左右才达到最高浓度，其消除速率更缓慢，180 min 后它已成为各组织器官中含[^{3}H]OMF 浓度最高的组织，所以如若在短期内多次给药，就有可能造成 OMF 在脂肪中的过量积累.

[^{3}H]OMF 与血浆蛋白的结合试验表明 OMF 与血浆蛋白的结合率在镇痛浓度范围(0.02 − 1 ng/ml)之内与浓度无关，这与芬太尼在大鼠、狗、人上的实验结果[9]一致.但是 OMF 的结合率比芬太尼稍低.据报道[9]芬太尼与血浆蛋白的结合随温度升高而增加，因此如果在 37℃条件下，[^{3}H]OMF 与血浆蛋白的结合率会更高些.考虑到我们没有测定组织蛋白与药物的结合率[8]，所以在计算分布容积时，没有用血浆蛋白的结合率进行校正.

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松果体和褪黑激素对小鼠痛觉敏感性的影响

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Effects of pineal body and melatonin on sensitivity to pain in mice

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ABSTRACT Sensitivity to painful stimuli was measured by hot plate, writhing tests and electric caudal stimulation in mice. The mice were kept under light-dark 12 / 12 cycle with light out at 18:00 for at least 2 wk. Both basal analgesia and meperidine (pethidine)-induced analgesic effect exhibited parallel circadian rhythms, with the marked peak and through occurring at mid-dark and mid-light phases, respectively. The day-night differences in pain threshold 10 d after pinealectomy were not evident, especially in the loss of dark time augmentation of analgesic responses, but persisted in sham operated mice. Melatonin (MT) 50−200 mg / kg ip during light phase produced analgesic activity. MT 250 mg / kg ip resulted in a loss of the righting reflex. In pinealectomized mice, the pre-treatment of MT 5 mg / kg potentiated levels of analgesia induced by

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meperidine 10 mg / kg or morphine 5 mg / kg. It is possible that MT is one of the endogenous nocifensor inhibitors in CNS.

KEY WORDS pain measurement; circadian rhythm; meperidine; morphine; melatonin; pineal body

提要 在光暗周期 12 / 12 条件下，小鼠的基础痛阈和哌替啶的镇痛效应均呈现昼夜节律，谷在 12:00，峰在 24:00.松果体切除术取消这两种昼夜差异.褪黑激素(MT 50–200 mg / kg, ip)以剂量依赖方式产生镇痛效应.小剂量(10 mg / kg) MT 可增强哌替啶和吗啡的镇痛效应.大剂量可致翻正反射消失.

关键词 疼痛测量; 生理昼夜节律; 哌替啶; 吗啡; 褪黑激素; 松果体

松果体褪黑激素(抗变黑激素，melatonin, MT)的生物合成在交替性光暗周期中呈现昼夜节律[1].在 MT 合成达高峰值的黑暗时相，吗啡的镇痛效应增强[2]，故痛阈的昼夜变化与光照–松果体–MT 系统似有联系.我们曾报道[3–5]MT 对大脑皮层和海马神经元的自发和诱发放电均有直接抑制作用，提示其参与镇痛过程.本文拟以痛反应为指标，探讨松果体和外源性 MT 对痛阈昼夜变化的影响，为阐明 MT 参与机体抗伤害反应和做为药物应用的可能性提供某些依据.

MATERIALS AND METHODS

昆明种小鼠由福建医学院实验动物中心供应，♀，体重 20.5 ± SD 1.8g，45–60 d 龄.光暗周期为 12 / 12h.采用白色荧光灯照明，在 06.00–18.00h 开灯.笼底水平的光照度为 600–700 lx(用 ST–2 照度计测定).小鼠在此环境适应至少 2wk.在暗时相操作时采用 15 W 红色安全灯.室温 20–22℃.

热板法 用 GJ–8402 热板测痛仪测定痛阈.热板温度维持在 55 ± 0.5℃.自动记录自小鼠足底接触热板至出现舔后足或跳跃的时间即热板反应潜伏期作为痛阈指标[2].先测二次潜伏期的 $\bar{x}$ 作为给药前对照.

扭体试验 每只小鼠 ip 0.4% 醋酸溶液 0.2ml, 10–15 min 内出现腹部内缩，后肢伸展，臀部高抬，身体扭曲者为阳性反应，否则为阴性.

