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Visceral chemical nociception in mice lacking μ-opioid receptors: effects of morphine, SNC80 and U-50,488

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Abstract

Writhing responses to intraperitoneal acetic acid administration and their modulation by μ-, κ- and δ-opioid receptor agonists were compared in wild-type and μ-opioid receptor knockout mice. Unpretreated homozygous knockout mice displayed less writhing than wild-type mice. U-50,488 [*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrolidinyl)cyclohexyl]-benzeneacetamide]) reduced writhing responses in wild-type and knockouts. Morphine and SNC80 [(+)-4-[9-α-*R*)-α-(2*S*,5*RO*-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide] were effective in wild-type mice but ineffective in knockouts. μ-opioid receptors appear to play important roles in responses to this visceral nociceptive stimulus and its modulation by μ- and δ-opioid receptor agonists.

Keywords: Visceral pain; Writhing test; μ-Opioid receptor knockout mouse

Visceral pain is commonly accompanied by physiological signs that can include prominent changes in tone of skeletal muscle groups. Studies in humans and in common animal models of visceral pain both support the idea that opiate drugs can influence various features of this type of pain. Agents active at each of the opiate receptor subtypes (Schmauss and Yaksh, 1984) can modulate mouse writhing responses to visceral pain. Data from knockout mice indicates a surprising μ-opioid receptor dependence for morphine effects on thermally-induced nociceptive responses (Matthes et al., 1996; Sora et al., 1997b). Responses to agonists at δ- and κ-opioid receptors can also be substantially reduced in mice that lack μ-opioid receptors, although results of μ-opioid receptor deletion can differ in different experimental settings (Sora et al., 1997b; Matthes et al., 1998; I.S., M.F. and G. U., unpublished observations).

To test the influences of μ-opioid receptor deletion on unmodulated and opiate drug modulated models of visceral pain, we tested influences of pretreatments with saline and prototypic δ-, κ- and μ-opioid receptor agonists on acetic acid writhing in wild-type and μ-opioid receptor knockout mice. The μ prototype agonist morphine, the δ selective compound SNC80, [(+)-4-[9-α-*R*)-α-(2*S*,5*RO*-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*, *N*-diethylbenzamide] (Bilsky et al., 1995), and the κ-selective drug U-50,488, [*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrolidinyl)cyclohexyl]-benzeneacetamide]) were administered as pretreatments prior to acetic acid injection into mice of each genotype, and the writhing behavior induced by this visceroperitoneal nociceptive stimulus was compared.

μ-Opioid receptor homozygous knockout mice and wild-type littermates were bred and housed as previously described (Sora et al., 1997b). Mice were unpretreated, or injected subcutaneously with saline, morphine sulfate (NIDA Drug Supply Program), SNC80 (Tocris Cookson, Ballwin, MO) or U-50,488 hydrochloride (RBI, Natick, MA) (10 mg/kg) and returned to their home cages. Ten minutes later, a 0.7% solution of glacial acetic acid was injected intraperitoneally (i.p.) in a volume of 10 ml/kg, and mice were placed in Plexiglass observation chambers

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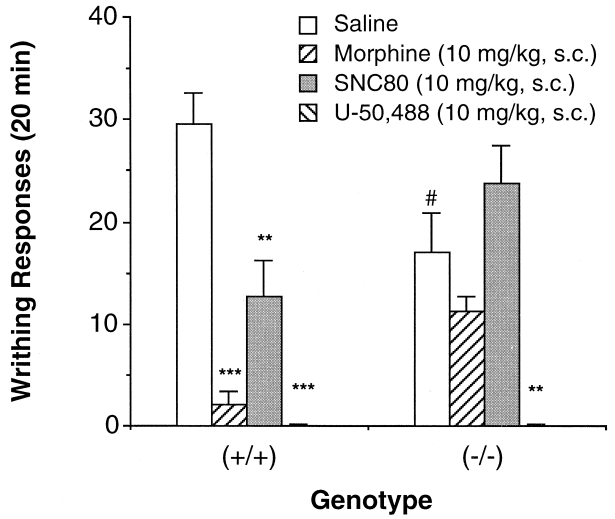

