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阿片类药物在豚鼠离体回肠中的依赖性和耐受性

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Opioid dependence and tolerance in the guinea-pig ileum *in vitro*

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ABSTRACT A rapid and simple method for the quantitative determination of opioid dependence and tolerance in the guinea pig ileum was introduced. Dependence, as indicated by a strong contraction of the ileum when challenged with naloxone, was produced by incubating ileum from native guinea pig with opioid at 37℃ for 1–6 h. The response of the ileum to naloxone was time-dependent and directly related to the normorphine concentration in the incubation fluid (10 – 1000 nmol / L) and to the challenge dose of naloxone (10–1000 nmol / L). There were lower naloxone-precipitated withdrawal responses in the guinea pig ileum incubated with pethidine, nalorphine and ohmefentanyl than with fentanyl, U50488H and morphine. Buprenorphine showed no withdrawal response.

In spite of rapid development of dependence, segments of ileum incubated with normorphine at low concentration (10–300 nmol / L) showed no or slight tolerance to normorphine (3-fold). Only by incubating with high concentration of normorphine (1 μmol / L) was high tolerance dectected. The results indicate that opioid dependence and tolerance do not develop in parallel.

KEY WORDS normorphine; ohmefentanyl; ileum; drug tolerance; substance dependence

提要 本文介绍用豚鼠回肠测定阿片类药物身体依赖性和耐受性的简便方法. 豚鼠回肠和阿片类药物在37℃孵育 1–6 h. 纳洛酮可使其挛缩, 成瘾性较弱的药物烯丙吗啡、哌替啶和羟甲芬太尼的戒断性收缩, 弱于芬太尼、U50488H 和吗啡. 低成瘾性药物丁丙诺啡无戒断性收缩. 依赖性和耐受性的产生并不平行.

关键词 去甲吗啡; 羟甲芬太尼; 回肠; 药物耐受性; 物质依赖性

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整体动物测定阿片类药物的身体依赖性和耐受性, 耗药多、费时长, 因此有必要建立体外模型用于药物初筛. 豚鼠回肠是评价阿片类药物作用性质的常用生物标本, 它和中枢神经系统具有相似的阿片受体特性[1]. 虽然已在豚鼠回肠上建立依赖性模型[2-3], 但仍耗药较多或费时较长. 本文目的是在豚鼠回肠上建立一个快速简便地测定阿片类药物身体依赖性和耐受性的体外模型, 并同时定量地比较依赖性和耐受性的关系.

MATERIALS AND METHODS

去甲吗啡(normorphine)、U50488H {反式-3,4-二氯-*N*-甲基-*N*-[2-(1-吡咯)环已烷基]苯乙酰胺}由英国阿伯丁大学成瘾性药物研究室 Prof Kosterlitz 惠赠. 烯丙吗啡(nalorphine)、哌替啶(pethidine)由美国加州大学罗浩教授惠赠. 羟甲芬太尼(我所第五研究室化学组). 纳洛酮(上海医科大学). 丁丙诺啡(buprenorphine)(英国 Reckitt & Colman 公司). 吗啡(青海制药厂). 芬太尼(宜昌制药厂). 氯化乙酰胆碱(上海东风生化厂).

标本制备 豚鼠, ♂, 体重 261 ± SD 42 g, 断颈处死, 取出回肠, 用 Kreb's 液冲去肠腔内容物, 弃去近回盲部约 10 cm 回肠, 其余回肠剪成 4–5 cm 的数段, 将每段回肠悬挂于 6 ml 含阿片类药物的 Kreb's 液的浴管中, 通 95%O_2+5%CO_2, 37 ± 1℃温孵, 每隔 10–15 min 换一次营养液. 记录前 15 min 将标本以 1 g 静止张力固定于张力换能器.

