No evidence for a protracted change in endogenous opioid activity following chronic opiate treatment in mice: parallel recovery of cross tolerance to stress and morphine antinociception

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Abstract. The involvement of central endogenous opioids in swim-induced antinociception in mice is well documented. The response is attenuated by central or systemic naloxone, displays two-way cross tolerance with morphine and is correlated with apparent occupation of central opiate receptors by endogenous ligands. Swim-induced antinociception was utilised as an in vivo model of endogenous opioid function to investigate a possible protracted functional change in endogenous opioid release or inactivation following chronic opiate treatment. Antinociceptive responses (tail-flick latency) to morphine (4.4 mg/kg, SC) and swimming were determined at various times following chronic methadone (24 days treatment, 102 mg/kg day in drinking water for the last 20 days) and chronic morphine (1 g/kg sustained release) treatment. In both experiments, parallel recovery from cross tolerance was observed for morphine- and swim-induced antinociception. These results were consistent with the view that no protracted functional change in the release or inactivation of endogenous opioids had occurred following chronic opiate treatment.

Key words: Opiate - Withdrawal - Stress - Morphine - Methadone - Endorphins

The discovery of endogenous opioid systems has encouraged much speculation as to their possible role in narcotic, and other drug dependence. One possibility is that the continued presence of an exogenous opiate could induce a negative feedback suppression and a subsequent disuse atrophy of endogenous opioid systems (Goldstein 1978). The behaviour of the drug-free ex-addict during the phase referred to as protracted abstinence (Wikler 1980) might then be attributed in part to a deficiency in the function of endogenous opioid systems.

However, to date investigations of the possible involvement of endogenous opioid systems in the mechanism of narcotic tolerance, dependence and protracted abstinence have yielded largely negative results. Possible changes in opiate binding sites and endogenous opioid levels have been equivocal (Hollt and Wuster 1978; Kitano and Takemori 1979; Dum et al. 1979; Childers et al. 1977; Fratta et al. 1977; Wuster et al. 1980). Hollt et al. (1980) reported decreased in potassium-evoked release of met-enkephalin in superfused striatal slices from morphine tolerant/dependent rats, suggesting that there is no functional degeneration of met-enkephalin release. Malfroy et al. (1978) reported enhanced activity of a high affinity enkephalin degrading peptidase in mouse striatal membranes following chronic morphine treatment, suggesting possible enhanced enkephalin inactivation.

An indirect method to assess in vivo endogenous opioid function is suggested by the well-documented involvement of endogenous opioids in stress-induced antinociception. In mice, warm water swimming produces an antinociceptive response (Chesher et al. 1980) which is partially antagonised by intracranial (Christie et al. submitted) or systemic (Chesher et al. 1980) naloxone, shows two-way cross tolerance with morphine (Christie et al. 1982) and is correlated with the apparent occupation of brain opioid receptors by endogenous ligands (Christie et al. 1981; Christie 1982). These results are consistent with the hypothesis that central endogenous opioids are involved in swim-induced antinociception in mice. Swim-induced antinociception in mice should therefore provide a useful model to investigate possible changes in the in vivo function of endogenous opioid systems.

If a protracted functional modification were to develop in the release, receptor or post-receptor interactions, or degradation of endogenous opioids following chronic narcotic treatment, then it should be reflected in the swim-induced antinociceptive response. For example, if there were no protracted functional change in release or inactivation of endogenous opioids following chronic opiate treatment, then cross tolerance to morphine-induced antinociception and its recovery over a period of days should closely parallel cross tolerance to swim-induced antinociception. On the other hand, if a long-term alteration in release or degradation of endogenous opioids occurs, then cross tolerance to swim-induced antinociception should outlast that which occurs to morphine-induced antinociception. These possibilities were tested in the present study following chronic regimens of both morphine and methadone.

Materials and methods

Experiment 1. Chronic methadone treatment

Random bred female QS mice (18 - 24 g) from the University animal farm were housed in groups of 15 at 22 ± 1°C (12 h
light 42 h dark cycle) and allowed unrestricted food except at times of behavioural observation.

