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Cross-tolerance between analgesia produced by xylazine and selective opioid receptor subtype treatments

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Abstract

Opioid receptor agonists produce analgesia through multiple systems activated by stimulation of μ_1, μ_2, δ_1, δ_2 and κ_1 opioid receptors. Morphine analgesia is modulated by stimulation of α_2 adrenoceptors. To understand how multiple opioid analgesic systems interact with α_2-adrenoceptor systems, analgesic cross-tolerance between the α_2 adrenoceptor agonist xylazine and opioid receptor agonists was studied using the mouse tail-flick assay. Mice received either xylazine (20 mg/kg, s.c.) or saline (1 ml/kg) for five days. On day six, mice received a dose of s.c. xylazine, i.c.v. [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO), i.t. Tyr-Pro-Trp-Gly-NH_2 (Tyr-W-MIF-1), i.c.v. or i.t. [D-Pen^2,D-Pen^5]enkephalin (DPDPE), i.t. [D-Ala^2]deltorphin II (deltorphin II), or s.c. *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl-cyclohexyl] benzeneacetamide (U50,488). Xylazine tolerant mice required 4.57-fold more xylazine to elicit the same response as saline treated animals and showed a 2.55-fold shift in i.c.v. DAMGO and a 3.37-fold shift in i.c.v. DPDPE antinociception. No cross-tolerance was seen with i.c.v. deltorphin II, i.t.Tyr-W-MIF-1, i.t. DPDPE, i.t. Tyr-W-MIF-1 or s.c. U50,488. These results implicate α_2 adrenoceptor systems in the modulation of supraspinal μ_1, and δ_1 opioid analgesic circuitry and raise the possibility that μ_2, δ_2 or κ_1 opioid receptor agonists may be alternated with α_2 adrenoceptor agonists to minimize tolerance or treat opioid-tolerant patients.

Keywords: Opioid receptor subtype; Xylazine; DAMGO; DPDPE; Tyr-W-MIF-1; U50,488; α_2 adrenoceptor

1. Introduction

Much research has been devoted to pain treatments that avoid or delay the development of tolerance to the analgesic effects of opiates and other drugs. One such proposed treatment employs a rotating schedule between two drugs that produce analgesia via different mechanisms, such as α_2 adrenoceptor agonists and opioid receptor agonists (Stevens, 1994; Paul and Tran, 1995). Although this issue has proven to be of little importance to most pain patients receiving opioid receptor agonists, the fact that many analgesics are cross-tolerant with opioids is critical for opioid drug abusers that are in pain. However, the issue of cross-tolerance between these two classes of drugs remains controversial.

Many investigators have reported cross-tolerance to morphine analgesia in subjects tolerant to the analgesic effects of α_2 adrenoceptor agonists (Paalzow, 1978; Bentley et al., 1983; Milne et al., 1985; Post et al., 1988; Paul and Tran, 1995), whereas others have reported no cross-tolerance (Ben-Zvi et al., 1987; Stevens et al., 1988). Differences in route of administration, degree of tolerance development, model of analgesia, species and efficacy of analgesics have all been invoked as explanations for these divergent results (Stevens, 1994), but none of these explanations has proven completely satisfactory.

Opioids produce their analgesic effects through several pharmacologically and anatomically distinct systems activated by the various opioid receptor subtypes. These subtypes have been designated μ_1, μ_2, δ_1, δ_2, κ_1 and κ_3. However, only three types of opioid receptors have been cloned (MOR1, DOR1, KOR1) and the relationship between these gene products and the pharmacologically defined subtypes has yet to be clearly delineated. The discrepancies among opioid and α_2 adrenoceptor agonist cross-tolerance studies might be explained by the existence of multiple opioid receptor subtypes and the multiple analgesic systems stimulated by each. Antinociception can

