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Intrathecal Tyr-W-MIF-1 produces potent, naloxone-reversible analgesia modulated by α_2-adrenoceptors

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Abstract

Spinal administration of morphine or [D-Ala^2,MePhe^4,Gly(ol)^5)]enkephalin (DAMGO) produces potent, naloxone-reversible analgesia that is modulated by α_2-adrenoceptors. Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) is a naturally occurring, amidated tetrapeptide that is structurally related to the melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1) family of endogenous peptides. Tyr-W-MIF-1 displays high selectivity for the μ-opioid receptor. We investigated the effect of spinal administration of Tyr-W-MIF-1 on analgesia using the mouse tail-flick assay. Intrathecal (i.t.) administration of Tyr-W-MIF-1 produced a dose-dependent analgesic response, with an ED_{50} of 0.41 μg, that was reversed by naloxone. Pretreatment with the μ-opioid receptor-selective antagonist β-funaltrexamine blocked the effect of i.t. Tyr-W-MIF-1. However, pretreatment with the μ_1-opioid receptor-selective antagonist naloxonazine did not antagonize the analgesia, indicating the effect was mediated through spinal μ_2-opioid receptors. Pretreatment with desipramine, an inhibitor of norepinephrine reuptake, potentiated the analgesic effect of i.t. Tyr-W-MIF-1, producing a 3.1-fold leftward shift in the dose-response curve. Spinal administration of yohimbine, an α_2-adrenoceptor-selective antagonist, significantly attenuated the analgesic effect of Tyr-W-MIF-1. Thus, the potent analgesic effect of i.t. Tyr-W-MIF-1 is mediated through spinal μ_2-receptors, and is modulated by norepinephrine and α_2-adrenoceptors.

Keywords: Analgesia; μ-Opioid receptor; Spinal cord; Desipramine; α_2-Adrenoceptor; Neuropeptide

1. Introduction

The intrathecal (i.t.) administration of morphine or the μ-opioid receptor-selective peptide [D-Ala^2,MePhe^4, Gly(ol)^5]enkephalin (DAMGO) produces analgesia that is mediated by μ-opioid receptors (Ward and Takemori, 1983; Heyman et al., 1988; Paul et al., 1989). There is considerable evidence implicating the neurotransmitter norepinephrine as a modulator of the antinociceptive effects of μ-opioid receptor-selective agonists in the spinal cord. The analgesic effects of spinally acting morphine are potentiated by spinal norepinephrine and α-adrenoceptor agonists (Hylden and Wilcox, 1983; Sullivan et al., 1987). I.t. injection of desipramine, a norepinephrine reuptake inhibitor, potentiates analgesia induced by i.c.v. morphine (Larsen and Arnt, 1984) and pretreatment with desipramine potentiates the analgesia induced by i.t. DAMGO (Paul and Hornby, 1995). Conversely, subcutaneous (s.c.) or i.t. injection of α-adrenoceptor antagonists attenuates morphine analgesia (Camarata and Yaksh, 1985; Jensen and Yaksh, 1986) and i.t. injection of yohimbine, an α_2-adrenoceptor-selective antagonist, attenuates antinociception induced by both norepinephrine (Hylden and Wilcox, 1983) and morphine (Wigdor and Wilcox, 1987).

Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) is a brain peptide recently isolated from human cortex (Erchegyi et al., 1992) and bovine hypothalamus (Hackler et al., 1993) that is structurally related to the melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1) family of endogenous peptides (Reed et al., 1994). It binds to opiate receptors with high selectivity for the μ-opioid site (Zadina et al., 1994a) as well as to specific nonopiate Tyr-MIF-1 sites (Zadina et al., 1990). Tyr-W-MIF-1 has been shown to produce prolonged, naloxone-reversible analgesia after i.c.v. administration in rats (Zadina et al., 1993; Gergen et al., 1994). The aim of the present study was to determine whether Tyr-W-MIF-1, like other μ-opioid receptor ago-

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nists, produces antinociception after i.t. administration that is mediated by μ-opioid receptors and modulated by norepinephrine.

2. Materials and methods

Male CD-1 mice (25–40 g; Charles River Breeding Laboratories, Wilmington, MA, USA) were maintained on a 12 h light/dark cycle with ad libitum access to Purina Mouse Chow and water. I.t. injections were made under light halothane anesthesia with a 10-μl Hamilton syringe fitted to a 30-gauge needle with PE10 tubing. I.t. injections were by lumbar puncture (Hylden and Wilcox, 1980) with an injection volume of 1 μl. S.c. injections were made in a volume of 1 ml/kg. All experimental groups were weight- and age-matched with controls.

