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The μ-opioid receptor is necessary for [D-Pen^2,D-Pen^5]enkephalin-induced analgesia

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Abstract

Interactions between δ-opioid receptors and morphine-preferring μ-opioid receptor subtypes have been suggested. Availability of transgenic μ-opioid receptor knockout mice allows assessment of μ-opioid receptor roles in the analgesia produced by the classical δ-opioid receptor agonist [D-Pen^2,D-Pen^5]enkephalin (DPDPE) in hot-plate and tail-flick tests. DPDPE analgesia was dramatically reduced in μ-opioid receptor knockout mice in a gene-dose-dependent fashion. The analgesia induced by this classic δ-opioid receptor agonist depends on intact μ-opioid receptors, suggesting that selective δ-opioid receptor drugs may require μ-opioid receptor occupancies for full efficacy.

Keywords: DPDPE ([D-Pen^2,D-Pen^5]enkephalin); δ-Opioid receptor; μ-Opioid receptor knockout mouse

Morphine-preferring μ- and enkephalin-preferring δ-opioid receptors recognize opiate drugs and opioid peptides and are expressed in multiple brain regions, including circuits associated with nociceptive modulation (Lord et al., 1977; Mansour et al., 1995). Consequences of μ- and δ-opioid receptor occupancies may interact. In several test systems, the consequences of μ-opioid receptor occupancies can depend on the extent of δ-opioid receptor occupancy and visa versa (Traynor and Elliott, 1993). Interactions could occur in coexpressing neurons. μ- and δ-opioid receptor expression on different neuronal populations could also render the consequences of activating an 'upstream' receptor depending on the state of activation of a receptor expressed 'downstream' in the same pathway.

[D-Pen^2,D-Pen^5]Enkephalin (DPDPE) is a δ-opioid receptor agonist with more than 1000-fold greater potency at δ- than at other opioid receptor subtypes that can provide naloxone-reversible antinociception when administered intracerebroventricularly or intrathecally (Goldstein and Naidu, 1989; Jiang et al., 1990). These data have been taken to indicate specific δ-opioid receptor-mediated analgesia distinct from classical μ-opioid receptor-mediated analgesia mediated by drugs such as morphine.

Recent availability of transgenic μ-opioid receptor knockout mice (Sora et al., 1997; Matthes et al., 1996) allows exploration of the role of the μ-opioid receptors in DPDPE-induced analgesia in heterozygote animals deleted in one copy of the μ-opioid receptor gene that express about 50% of wild-type μ-opioid receptor levels and in homozygous knockout mice that lack detectable μ-opioid receptors.

μ-Opioid receptor knockout transgenic mice were maintained on C57/129Sv F2 or F3 genetic backgrounds as described (Sora et al., 1997). DPDPE (Research Biochemical International, 60 nmol in 5 μl distilled water) or vehicle was injected into the lateral cerebral ventricle by Hamilton syringe 15 min prior to behavioral testing (Haley and McCormick, 1957). Analgesia was tested using 55°C hot-plate and 53°C tail-flick assays as previously described, with values converted to percent maximal possible analgesic effects (%MPE) on each test to allow comparisons (Sora et al., 1997). Statistical comparisons were made with analyses of variance following by Scheffe post-hoc analyses for analgesia test data.

DPDPE induced antinociception in both hot-plate and tail-flick tests in wild-type mice (Fig. 1). No injected animal displayed signs of gross behavioral toxicity. No

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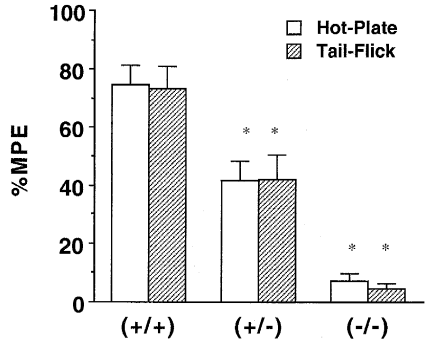

Fig. 1. The percent of the maximum possible analgesic effect induced by intracerebroventricular injection of DPDPE (60 nmol) 15 min before testing on 55°C hot-plate (open bars) and 53°C tail-flick (filled bars) testing in wild-type ($+/+$, $n=17$), heterozygous ($+/-$, $n=18$) and homozygous ($-/-$, $n=16$) μ-opioid receptor knockout animals. * $P<0.01$ statistically significant between wild-type, heterozygous and homozygous mice.

significant hemorrhage or gross injection trauma was noted in any of four initial injected mice when they were examined postmortem. DPDPE analgesia was reduced to about 50% in heterozygote mice with one μ receptor copy in both hot-plate and tail-flick tests ($P<0.01$). DPDPE analgesia was virtually eliminated in homozygotes ($P<0.01$). Vehicle injections produced no significant effects on hot-plate or tail-flick latencies in mice of any of the three genotypes (data not shown).

The present data suggest a virtually absolute requirement from μ-opioid receptor expression to allow expression of DPDPE analgesia. These results cannot distinguish whether the μ-opioid receptor/δ-opioid receptor interactions required to allow effective DPDPE analgesia occur in coexpressing neurons or in circuits expressing 'downstream' μ-opioid receptors and 'upstream' δ-opioid receptors. Interestingly, data from Jiang et al. (1990) also indicate that some δ-opioid receptor occupancy can be necessary for full μ-opioid receptor-mediated analgesia.

Some DPDPE analgesia might conceivably be due to interactions of DPDPE or its metabolites with μ-opioid receptors. However, DPDPE's potency at δ-opioid receptors, the other features of its pharmacological profile, and preliminary reports by Matthes et al. (1996) that another selective δ-opioid receptor agonist, Tyr-D-Ser(*O*-tert-butyl)-Gly-Phe-Leu-Thr(*O*-tert-butyl) (BUBU), also requires μ-opioid receptor expression for its analgesic function all suggest that the current results are unlikely to arise solely due to occult DPDPE analgesia acting directly at μ-opioid receptors.

Analgesic actions of agonists at both δ-opioid receptors and μ-opioid receptors are thus highly dependent on μ-opioid receptor expression. Other recent results also demonstrate substantial dependence of κ-opioid receptor-mediated analgesia on μ-opioid receptor expression (I.S., M.F. and G.R.U, data not shown). Drugs acting at μ-opioid receptors appear likely to remain mainstays of opiate-based pain relief.

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