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be produced by stimulating supraspinal μ_1, δ_1, δ_2 or κ_3 opioid receptors or spinal μ_2, δ_2, or κ_1 opioid receptors in mice (Heyman et al., 1987; Porreca et al., 1987; Heyman et al., 1988; Gistrak et al., 1989; Paul et al., 1989,1990; Millan, 1990; Jiang et al., 1991; Mattia et al., 1991; Gergen et al., 1996). Thus, there is the possibility that α_2 adrenoceptor agonists may be cross-tolerant with only some of these opioid receptor mediated systems. Accordingly, the current studies investigated shifts in the dose–response curves for i.c.v. [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO), i.c.v. and i.t.[D-Pen^2,D-Pen^5]enkephalin (DPDPE), i.c.v. deltorphin II, i.t.Tyr-Pro-Trp-Gly-NH_2 (Tyr-W-MIF-1) and s.c. *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl-cyclohexyl] benzeneacetamide (U50,488) after development of tolerance to the α_2 adrenoceptor agonist, xylazine.

2. Materials and methods

2.1. Subjects

Male, CD-1 mice (25–30 g; Charles River Breeding Laboratories, Wilmington, MA) were maintained on a 12-h light/dark cycle with ad libitum access to Purina Mouse Chow and water. I.t. and i.c.v. injections were made under light halothane anesthesia using Hamilton 10 μl syringe fitted to a 30-gauge needle with PE10 tubing. I.c.v. injections were administered approximately 2 mm caudal and 2 mm lateral to bregma at a depth of 3–4 mm (Haley and McCormick, 1957). I.t. injections were by lumbar puncture (Hylden and Wilcox, 1980). The injection volume for i.c.v. and i.t. injections was 1 μl and s.c. injections were 1 ml/kg.

2.2. Drugs and chemicals

Xylazine and U50,488 were purchased from Research Biochemicals (Natick, MA), deltorphin II, DAMGO and DPDPE from Peninsula (Belmont, CA), and yohimbine from Sigma (St. Louis, MO). Tyr-W-MIF-1 was a generous gift from Dr. James Zadina. Routes of administration were the same as those used previously to characterize the analgesic systems. Xylazine, yohimbine and U50,488 were administered s.c. Deltorphin II, DAMGO and DPDPE were all injected i.c.v. DPDPE and Tyr-W-MIF-1 were administered i.t.

2.3. Antinociception assay

Antinociception was determined using the radiant heat tail-flick technique (D'Amour and Smith, 1941; Paul et al., 1989, 1990). Briefly, the tail of each animal was exposed to a focussed light beam and the time to remove the tail measured electronically using a photocell. Baseline latencies (2.5–4.9 s) were determined before experimental treatments as the mean of two trials. Animals whose test latency was at least double their baseline latency were considered analgesic. A cut-off latency of 10 s was used to minimize tissue damage.

2.4. Tolerance development and testing

To develop tolerance to the analgesic effect of xylazine, subjects received five days of once daily s.c. administration of xylazine (20 mg/kg, s.c.). Control mice were administered saline (10 ml/kg, s.c.) for five days. Baseline tail-flick latencies were measured each day with testing for antinociception by probe doses on days one and five to measure tolerance while minimizing tissue damage. On the sixth day, baselines were recorded and various doses of an opioid receptor agonist were injected either i.t. (DPDPE: δ_1; Tyr-W-MIF-1: μ_2), i.c.v. (DAMGO: μ_1; DPDPE: δ_1; deltorphin II: δ_2) or s.c. (U50,488: κ_1; xylazine: α_2 adrenoceptor). Post-treatment tail-flick latencies were determined 30 min after s.c. injections or 15 min after i.c.v. or i.t. injections. These testing intervals were determined by pilot experiments. The analgesic dose–response curve for the opioid receptor agonist in xylazine tolerant mice was then compared to that of the mice treated with saline.