尾刺激法 参考文献[6]，并加以改进，先将小鼠装入自制固定器中，在尾部插入两根外径为 0.3mm 不锈钢电极，待小鼠安静时，经刺激器输出矩形脉冲，并调节刺激强度至小鼠发出与刺激频率一致的嘶叫声为止.刺激参数通常为 8–10V，2Hz，波宽为 5ms.每次刺激持续 5s，采用录音机记录嘶叫次数并在 SBR–1 双线示波器上同时观察声音信号和刺激信号.比较给药前后的嘶叫次数.

松果体切除术 用乙醚吸入麻醉小鼠，在顶骨人字缝上用自制的开颅器钻一直径为 4 mm 圆孔，取出骨片，在横窦与矢状窦汇合处夹出松果体(直径约 0.5mm)，仔细止血，放回骨片后缝合皮肤，并给予抗感染.术后 d 10 开始测痛阈.实验结束时断头取脑，检查松果体切除情况并进行松果体切片的组织学鉴定，如果发现松果体未被切除者，不予统计，对照组采用松果体假切除术，手术操作同上.

药物的配制 盐酸褪黑激素(Sigma)溶于 10%丙二醇的生理盐水中，临用前配制.ip 后 20 min 测痛阈.对照组 ip 等量溶媒.盐酸吗啡和盐酸哌替啶(pethidine, meperidine)均为沈阳第一制药厂产品,分别作 ip 和 sc.对照组 ip 等量生理盐水.采用方差分析和 t 检验进行统计学处理.

RESULTS

基础痛阈和哌替啶镇痛效应的昼夜节律 以热板法测痛阈，每 4 h 测一次 ($n = 10$)，24 h 共测 6 次，生理盐水组($n = 60$) 和哌替啶组($n = 60$)的潜伏期夜间峰值与白天谷值之间的差异显著($P < 0.01$，Fig 1)，两组的峰和谷与各自的 24 h 平均值(18 ± 3 s; 54 ± 15 s)比较也

有显著差异($P<0.05$).

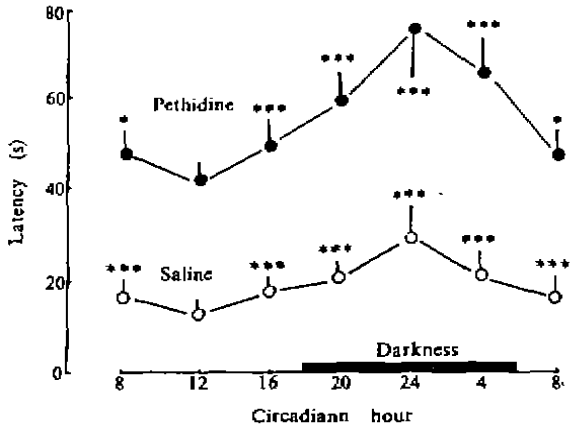

Fig 1. Circadian rhythm in thermal response latencies of mice treated with pethidine (30 mg / kg, sc) under light–dark 12 / 12 cycle. $n=10$, $\bar{x}$ ± SD. $^{*}P>0.05$, $^{***}P<0.01$ *vs* 12.00 h.

松果体切除术对痛觉昼夜差异的影响 在 12:00 和 24:00 所测的潜伏期(Fig 2)显示，松果体切除组的基础痛阈和哌替啶镇痛的昼夜差异均不显著($P>0.05$, $n=40$).假手术组和松果体完整(非手术)组的潜伏期的昼夜差异仍然显著($P<0.01$).

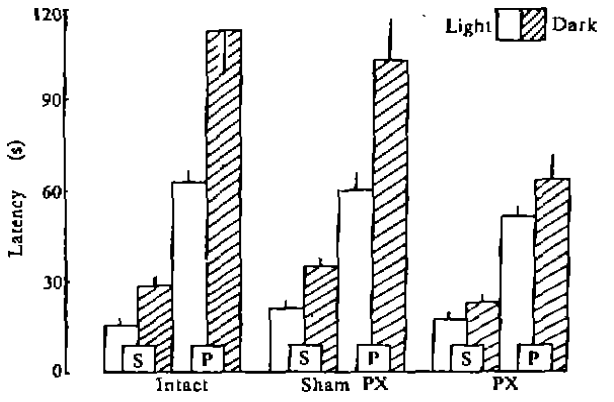

Fig 2. Effects of pinealectomy (PX) on basal pain threshold (saline, S) and pethidine (P) analgesia (40 mg / kg, sc) Day–night variations were abolished in pinealectomized mice but persist in intact or sham–operated animals kept under light–dark 12:12 cycle with light off at 18:00. Tests made during mid–light and mid–dark phases. $n=20$, $\bar{x}$ ± SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* mid–dark phase.

