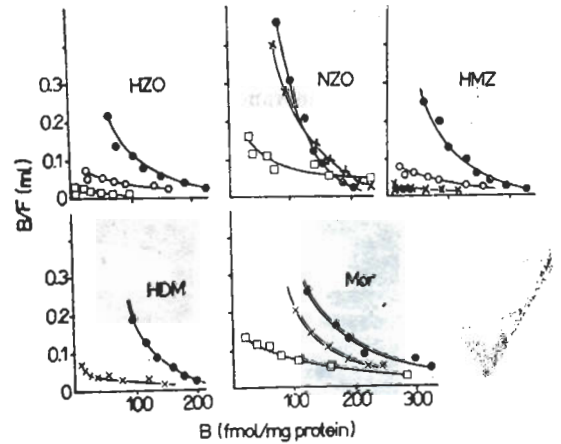


Fig 2. Scatchard plots showing recovery of reactivity after washing of opiate receptors bound with hydrazone derivatives of opiates. Method of treatment of P₂ membrane preparation with drugs and washes see legend of Fig 3. The curves were plotted by a PS-80 computer. (□: 2 washes, ×: 4 washes, ○: 6 washes, ●: control. n = 2)

涤,受点总结合力的恢复情况并与 NZO 及 Mor 进行了对比(图 3)看到 Mor 及 NZO 结合后洗涤 3-4 次, HMZ 洗涤 10 次, 受点活性可以完全恢复(图 3), 说明所试阿片类化合物与阿片受点的结合是可逆的。

对离体 GPI 的作用 NZO(0.63 μM)可完全对抗 0.63 μM Mor 对 GPI 电刺激收缩的抑制作用。洗涤 3 次, 这种作用即可被除去。HZO(0.075 μM), HMZ(0.1 μM)可以抑制 GPI 的电刺激收缩, 经 3-4 次洗涤亦可被去掉,

并且能被 N_x 逆转, 这些都与 Mor 的作用情况

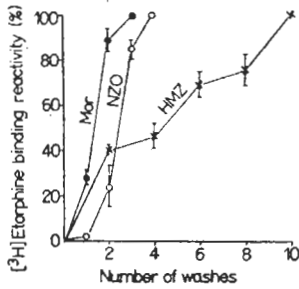


Fig 3. Recovery of binding reactivity of opiate receptors by repeated washing of the receptor bound with opiates. P₂ membrane preparation was incubated with HMZ (10 μM), NZO (5 μM) or Mor (10 μM) at 30°C for 15 min and then washed repeatedly (each washing consisted of centrifugation, 1000 × g for 20 min at 4°C, suspension with Tris buffer and incubation at 30°C for 15 min), then the [³H]Etor binding reactivity of opiate receptors was determined. Binding reactivity of P₂ membrane not treated with drugs (control) was taken as 100%. n = 4.



Fig 4. Effects of hydrazone derivatives of opiates on the longitudinal muscle of guinea pig ileum (GPI). A) Antagonistic effect of Nx or NZO against Mor was abolished by 3-6 washes (W × 3 or 6). B) Inhibition of GPI contraction by Mor, HZO or HMZ was abolished by 3 washes. C) Inhibition of GPI contraction by Mor, or HMZ was readily reversed by Nx.

相似(图 4), 表明阿片类对 GPI 的作用较易逆转。

镇痛作用 与阿片受体结合的牢固程度相一致, 阿片类镇痛持续时间亦较长(图 5, 表 1)。小鼠 sc 约可使 90% 产生镇痛作用的剂量后, 镇痛有效率下降到 50% 所需时间约比 Mor 长 2 倍。

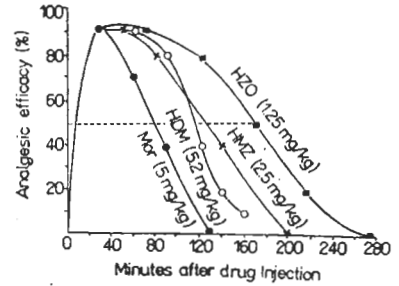


Fig 5. Duration of analgesia produced by hydrazone derivatives of opiates. Duration of analgesic effect of drug was tested on 10 mice by heating the tail at different intervals after sc injection. Time intervals needed for the analgesic efficacy to fade to 50% were compared and taken to be duration of analgesia. Number of repetitions of the experiment see Tab 1, the result shown here is that of 1 experiment.

小鼠 icv 毒性 小鼠 icv 阿片类化合物 HDM, HMZ, HZO 或 Mor (皆为 180 nmol/鼠), 每种药各 10 只均无死亡, 表明阿片类的毒性不大。

讨 论

本文试验看到 NZO 与阿片受体结合后可被洗脱, 结合的牢固程度与 Mor 相差不多, 但 Pasternak 等⁽³⁾ 则认为 NZO 对阿片受体高亲和力结合点有不可逆结合。最近他们报告⁽⁶⁾, 这主要是 NZO 的双分子结合产物 naloxonazine 的作用, 它对阿片受体的结合能力要比 NZO 强 20-30 倍。本文试验所用 NZO 在合成时曾反复纯化且经红外光谱及质谱鉴定, 在药理试验中, 药液又是在使用当天配制的, NZO 转变为双分子聚合物的数量不会很多, 这也可能是试验结果与他们报道不同的主要原因, 我们曾看