Methadone was administered to mice by its addition to normal drinking water. Methadone was considered a suitable opiate for oral administration, since it is well absorbed orally and has a long plasma half-life (Singh 1975). On the first day of treatment, a 0.4 mg/ml solution of methadone HCl was substituted for normal drinking water. This was increased to 0.8 mg/ml on the third day. Previous studies have suggested that methadone represents an aversive taste stimulus (Chipkin and Rosecrans 1978). Preliminary experiments indicated that animals would not ingest higher daily doses of methadone when the concentration was increased at 1.2 mg/ml. Treatment was therefore maintained at 0.8 mg/ml for a total of 21 days. No mortality was observed with this dose regimen. The daily dose (± SEM) for the last 20 days of treatment was 102 ± 2.8 mg/kg.

Independent groups of mice (18—26 animals/group) were tested for baseline, morphine-induced and swim-induced tail flick latency (TFL) responses 5, 10 and 20 days after withdrawal from oral methadone. TFL was determined as previously described (Chesher et al. 1980). Mice were restrained in a plexiglas cylinder (2.5 x 8.0 cm) closed at one end, with the tail protruding from the open end. The tail was immersed in water maintained at 55 ± 1°C and the time taken to withdraw the tail was recorded with a stopwatch. Each mouse was tested only once. TFL was determined blind as far as was possible (this could not be achieved for swum animals). For baseline TFL, mice were tested immediately on removal from the home cage. For morphine-induced antinociception, TFL was determined 30 min after injection of morphine HCl (4.4 base mg/kg, 10 ml/kg, SC). This dose of morphine was selected to produce a similar extent of antinociception to swimming and to approximate the ED₅₀ of morphine (Christie et al. 1982). Warm water swimming was performed as previously described (Chesher et al. 1980). Mice were swum in groups of six for 3 min in water at 32 ± 1°C (container; 29 x 20 x 15 cm, water depth 10 cm). TFL was determined immediately following swimming.

**Experiment 2. Chronic morphine treatment**

Mice were housed and tested for morphine and swim-induced antinociception as described in experiment 1. A sustained release preparation of morphine free base was utilised for chronic morphine treatment (Collier et al. 1972; Marshall et al. 1981). Animals were injected with a preparation (10 ml/kg SC) consisting of 7.5 % Arlacel A (Sigma, St. Louis, MO, USA), 42.5 % paraffin oil, 50 % normal saline and 1 g/kg morphine free base, or vehicle. Mortality for this dose was 3 %. Control animals received the same volume of vehicle. Baseline TFL responses were tested in independent groups of mice (15 animals/group) at various times after injection. Significant antinociception was observed up to 2 days after treatment (data not shown). The TFL response of morphine-pretreated mice did not significantly differ from vehicle pretreatment mice 4 days after treatment (data not shown) and was the same as vehicle-pretreated mice 7 days after treatment (see Fig. 2). Mice (30 animals/group) were then randomly assigned to one of eight treatment groups. Morphine-induced and swim-induced TFL responses were tested in morphine and vehicle-pretreated mice 8 and 16 days after injection. Animals tested on day 16 were tested again 24 days after injection.

**Experiment 1. Chronic methadone treatment**

Results for baseline, morphine-induced and swim-induced TFL response are shown in Fig. 1. These data were analysed by a 3-factor ANOVA (2 x 3 x 3, see Table 1) of the following design: Factor A, methadone, control; Factor B, naive, swim, morphine; Factor C, day 5, day 10, day 20. The following planned contrasts were analysed within factors B and C: B1, naive versus the average of swim and morphine; B2, swim versus morphine; C1, linear trend component (day 5 vs day 20); C2, quadratic trend component (day 10 versus average of days 5 and 20).

The ANOVA summary table is shown in Table 1. Significance levels were calculated from critical Bonferroni F values (Miller 1966). Significant contrast of interest were: B1, i.e. both swim and morphine produced antinociception; AB2, i.e. swim versus morphine was different in methadone-versus vehicle-pretreated animals; AC1, i.e. linear trend differed in methadone versus vehicle, demonstrating recovery from cross tolerance during the test period.

Nonsignificant contrasts of interest were: B2, i.e. there were no differences between morphine and swim; AB2C1, i.e. there were no differences in the linear trend of swim versus morphine between methadone- and vehicle-pretreated animals; AB2C2, i.e. there were no differences in the quadratic trend of swim versus morphine between methadone- and vehicle-pretreated animals.

**Experiment 2. Chronic morphine treatment**

Results for baseline, morphine-induced and swim-induced TFL responses are shown in Fig. 2. The results for day 8 and 16 were analysed by a 3-factor ANOVA (2 x 2 x 2, see Table 2) of the following design: Factor A, morphine, vehicle pretreatment; Factor B, swim, morphine; Factor C, day 8, day 16.