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No heroin or morphine 6β-glucuronide analgesia in μ-opioid receptor knockout mice

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Abstract

Recent reports suggest that heroin and its metabolite morphine 6β-glucuronide can produce analgesia independent of the morphine-preferring μ-opioid receptor. We have tested heroin and morphine 6β-glucuronide analgesia in wild-type, homozygous and heterozygous μ-opioid receptor knockout mice. Homozygotes display no heroin or morphine 6β-glucuronide analgesia. Heterozygous mice with one μ-opioid receptor gene copy reveal reduced heroin and morphine 6β-glucuronide analgesia. The μ-opioid receptor-dependence of heroin and morphine 6β-glucuronide fails to support a requirement for a heroin-specific opiate receptor subtype.

Keywords: Morphine; Pain; Opiate

Morphine is commonly used to treat pain. Heroin (3,6-diacetylmorphine) and its major metabolite morphine 6ß-glucuronide are also analgesic, although the mechanisms underlying the actions of these three compounds may be complex. In binding studies using rat brain membranes, for example, morphine 6β-glucuronide can displace binding of radiolabeled opiates more potently than morphine (Abbott and Palmour, 1988).

Recent studies in opiate receptor knockout mice (Matthes et al., 1996; Sora et al., 1997) demonstrate that morphine fails to induce analgesia without μ-opioid receptors. Heroin and its metabolites could work at these same μ-opioid receptors. Differences between the pharmacologies of morphine and morphine 6β-glucuronide are noted, however. Although they may be derived from differences in pharmacodynamic features including speed of in vivo compartmentalization (Stain et al., 1995), they could also conceivably be due to involvement of novel receptor types. A recently proposed novel opiate receptor subtype with especial affinity for morphine 6β-glucuronide could perform this function, for example (Rossi et al., 1996). Transgenic mice provide a useful approach to elucidating the roles of specific proteins in inducing antinociception (Matthes et al., 1996; Sora et al., 1997; Simonin et al., 1998). We have thus tested the influences of heroin and morphine 6β-glucuronide in thermal nociceptive tests in wild-type mice and in μ-opioid receptor knockout mice to assess possible differences in the μ-opioid receptor-dependence of the analgesic actions of these compounds.

Ten- to twelve-week-old μ-opioid receptor knockout mice and littermate controls were bred on a mixed C57/129Sv genetic background, genotyped and housed as described (Sora et al., 1997). Antinociceptive responses to heroin and morphine 6β-glucuronide were determined using hot-plate and tail-flick tests, and compared to values for morphine analgesia previously determined using the same tests (Sora et al., 1997). Antinociceptive effects were assessed in mice injected at 20-min intervals with saline and then with ascending heroin doses producing total drug doses of 0.1, 0.3, 1 and 3 mg/kg. Antinociceptive effects were also assessed in mice injected at 30-min intervals with saline and then with ascending morphine 6β-glucuronide doses producing total drug doses of 1, 3, 10 and 30 mg/kg. Statistical comparisons were made with

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ANOVAs (analysis of variance) and Scheffe post-hoc comparisons using the Statistical Package for Social Science (SPSS, Chicago, IL, USA).

In wild-type mice, heroin and morphine 6β-glucuronide induced maximal antinociception at 3 and 30 mg/kg in 55°C hot-plate and 53°C tail-flick thermal analgesia testing, respectively (Fig. 1). Heroin was 10-fold more potent than morphine, while morphine 6β-glucuronide was twice as potent (in comparisons with data from Sora et al., 1997). Neither heroin nor morphine 6β-glucuronide produced any significant analgesia in homozygous μ-opioid receptor knockout mice (Fig. 1). Both compounds produced slight hyperalgesic responses in μ-opioid receptor homozygotes, responses not found following morphine treatments (Sora et al., 1997). Heterozygous mice with one μ-opioid receptor gene copy displayed right- and downward shifts in heroin and morphine 6β-glucuronide antinociceptive dose–effect relationships (Fig. 1). These shifts were similar to previous observations concerning morphine-induced analgesia in these animals (Sora et al., 1997).