依赖性测定[2] 豚鼠回肠在含阿片类药物的 Kreb's 液中孵育 4 h 后, 在无电刺激的条件下, 在浴管中加入纳洛酮, 记录回肠的收缩高度. 洗去纳洛酮后加入氯化乙酰胆碱

(ACh) 100 nmol/L，记录 ACh 引起的收缩高度．纳洛酮和 ACh 引起的收缩高度比值，作为戒断性收缩的指标．

耐受性测定　取同一豚鼠的两段回肠分别在含(处理组)和不含(对照组)阿片类药物的 Kreb's 液中培养 4 h，然后加入不同浓度的阿片类药物，测定其抑制电刺激收缩的高度，根据剂量反应曲线求得 IC_{50}．电刺激参数(1 ms, 60V, 15 s)．处理处测定 IC_{50} 时营养液中仍保留孵育浓度的阿片类药物．耐受性指标以 Q 表示，Q 为处理组和对照组 IC_{50} 的比值[6]．

RESULTS

豚鼠回肠对去甲吗啡的依赖性　豚鼠回肠和去甲吗啡在 37℃ 共同孵育 4 h 后，纳洛酮可使其挛缩(Fig 1)．在含去甲吗啡 100 nmol/L 的营养液中培养的豚鼠回肠，其纳洛酮催瘾戒断性收缩随培养时间延长而增加．然而在 37℃ 培养 6 h 以上的豚鼠回肠对 ACh 的敏感性降低．因此培养时间不应大于 6 h．

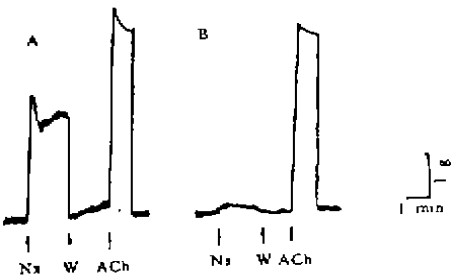

Fig 1. Naloxone (Nx, 100 nmol/L)-induced contraction in guinea pig ileum incubated with (A) or without (B) normorphine 100 nmol/L at 37 ℃ for 4 h. ACh 100 nmol/L. W: Wash.

豚鼠回肠的戒断性收缩也直接依赖于培养液中去甲吗啡浓度和催瘾的纳洛酮浓度(Fig 2)．对于依赖去甲吗啡的豚鼠回肠，我们选用纳洛酮 100 nmol/L 催瘾．

在含去甲吗啡 100 nmol/L 的营养液中培养 4 h 的豚鼠回肠，纳洛酮 100 nmol/L 可使其明显挛缩，平均张力比值为 0.70±0.07(n=7)；而对照组纳洛酮仅引起轻微收缩．平均张力比值为 0.10± 0.03(n=3)，P<0.01．如果豚鼠回肠同时和去甲吗啡 100 nmol/L、纳洛酮 100 nmol/L 共同孵育 4 h，则不出现戒断性收缩．

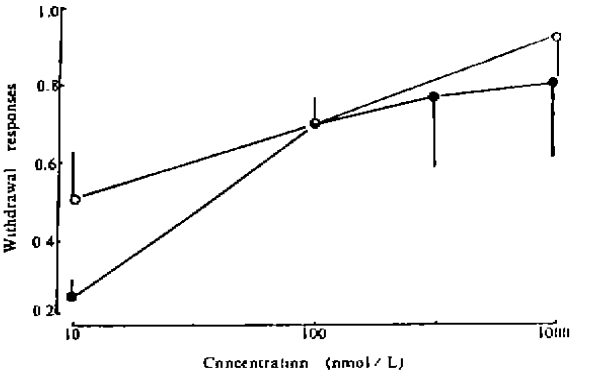

Fig 2. Withdrawal responses of guinea pig ileum after incubation with normorphine 100 nmol/L for 4 h to naloxone (○) or with normorphine (●) for 4 h to naloxone 100 nmol/L. Withdrawl responses are expressed as ratio of ACh 100 nmol/L responses. n=5–7 guinea pigs. $\bar{x}$ ±SD.