Fig. 1. Chemical antinociception induced by morphine, SNC80 or U-50,488. After saline pretreatment, μ-opioid receptor homozygous knockout ($-/-$, $n=10$) mice had significant less writhing induced by acetic acid in wild-type ($+/+$, $n=8$) mice ($\#P<0.05$). Morphine, SNC80 or U-50,488 (10 mg/kg, s.c.) induced significant antinociception against chemical stimuli in wild-type ($+/+$, $n=7$) animals ($***P<0.001$, $**P<0.01$, $***P<0.001$ vs. saline pretreated group, respectively). Homozygous ($-/-$, $n=15$) mice displayed significant antinociception induced by U-50,488 ($**P<0.01$ vs. saline pretreated group), failed to show antinociception induced by Morphine or SNC80, however. Data are presented as a mean ± S.E.M.

($18\times18\times18$ cm^3). The number of abdominal constrictions noted over the next 20 min were counted by an experienced observer blinded to drug pretreatment and genotype, as previously described (Collier et al., 1968). Statistical comparisons were made by the Statistical Package for Social Science (SPSS, Chicago, IL) with *t*-tests.

Writhing induced by acetic acid in naive μ-opioid receptor homozygous knockout mice (23.7 ± 3.8 per 20 min, $n=7$) was significantly less frequent than in wild-type mice (41.3 ± 5.5, $n=7$, $P<0.05$). Saline-pretreated homozygous mice also displayed significantly fewer writhing responses than unpretreated wild-type mice ($P<0.05$, Fig. 1). Deletions of μ-opioid receptors thus reduce this visceral chemical nociceptive response. Previous observations that writhing is enhanced by acute administration of opioid antagonists (Calvino and Le, 1986) contrast with our observation that lifelong absence of μ-opioid receptors decreases this viscero-chemical nociceptive response.

In wild-type mice, writhing was eliminated by pretreatment with the selective κ-opioid receptor agonist, U-50,488. It was reduced dramatically by the prototype μ agonist morphine, and reduced by 43% by the very highly selective δ-opioid receptor agonist, SNC80 (Bilsky et al., 1995, $P<0.001$ vs. the saline pretreated group for each, Fig. 1). By contrast, neither morphine nor SNC80 reduced writing responses in homozygous knockout mice (Fig. 1).

These data underscore and add to a growing body of evidence that support specific but complex interactions between exogenous and endogenous opioids and visceral nociceptive systems and responses. The data are in accord with previous reports suggesting that μ- and κ-opioid receptor agonists can powerfully suppress writhing responses, while δ-opioid receptor agonists have more modest efficacies (Schmauss and Yaksh, 1984). Thermal antinociception induced by morphine (Matthes et al., 1996; Sora et al., 1997b), the selective δ-opioid receptor agonist, [D-Pen^2, D-Pen^5] enkephalin (DPDPE; Sora et al., 1997a) and SNC80 (I.S. and G.U., unpublished observations) are also reduced in μ-opioid receptor knockout mice. The current results thus indicate that μ- and δ-opioid receptor agonists fail to reduce visceral chemical nociception, as they fail to reduce thermal nociception in these μ-opioid receptor knockouts.

The κ-opioid receptor agonist, U-50,488 retained significant antinociception against chemical stimuli in mice without μ-opioid receptors ($P<0.01$ vs. saline pretreated group, Fig. 1). Thus, μ-, δ- and κ-opioid receptor agonists each reduced writhing in wild-type mice, but in μ-opioid receptor knockout mice, only the κ-opioid receptor agonist was active. Agents with significant affinity for κ-opioid receptors have been reported to be active in suppressing responses to visceral chemical stimuli, while exerting smaller effects on thermally-evoked cutaneous reflexes (Schmauss and Yaksh, 1984). Since κ-opioid receptor agonists induced significant analgesia against visceral chemical stimuli in homozygous mice lacking μ-opioid receptors, κ-opioid receptor agonists could play special roles in modulating this sort of visceral pain.

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