2.5. Data analysis

Dose–response curves were analyzed using a modification of the BLISS 20 program to determine the ED_{50} for each drug tested. This program maximizes the log-likelihood function to fit a parallel set Gaussian normal sigmoid curves to quantal dose–response data (Umans and Inturrisi, 1981). This program also uses the χ^2 statistic to test for

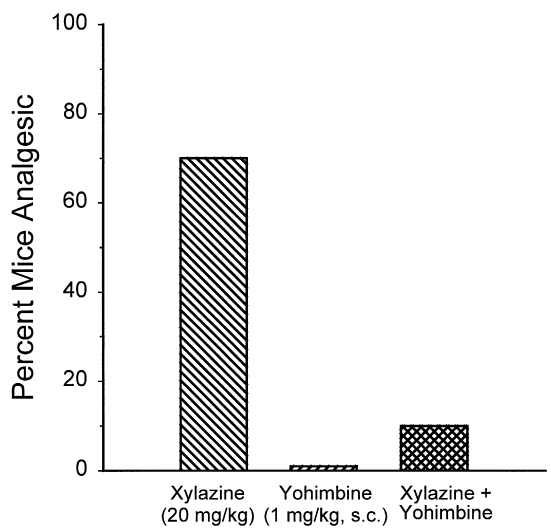

Fig. 1. Yohimbine blockade of xylazine antinociception. Groups of mice ($n = 10$) were injected with 20 mg/kg xylazine + saline, 1 mg/kg yohimbine + saline or xylazine + yohimbine and were tested for tail-flick antinociception 30 min later. Yohimbine blocked the antinociception produced by xylazine.

divergence from a parallel model and computes the 95% confidence interval for the relative potency of the control vs. experimental dose–response curves. Single dose comparison (yohimbine reversal study) was by the Fisher Exact test.