豚鼠回肠对不同阿片类药物的依赖性　U50488H 是高度选择性的 κ 配体，而纳洛酮对 μ 受体选择性较高．为了使豚鼠回肠对 U50488H 的依赖性充分表达，我们选用纳洛酮 1μmol/L 催瘾．豚鼠回肠在 10 倍 IC_{50} 浓度的各种阿片类药物中培养 4 h，其戒断性收缩见 Tab 1．吗啡、芬太尼、U50488H 均能产生较强的戒断性收缩．其张力比值分别为 0.71±0.13，0.97±0.04，0.86±0.18；羟甲芬太尼、哌替啶、烯丙吗啡均产生较弱的戒断性收缩．其张力比值分别为 0.30±0.06，0.52±0.20，0.48±0.18．而丁丙诺啡无戒断性收缩．

豚鼠回肠对去甲吗啡的耐受性　将豚鼠回肠培养在含去甲吗啡 100 nmol/L 的 Kreb's 液中，培养 1, 2, 4 h 后，去甲吗啡的 Q 值分别为 0.90±0.29，1.06±0.24，0.90±0.15，说明无耐受产生(Fig 3A)．在含去甲吗啡 100

Tab 1. Naloxone (1 μmol / L)-precipitated withdrawal responses of guinea pig ileum incubated with opioids at 37 ℃ for 4 h. Concentration of opioids was 10 × IC_{50}. Withdrawal responses were expresses as ratio of ACh 100 mmol / L responses. $\bar{x}$ ±SD.

Opioids (nmol / L)	Guinea pigs	Withdrawal responses
Morphine (3000)	4	0.71 ± 0.13
Fentanyl (10)	4	0.97 ± 0.04
U50488H (50)	6	0.86 ± 0.18
Ohmefentanyl (1)	6	0.30 ± 0.06
Pethidine (20000)	6	0.52 ± 0.20
Nalorphine (300)	6	0.48 ± 0.18
Buprenorphine (10)	6	0 ± 0

nmol / L 的 Kreb's 液中培养 4 h 的豚鼠回肠，去甲吗啡的 IC_{50} 为 219± 49 nmol / L，而对照组的 IC_{50} 为 245± 88 nmol / L，(n = 3)，两者无显著差异.

在低浓度去甲吗啡(10, 100 nmol / L)中培养 4 h 的豚鼠回肠不出现耐受；在去甲吗啡 300 nmol / L 中培养 4 h 仅出现轻度耐受 (Q = 2.9± 0.4)(Fig 3 B)，而在高浓度去甲吗啡 (1 μmol / L)中培养 4 h，则出现显著耐受. 该条件下高达 300 nmol / L 和 3μmol / L 的去甲吗啡仅分别抑制电刺激引起收缩的 16± 3%和 30± 4%. 由于更高浓度的去甲吗啡不能进一步抑制电刺激引起的收缩. 所以无法直接测定 IC_{50}，只能推测 Q 值大于 10.

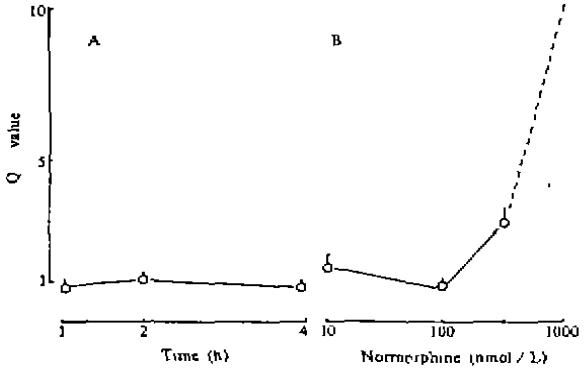

Fig 3. Q value after incubation (A) with normorphine 100 mmol / L for different time; (B) with normorphine for 4 h. Q value is index of tolerance. Q = $IC_{50(treated)}$ / $IC_{50(control)}$. (——) indicates Q > 10 with normorphine 1 μmol / L. n = 3–4 guinea pigs. $\bar{x}$ ± SD.