The results document a near-total dependence of the analgesic actions of heroin and its active metabolites on the presence of μ-opioid receptors. In recent preliminary data, cumulative heroin doses as high as 10 mg/kg also fail to induce any significant analgesia in homozygous knockout mice (Kitanaka, N., Sora, I. and Uhl, G., unpublished observations). Taken together, these results fit well with an emerging picture that suggests that much opiate and opioid analgesia depends on intact μ-opioid receptors. These data contrast with those of Schuller et al. (1997), however. These workers found that about 40% of homozygous μ-opioid receptor knockout mice displayed heroin- and morphine 6β-glucuronide-induced analgesia at doses lower than those used in the present studies. While these workers suggested that some heroin and morphine 6β-glucuronide analgesia might be mediated by receptors and/or pathway(s) differing from those that mediate morphine analgesia (Abbott and Palmour, 1988; Rossi et al., 1996), the present results provide no support for this view. The current data suggests that the antinociceptive responses from heroin, morphine 6β-glucuronide as well as

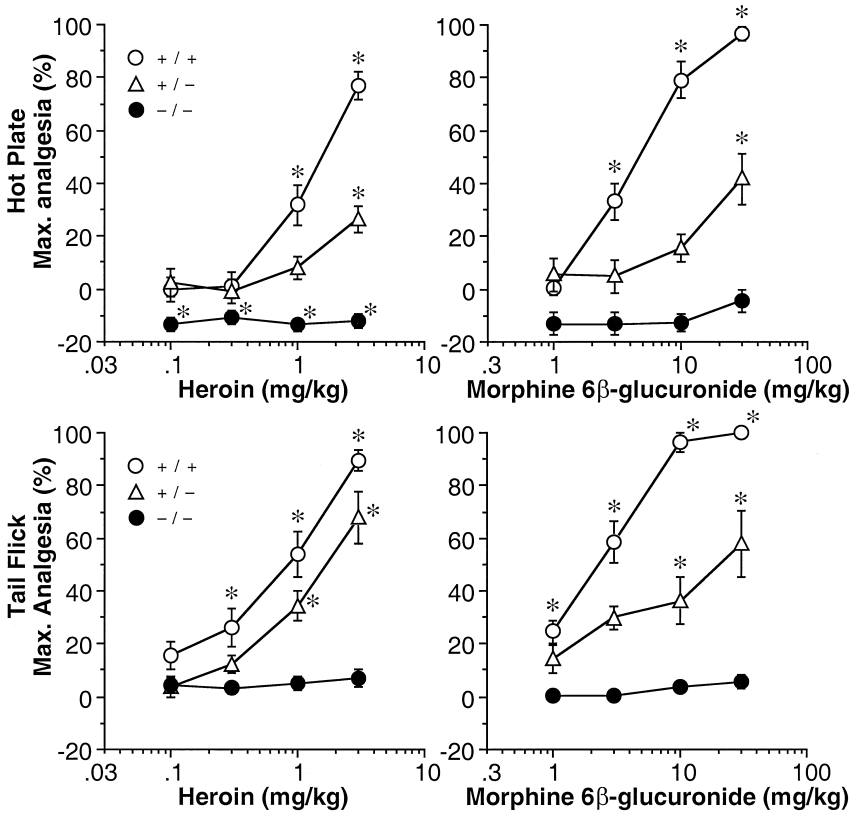

Fig. 1. Heroin- and morphine 6β-glucuronide-induced analgesia in μ-opioid receptor knockout mice. Mice, aged about 10 weeks, were injected s.c. with heroin or morphine 6β-glucuronide at the indicated concentrations. The percent maximal analgesia was calculated for each mouse as: 100 × {[(latency to hot plate or tail flick after drug) − (latency to hot plate or tail flick at baseline)]/[30 or 15 s cutoff time) − (baseline latency)]}. The results are indicated as the mean ± S.E.M. of 10–26 independent experiments. ∗ $P < 0.05$ compared with preinjection control values (termed 'zero' in figure) for the appropriate genotype. Latencies for hot plate and tail flick responses for heroin (3 mg/kg) were 24.6 and 14.2 s for (+/+), 11.6 and 11.6 s for (+/−) and 2.8 and 3.5 s for (−/−), respectively. The baseline values were 7.5 and 4.0 s for (+/+), 5.0 and 6.7 s for (+/−) and 5.7 and 2.6 s for (−/−), respectively. Latencies for hot plate and tail flick responses for morphine 6β-glucuronide (30 mg/kg) were 29.1 and 15 s for (+/+), 15.3 and 10.4 s for (+/−) and 5.8 and 3.3 s for (−/−), respectively. The baseline values were 7.3 and 3.6 s for (+/+), 4.8 and 4.1 s for (+/−) and 6.7 and 2.5 s for (−/−), respectively.

morphine all require the protein product of the μ-opioid receptor gene. These observations continue to motivate searches for μ-opioid receptor-specific sites and mechanisms as some of the best current candidate targets for the development of analgesic compounds.

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