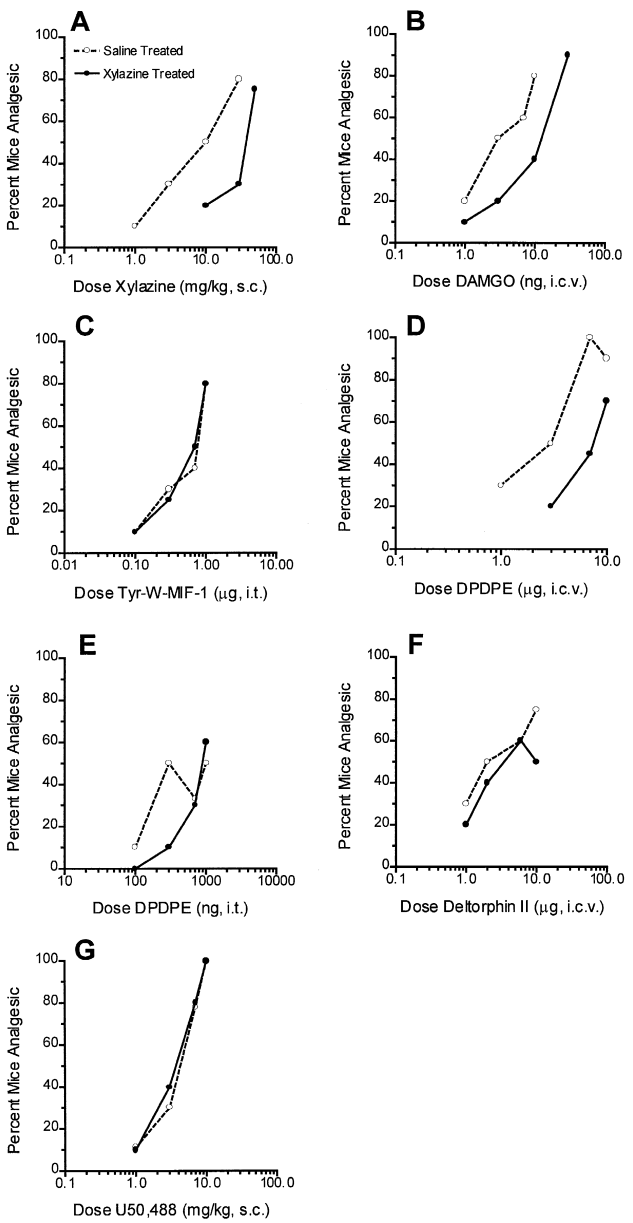

Fig. 2. Tolerance to xylazine and cross-tolerance to selective opioid agonist treatments. Groups of mice ($n = 10$) received five daily injections of either saline or xylazine s.c. On day six, mice received the indicated dose of (A) s.c. xylazine, (B) i.c.v. DAMGO, (C) i.t. Tyr-W-MIF-1, (D) i.c.v. DPDPE, (E) i.t. DPDPE, (F) i.c.v. [D-Ala^2]deltorphin II, or (G) s.c. U50,488. Mice were tested for tail-flick antinociception 30 min, thereafter, for drugs injected s.c. and 15 min thereafter for drugs injected i.c.v. or i.t. Five days of xylazine treatment shifted the xylazine dose–response curve 4.57 (c.i. 0.978–33.19)-fold rightward, the i.c.v. DAMGO dose–response curve 2.55 (c.i. 0.715–13.13)-fold rightward, and the i.c.v. DPDPE dose–response curve 3.37 (c.i. 1.39–15.74)-fold rightward. Other opioid dose–response curves were not affected.

3. Results

3.1. Yohimbine antagonism of xylazine antinociception

To confirm the selectivity of 20 mg/kg s.c xylazine for α_2 adrenoceptors, groups of mice ($n = 10$) were injected with this dose of xylazine with or without 1 mg/kg of yohimbine. This dose of yohimbine is selective for α_2 adrenoceptors (Hsu, 1982; Luttinger and Ferrari, 1995). Mice receiving both xylazine and yohimbine were injected with yohimbine 5 min prior to xylazine. Xylazine control mice were injected with saline 5 min prior to xylazine, whereas yohimbine control mice received saline 5 min after yohimbine. All mice were tested 30 min after the second injection. Saline + xylazine produced antinociception in 70% of mice (Fig. 1). In contrast, only 10% of mice treated with both yohimbine and xylazine were analgesic. As expected, none of the yohimbine + saline treated mice were analgesic.

3.2. Xylazine tolerance and cross-tolerance to opioids

To demonstrate tolerance development to the analgesic effect of xylazine, and assess cross-tolerance to antinociception produced by selective opioid treatments, groups of mice ($n = 10$) were injected for five days with either xylazine (20 mg/kg s.c.) or saline. Five days of treatment with xylazine produced tolerance to xylazine's analgesic effect (Fig. 2A). On day six, the dose–response curve for xylazine was shifted 4.57 (c.i. 0.978–33.19)-fold to the right. Five days of treatment with xylazine also produced a 2.55 (c.i. 0.715–13.13)-fold rightward shift in the analgesic dose–response curve for i.c.v. administration of the μ opioid receptor agonist DAMGO (Fig. 2B). However, xylazine treatment did not affect the dose–response curve for i.t. administration of the selective μ_2 opioid receptor agonist Tyr-W-MIF-1 (Fig. 2C). In xylazine tolerant mice, the dose–response curve for i.c.v. administration of the δ opioid receptor agonist DPDPE (Fig. 2D) was shifted 3.37 (c.i. 1.39–15.74)-fold shift to the right. In contrast, this treatment produced no shift in the dose–response curve for i.t. DPDPE (Fig. 2E) or i.c.v. administration of the δ_2 opioid receptor agonist, deltorphin II (Fig. 2F). Finally, five days of xylazine treatment did not affect the analgesic dose–response curve for s.c. administration of the κ_1 opioid receptor agonist, U50,488 (Fig. 2G).

4. Discussion

With the results of this series of studies, we demonstrated that the antinociception produced by α_2 adrenoceptor stimulation was cross-tolerant to the antinociception produced by stimulation of supraspinal μ_1 and δ_1 opioid

receptors, but not to the antinociception produced by supraspinal δ_2, and spinal μ_2, δ_1, and κ_1 opioid receptors. This supports the hypothesis that cross-tolerance between α_2 adrenoceptor and opioid receptor agonist antinociception is dependent upon the subtype of opioid receptor stimulated.

To confirm that the xylazine treatment produced antinociception via stimulation of α_2 adrenoceptors, we demonstrated that a dose of yohimbine that is selective for α_2 adrenoceptors (Wigdor and Wilcox, 1987) blocked the xylazine-produced antinociception.