DISCUSSION

本文采用短时间孵育方法测定药物依赖性. 实验表明这种改良方法耗药省，实验周期短，条件易于控制，可广泛用于初筛阿片类药物的身体依赖性. 本文结果显示不同阿片类药物在豚鼠回肠上的戒断性收缩，和它们在人或动物上产生的身体依赖性程度基本一致. 如烯丙吗啡、哌替啶在人或动物上成瘾性较吗啡弱[7]. 在豚鼠回肠上的戒断性收缩也较小. 丁丙诺啡在大鼠上无戒断性症状[8]，在豚鼠回肠上也无戒断性收缩. 应该指出，吗啡的成瘾性在人或动物上大于芬太尼，但在豚鼠回肠上吗啡的戒断性收缩却比芬太尼低. 值得注意的是，我所合成的强效镇痛剂羟甲芬太尼的戒断性收缩较弱，该实验结果和猴子的成瘾性结果基本一致. 羟甲芬太尼在猴子上的成瘾性明显低于吗啡，稍弱于芬太尼(未发表资料). 也许该化合物对人体的成瘾性也较低，有待研究.

本实验结果也表明，耐受性和依赖性的产生缺乏平行性. 低浓度去甲吗啡(10, 100, 300 nmol / L)中孵育的豚鼠回肠，虽然已产生明显的依赖性，但是对去甲吗啡无耐受或耐受性很小.只有在高浓度去甲吗啡(1 μmol / L)中培养的豚鼠回肠，才对去甲吗啡既有依赖性，又有显著耐受. 这提示耐受性的产生可能与阿片类药物浓度有关，高浓度药物使豚鼠回肠较早产生耐受，而低浓度药物可能需要较长时间才能出现耐受. 实验中处理组的 IC_{50} 是在营养液中继续含有去甲吗啡的条件下测定的，因此不存在耐受性丧失问题. Schulz[9]曾提出当豚鼠回肠尚未出现耐受时，依赖性就迅速达到最大值，它不随耐受程度的增加而进一步加深. 我们的实验结果支持 Schulz 的观点，说明耐受性和依赖性两者可以分离.

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黄花夹竹桃次甙甲和乙对 Na^+,K^+–ATP 酶的抑制作用

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Inhibitory action of peruvoside and neriifolin on Na^+, K^+–ATPase

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ABSTRACT Effects of peruvoside and neriifolin, main components of neriperside, a tevetoside extracted from *Thevitia neriifolia* Juss, on Na^+, K^+–ATPase activities and on [^{3}H] ouabain binding to the Na^+, K^+–ATPase isolated from hearts of guinea pigs, dogs and cats and kidneys of guinea pigs and cats were compared with digoxin and ouabain. It was found that peruvoside and neriifolin inhibited Na^+, K^+–ATPase activities and they showed a strong competitive inhibition on [^{3}H] ouabain binding to the enzymes isolated from various tissues. A marked species difference existed as great as that of digitalis. The mechanism of action of these 2 drugs may be similar to that of digitalis. Their inhibitory effects on the enzyme activity were stronger than their positive inotropic effects, while both actions of digitalis were parallel quantitatively. There may be some differences in the modulation of the intracellular Ca^{2+} between neripersides and digitalis.

KEY WORDS peruvoside; neriifolin; digoxin; sodium, potassium adenosine triphosphatase; ouabain

提要 本文比较了 Per 和 Ner, Dig 和 Oua 对豚鼠和犬心等组织 Na^+, K^+–ATP 酶活力和[^{3}H]Oua 与其受点结合的影响. 发现 Per 和 Ner 对酶活力和[^{3}H]Oua 结合有抑制作用, 其种属差异性和洋地黄相同. 表明其作用机理与洋地黄相似. 但 Per 和 Ner 的抑酶作用超过 Oua, 而正性肌力作用并不超过 Oua, 此现象提示两类药作用机理, 尤其在调节细胞内 Ca^{2+} 方面可能存在某些不同.

关键词 黄夹次甙甲; 黄夹次甙乙; 地高辛; 钠,钾腺苷三磷酸酶; 哇巴因

黄夹甙(neriperside, 强心灵)是由黄花夹竹桃(*Thevitia neriifolia* Juss)果实中提取的混合甙[1]. 临床上用于治疗各种心力衰竭, 具有效果好、副作用轻、且资源丰富等优点[2,3]. 近年来发现其主要成分黄夹次甙甲

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