MT 对痛觉的影响 在 12:00 时 ip MT 潜伏期随剂量的递增而延长(Fig 3). 剂量在 250 mg / kg 除镇痛效应外，还伴活动减少，甚至丧失翻正反射，进入睡眠状态. 这通常发生于给药后 5–10 min，并于 40 min 后清醒.镇痛时程为 1.5–2.0 h.等容量 MT 溶媒对痛阈无明显影响($P>0.05$).

在扭体试验，ip MT 150 mg / kg 后发生阳性反应的鼠数(2 / 20)少于对照组(20 / 20, $P<0.01$).

MT 150 mg / kg 抑制电刺激鼠尾所致嘶叫. 由给药前 8.6 ± 0.6 次 / 5 s 减至 3.6 ± 1.6 次 / 5 s, $P<0.05$.

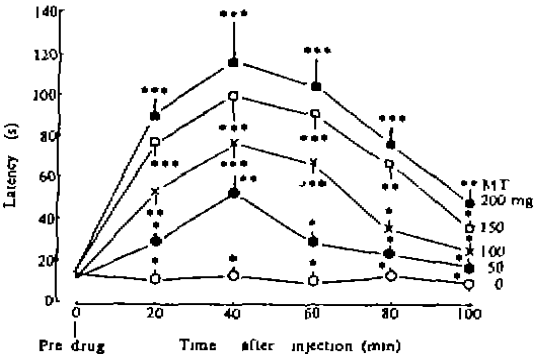

Fig 3. Effects of melatonin (MT, ip) on hot plate response latencies in intact mice. Administration made during mid–light phase (12:00). $n=10$, $\bar{x}$ ± SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* pre–drug. MT vehicle: 10 % propylene glycol.

MT 对阿片类药物镇痛效应的影响 在 12:00 测定松果体切除小鼠的潜伏期，结果如 Tab 1 所示，单独 ip 小剂量 MT 或哌替啶对痛阈无明显影响($P>0.05$). 当 MT 和哌替啶联合应用时，所产生的镇痛效应与对照组比较，差异显著($P<0.01$).镇痛时程持续 2 h，以同样方法观察 MT 对吗啡的镇痛效应的影响，发现 MT 也增强吗啡的效应($P<0.01$).

Tab 1. Effects of melatonin (MT) on pethidine (P) and morphine (Mor) analgesia in pinealectomized mice. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug (mg / kg)	mice	Latency (s)
Control	30	21.2 ± 4.3
P (10)	10	30.6 ± 4.5*
MT (5)	10	22.4 ± 2.6*
Mor (5)	10	50.9 ± 4.1**
MT (5) + P(10)	10	76.5 ± 4.8***
MT (5) + Mor (5)	10	80.8 ± 9.8***

DISCUSSION

在哺乳类动物，无论是昼行性或者夜行性的，MT 水平总是伴随黑夜升高[1,7,8].本研究观察到，小鼠的基础痛阈和哌替啶的镇痛效应均具有明显的昼夜节律，并与已知的 MT 节律基本同步.切除松果体则导致痛阈的昼夜节律消失. 这些提示，伴随黑夜而升高的痛阈与松果体的分泌活动增强有关.

在光照时相给予 MT 可明显提高小鼠对 3 种伤害性刺激的感受阈值，并且随着 MT 剂量的增加，其对 CNS 的抑制也相应加强.在松果体切除的小鼠，小剂量 MT 与阿片类镇痛药哌替啶和吗啡有协同镇痛作用，大剂量 MT 可致翻正反射消失. 据报道[9]，内源性阿片系统可促进 MT 释放.这些提示，MT 可能是 CNS 的一种内源性抗伤害性抑制物，并具有广泛的临床应用前景.

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