Five days of α_2 adrenoceptor stimulation with xylazine produced tolerance to the analgesic effects of xylazine. In mice, antinociception can be produced by stimulation of supraspinal μ_1, δ_1 or δ_2 opioid receptors, or spinal μ_2, δ_1 and κ_1 opioid receptors. Mice that received five days of xylazine treatment were cross-tolerant to supraspinal μ_2 and δ_1 opioid receptor mediated antinociception. However, mice receiving the five-day xylazine treatments, although tolerant to xylazine, were not cross-tolerant to the antinociception produced by supraspinal δ_2 or spinal μ_2, δ_1 and κ_1 opioid receptors.

The finding that the antinociception produced by xylazine is cross-tolerant with the antinociception produced by i.c.v. DAMGO and i.c.v. DPDPE implicates the involvement of α_2 adrenoceptors in the mediation of descending inhibitory systems driven by activation of μ_1 and δ_1 opioid receptors. This mechanism has been proposed for cross-tolerance between antinociception produced by morphine and α_2 adrenoceptor agonists (e.g., Stevens, 1994). In this scenario, the administration of the opioids activates descending inhibitory systems that use norepinephrine as a neurotransmitter. The release of norepinephrine stimulates α_2 adrenoceptors in the spinal dorsal horn, resulting in a direct or indirect inhibition of projection neurons that mediate pain. When these α_2 adrenoceptors are repeatedly stimulated with xylazine, tolerance is produced through pharmacodynamic (Mutlib et al., 1992; Portenoy, 1994) or associative (Goudie and Griffiths, 1986) mechanisms. Because tolerance is developed for the norepinephrine/α_2 adrenoceptor intermediary, the stimulation of μ_1 or δ_1 opioid receptors is less effective in producing antinociception.

No cross-tolerance between the antinociception produced by xylazine and i.c.v. deltorphin II was observed in these studies. This may be explained by the possibility that i.c.v. deltorphin II antinociception may involve descending inhibition mediated by a neurotransmitter other than norepinephrine, such as 5-hydroxytryptamine. A second possibility may be that deltorphin II does not involve descending inhibition and produces antinociception through cerebrofugal mechanisms. In either case, this novel finding is important for two additional reasons. First, this finding disproves the hypothesis that opioid cross-tolerance to α_2 adrenoceptor agonists depends upon whether the opioid receptors that are stimulated are spinal or supraspinal. Second, it is evident that the analgesic systems that are driven by activation of the two δ-opioid receptor subtypes involve different neural mechanisms.

Current theory for local dorsal horn circuitry suggests descending inhibitory neurons are able to directly inhibit neurons as they synapse with the ascending pain pathways. This theory also holds that enkephalinergic inhibitory neurons link descending inhibitory neurons and nocisponsive projection neurons. These enkephalinergic inhibitory neurons are then believed to pre- and post-synaptically inhibit stimulation of projection neurons by primary afferents.

The finding that tolerance to the analgesic effect of xylazine did not produce cross-tolerance to the antinociception produced by i.t. injection of opioid receptor agonists is in agreement with this theory of local dorsal horn circuitry. If these spinal opioidergic interneurons are either not involved with or post-synaptic to the norepinephrine/α_2 adrenoceptor descending inhibitory system, then development of tolerance to an α_2 adrenoceptor agonist should have no effect on spinal μ_2, δ_2 or κ_1 opioid receptor mediated antinociception.

In addition to the issue of cross-tolerance, there has been considerable attention to the analgesic synergy of opioid receptor–α_2 adrenoceptor agonist combinations. The analgesia produced by combinations of morphine or DPDPE with clonidine is greater than would be expected by an additive model (Roerig and Fujimoto, 1989; Roerig et al., 1992). These results are in agreement with the interpretation that μ- and δ opioid systems are neuroanatomically linked to NE systems in the spinal cord. In mice made tolerant to morphine analgesia using a pellet implantation procedure, Roerig (1995) demonstrated a reduction in morphine's analgesic synergy with clonidine. Roerig proposed that tolerance may be related to a reduction in the synergy between adrenergic systems and morphine. In contrast, Fairbanks and Wilcox (1999) reported that development of tolerance to morphine does not attenuate the ability of this drug to produce synergy with clonidine. Interestingly, Roerig reported cross-tolerance to clonidine whereas Fairbanks and Wilcox reported no observable cross-tolerance. Although many factors may be invoked to explain these disparate results, it seems evident that synergy and cross-tolerance are closely related.