到 Nx 的胍衍生物 NZI 与阿片受点的结合也是可逆的, 这与我们用真正的不可逆配基奥派文 7 氮芥衍生物 α -CAO(即 14_a) 所得实验结果迥异⁽⁷⁾。

虽然 NZI 及 NZO 与阿片受点结合的牢固程度相近似, 但它们的 N-甲基类似物则不同。NZI 的 N-甲基类似物 HZI 与阿片受点结合并不牢固, 镇痛持续时间也与 Mor 相差无几⁽⁴⁾, 而 NZO 的 N-甲基类似物 HDM, HMZ, HZO 等与阿片受点结合则很牢固, 镇痛作用也比 Mor 长得多。

Portoghese 等^(8,9)曾合成过一些带有长效作用基团的阿片类化合物, 其中并无满意的长效镇痛剂, 他所报道的⁽⁸⁾纳曲酮 6 氮芥衍生物 CNA 及其相应的 N-甲基类似物 COA 毒性较大, 小鼠 icv 注射 CNA (4.8 nmol/鼠) 后有 12% 死亡。本报道中的阿片类化合物毒性小得多, 小鼠 icv 给药 (180 nmol/鼠) 后无死亡, 似为较有希望的镇痛剂类型。虽然阿片类镇痛时间较长, 但强度不高, 最强的 HZO 只有 Mor 的 11 倍。若能将胍基引入到芬太尼或奥派文等强效镇痛剂分子中去或可获得作用更强的长效镇痛剂。

阿片类与阿片受点高亲和力部分的结合较牢固, 相应地其镇痛作用也较持久。因之镇痛作用与高亲和力部分有关。一般认为镇痛作用与 μ 亚型阿片受点有关, 是否阿片受点的高亲和结合部分与 μ 亚型受点相当。张安中等⁽¹⁰⁾曾报道过与此一致的看法。

阿片类虽非阿片受点不可逆配基, 但当洗涤次数适宜时, 在低亲和力结合点活性恢复后高亲和力结合点仍被占据, 故可作为分辨高、低亲和力结合点的工具药。尤其是 NZO 的 N-甲基类似物与高亲和力部分的结合更为牢固, 并且又是激动剂, 用做工具药更有其特殊的价值。

致谢 田民同志参加镇痛测定方法的建立, 索彩玲同志进行了镇痛试验, 李北波同志编制了受点分析的计算机程序且进行了计算。

参 考 文 献

- 1 Snyder SH, Childers SR, Pasternak GW. Opiate receptors. In: Jacob J, ed. *Advances in pharmacology and therapeutics; 7th Int Cong Pharmacol*; vol 1. Oxford: Pergamon, 1979: 39-66
- 2 Pasternak GW, Childers SR, Snyder SH. *J Pharmacol Exp Ther* 1980; 214: 455
- 3 Pasternak GW, Hahn EF. *J Med Chem* 1980; 23: 674
- 4 李灵源、叶菜英、张佩文、田民、金荫昌. 药学报 1984; 19: 251
- 5 Scatchard G. *Ann NY Acad Sci* 1949; 51: 660
- 6 Hahn EF, Pasternak GW. *Life Sci* 1982; 31: 1385
- 7 李灵源、叶菜英、金荫昌. 科学通报 1984; 29: 385
- 8 Portoghese PS, Larson DL, Jiang JB, Takemori AE, Caruso TP. *J Med Chem* 1978; 21: 598
- 9 Portoghese PS, Larson DL, Jiang JB. *Ibid* 1979; 22: 168
- 10 Zhang AZ, Pasternak GW. *Eur J Pharmacol* 1980; 67: 323

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FOUR LONG ACTING ANALGESIC HYDRAZONE DERIVATIVES OF OPIATES WHICH BIND MORE FIRMLY WITH OPIATE RECEPTORS

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ABSTRACT Like morphine (Mor), the reaction of naloxazone (NZO) with opiate recep-

tors was found to be reversible. Rat brain (without cerebellum) P₂ membrane preparation

was incubated with NZO (5 μ M) for 15 min and washed 4 times and tested for [3 H]etorphine ([3 H]Etor) binding; the reactivities of both high and low affinity binding sites of opiate receptors were restored. Similar results was obtained with Mor (0.1 mM). The *N*-methyl analog of NZO especially those with double bond at 7-8, e.g, 14-hydroxymorphazone(HMZ) bound more firmly to opiate receptors. But even in the case of HMZ, the binding reactivity of the pretreated P₂ preparation could be recovered entirely after 10 washes. The effect of hydrazone derivatives on the longitudinal

muscle of guinea pig ileum (GPI) was readily abolished by 3-6 washes and was easily reversed by naloxone (Nx), showing the reversibility of this effect.

The *N*-methyl analogs are agonists. Their analgesic action lasted twice as long as that of Mor and their toxicity was quite low, with no death after icv injection of 180 nmol/mouse. They may be promising long-acting analgesics.

KEY WORDS narcotics, hydrazones, morphine, naloxone, endophin receptors