Alternating between opioid and α_2 adrenoceptor agonists has been proposed for the treatment of chronic pain patients (Stevens, 1994; Stevens et al., 1988; Paul and Tran, 1995). However, this may be a problem only in patients who are treated for years and in patients with a history of opioid abuse (Portenoy, 1994). In the latter group, the use of an analgesic that is not cross-tolerant with the abused drug may be the only way these patients can achieve satisfactory pain management. The present studies emphasize the importance of understanding multiple opioid analgesic systems and how they interact with α_2 adrenoceptor agonists. If the findings of these experiments are confirmed in clinical trials, the use of a rotating

schedule between an α_2 adrenoceptor agonist and a non-cross-tolerant opioid receptor agonist should be employed to avoid adverse drug effects and tolerance in the treatment of chronic pain patients who develop tolerance. For example, in chronic pain patients, a rotating schedule of α_2 adrenoceptor agonist and a δ_2, κ_1 or μ_2 opioid receptor agonist would be likely to minimize tolerance while providing satisfactory pain relief. Similarly, a heroin addict may achieve some level of analgesia using α_2 adrenoceptor agonists.

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References

Ben-Zvi, Z., Graham, C.E., Hurwitz, A., 1987. Tolerance to effects of clonidine and morphine on sulfobromophthalein disposition in mice. Life Sci. 40, 1617–1623.

Bentley, G.A., Newton, S.H., Starr, J., 1983. Studies on the antinociceptive action of alpha-agonist drugs and their interactions with opioid mechanisms. Br. J. Pharmacol. 7, 125–134.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Fairbanks, C.A., Wilcox, G.L., 1999. Spinal antinociceptive synergism between morphine and clonidine persists in mice made acutely or chronically tolerant to morphine. J. Pharmacol. Exp. Ther. 288, 1107–1116.

Gergen, K.A., Zadina, J.E., Kasten, A.J., Paul, D., 1996. Intrathecal Tyr-W-MIF-1 produces potent, naloxone-reversible antinociception modulated by alpha$_2$-adrenoceptors. Eur. J. Pharmacol. 298, 235–239.

Gistrak, M.A., Paul, D., Hahn, E.F., Pasternak, G.W., 1989. Pharmacological actions of a novel mixed opioid agonist/antagonist: naloxone benzoylhydrazone. J. Pharmacol. Exp. Ther. 251, 469–476.

Goudie, A.J., Griffiths, J.W., 1986. Behavioral factors in drug tolerance. Trends Pharmacol. Sci. 7, 192–196.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. Chemother. 12, 12–15.

Heyman, J.S., Mulvaney, S.A., Mosberg, H.I., Porreca, F., 1987. Opioid delta receptor involvement in supraspinal and spinal antinociception in mice. Brain Res. 420, 100–107.

Heyman, J.S., Williams, C.I., Burks, T.F., Mosberg, H.I., Porreca, F., 1988. Dissociation of opioid antinociception and central gastrointestinal propulsion in the mouse: studies with naloxonazine. J. Pharmacol. Exp. Ther. 245, 238–243.

Hsu, W.H., 1982. Xylazine-induced delay of small intestinal transit in mice. Eur. J. Pharmacol. 83, 55–60.

Hylden, J.L.K., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Jiang, Q., Takemori, A.E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, H.I., Porreca, F., 1991. Differential antagonism by [D-Ala^2,Cys^6]enkephalin and naltrindole 5′-isothiocyanate: evidence for subtypes. J. Pharmacol. Exp. Ther. 257, 1069–1075.

Luttinger, D., Ferrari, R., 1995. Pharmacological analysis of alpha-2 adrenergic mechanisms in nociception and ataxia. J. Pharmacol. Exp. Ther. 232, 883–889.

Mattia, A., Vanerhah, T., Mosberg, H.I., Porreca, F., 1991. Lack of antinociceptive cross-tolerance between [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2]deltorphin II in mice: evidence for delta receptor subtypes. J. Pharmacol. Exp. Ther. 258, 583–587.

Millan, M.J., 1990. κ-opioid receptors and antinociception. Trends Pharmacol. Sci. 11, 70–75.

Milne, B., Cervenko, F., Jhamandas, K., Loomis, C., Sutak, M., 1985. Antinociception and tolerance to intrathecal morphine and norepinephrine infusion via implanted mini-osmotic pumps in the rat. Pain 22, 165–172.

Mutlib, A.E., Chui, Y.C., Young, L.M., Abbott, F.S., 1992. Characterization of metabolites of xylazine produced in vivo and in vitro by LC/MS/MS and by GC/MS. Drug Metab. Dispos. 20, 840–848.

Paalzow, G., 1978. Development of tolerance to the analgesic effect of clonidine in rats: cross-tolerance to morphine. Naunyn-Schmiedeberg's Arch. Pharmacol. 304, 1–4.

Paul, D., Bodnar, R.J., Gistrak, M.A., Pasternak, G.W., 1989. Different mu receptor subtypes mediate spinal and supraspinal analgesia in mice. Eur. J. Pharmacol. 168, 307–314.

Paul, D., Levison, J.A., Howard, D.G., Pick, C.G., Hahn, E.F., Pasternak, G.W., 1990. Naloxone benzoylhydrazone (NalBzoH) antinociception. J. Pharmacol. Exp. Ther. 255, 769–774.

Paul, D., Tran, J.G., 1995. Differential cross-tolerance between antinociception produced by alpha$_2$-adrenoceptor agonists and receptor subtype selective opioid treatments. Eur. J. Pharmacol. 272, 111–114.

Porreca, F., Heyman, J.S., Mosberg, H.I., Omnaas, J.R., Vaught, J.L., 1987. Role of mu and delta opioid receptors in the supraspinal and spinal analgesic effects of [D-Pen^{2,5}]enkephalin in the mouse. J. Pharmacol. Exp. Ther. 241, 393–398.

Portenoy, R.K., 1994. Opioid tolerance and responsiveness: research findings and clinical observations. In: Gebhart, G.F., Hammond, D.L., Jensen, T.S. (Eds.), Proceedings of the 7th World Congress on Pain. IASP, Seattle, pp. 595–619.

Post, C., Archer, T., Minor, B.G., 1988. Evidence for cross-tolerance to the analgesic effects between morphine and selective α_2-adrenoceptor agonists. J. Neural Transm. 72, 1–9.

Roerig, S.C., Fujimoto, J.M., 1989. Multiplicative interaction between intrathecally and intracerebroventricularly administered mu opioid agonists but limited interactions between delta and kappa agonists for antinociception in mice. J. Pharmacol. Exp. Ther. 249, 762–768.

Roerig, S.C., Lei, S., Kitto, K., Hylden, J.K.L., Wilcox, G.L., 1992. Spinal interactions between opioid and noradrenergic agonists in mice: multiplicativity involves delta and alpha-2 receptors. J. Pharmacol. Exp. Ther. 262, 365–374.

Stevens, C.W., 1994. Perspectives on opioid tolerance from basic research: behavioural studies after spinal administration in rodents. Cancer Surv. 21, 25–98.

Stevens, C.W., Monasky, M.S., Yaksh, T.L., 1988. Spinal infusion of opiate and alpha-2 agonists in rats: tolerance and cross-tolerance studies. J. Pharmacol. Exp. Ther. 244, 63–70.

Umans, J.G., Inturrisi, C.E., 1981. Pharmacodynamics of subcutaneously administered diacetylmorphine, 6-acetyl morphine and morphine in mice. J. Pharmacol. Exp. Ther. 218, 409–415.

Wigdor, S., Wilcox, G.L., 1987. Central and systemic morphine-induced antinociception: contribution of descending serotonergic and noradrenergic pathways. J. Pharmacol. Exp. Ther. 242, 90–95.