Tyr-W-MIF-1 was synthesized as described previously (Erchegyi et al., 1992). Desipramine and naloxone hydrochloride were purchased from Sigma (St. Louis, MO, USA), and yohimbine hydrochloride from Research Biochemicals International (Natick, MA, USA). β-Funaltrexamine was obtained from the Research Technologies Branch of NIDA. Naloxonazine was a gift from Dr. Gavril Pasternak. β-Funaltrexamine (40 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were injected 24 h before treatment. Desipramine (5 mg/kg, s.c.) was injected 1 h before treatment. Naloxone hydrochloride (0.1, 0.3, 0.6 and 1.0 mg/kg, s.c.) was injected 15 min before treatment whereas yohimbine hydrochloride (1 μg, i.t.) was injected simultaneously with i.t. Tyr-W-MIF-1. All drugs were dissolved in saline, except β-funaltrexamine and naloxonazine, which were dissolved in water.

Under the conditions used in this study, β-funaltrexamine (40 mg/kg, s.c.) blocks both μ_1- and μ_2-opioid receptors (Recht and Pasternak, 1987) and antagonizes both spinal and supraspinal analgesia (Pick et al., 1991) whereas naloxonazine (35 mg/kg, s.c.) selectively blocks μ_1-opioid receptors and supraspinal analgesia, but does not block spinal analgesia mediated through μ_2-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989; Pick et al., 1991). The subanalgesic dose of desipramine (5 mg/kg, s.c.) used was chosen based on the selectivity of desipramine for the inhibition of norepinephrine reuptake and the finding that this dose of desipramine produces a 100-fold leftward shift in the dose-response curve for i.t. norepinephrine without affecting analgesia induced by i.t. serotonin (Paul and Hornby, 1995). Yohimbine hydrochloride (1 μg, i.t.) has been shown by Wigdor and Wilcox (1987) to produce a 75% antagonism of the antinociceptive effect of 2 μg of morphine given i.c.v.

Antinociception was determined using the radiant heat tail-flick technique (D'Amour and Smith, 1941). The latency to withdraw the tail from a focused light stimulus was measured using a photocell. Baseline latencies were determined before experimental treatment as the mean of two trials, and a maximal latency of 12 s was used to minimize tissue damage. Mice recovered from halothane anesthesia in 2–5 min and post-treatment latencies were determined at 15 min after i.t. injection. The analgesic response was assessed quantitatively as the percentage of mice at least doubling their individual baseline latencies.

Dose-response curves were analyzed using the Prism computer program (Graph Pad). This program maximizes the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose-response data. Statistical comparisons were made using the Fisher Exact test. The level of significance was set at $P < 0.05$.

3. Results

The analgesic dose-response curves for i.t. Tyr-W-MIF-1 alone and 1 h after pretreatment with desipramine (5 mg/kg, s.c.) are shown in Fig. 1. The ED_{50} value for the production of antinociception after i.t. Tyr-W-MIF-1 was 0.41 μg (CI_{95} = 0.38–0.45). Pretreatment with desipramine, an inhibitor of norepinephrine reuptake, produced a significant, 3.1-fold leftward shift in the dose-response curve for Tyr-W-MIF-1 [ED_{50} = 0.13 μg, CI_{95} = 0.11–0.15; $P < 0.05$]. Control animals ($n = 5$) given i.t. saline after desipramine pretreatment showed no elevation in their individual baseline tail-flick latencies.

The dose-response curve for the inhibition of the analgesic effect of 0.75 μg of i.t. Tyr-W-MIF-1 by naloxone is shown in Fig. 2. Naloxone (0.1, 0.3, 0.6 and 1.0 mg/kg, s.c.) injected 15 min before the 57% effective dose of Tyr-W-MIF-1 caused a dose-dependent inhibition of the

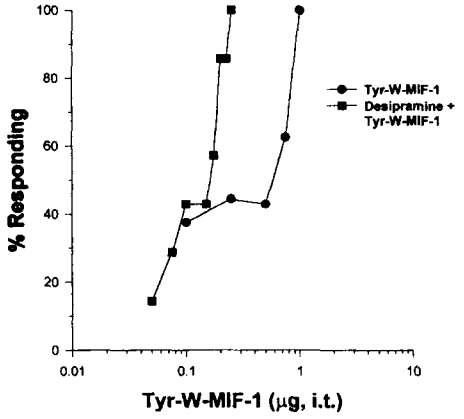

Fig. 1. Log dose-response curves showing the potentiation of Tyr-W-MIF-1 analgesia by pretreatment with desipramine (5 mg/kg, s.c). Groups of mice (n = 7–9) received i.t. Tyr-W-MIF-1 either alone (●) or 1 h after pretreatment with desipramine (■). All animals were tested for analgesia before and 15 min after injection of Tyr-W-MIF-1. Tyr-W-MIF-1 produced antinociception dose dependently (ED_{50} = 0.41 μg). Pretreatment with desipramine produced a 3.1-fold leftward shift in the analgesic dose-response curve for Tyr-W-MIF-1 (ED_{50} = 0.13 μg; $P < 0.05$). Control mice ($n = 5$) administered saline (1 μl, i.t.) 1 h after desipramine showed no analgesic response.

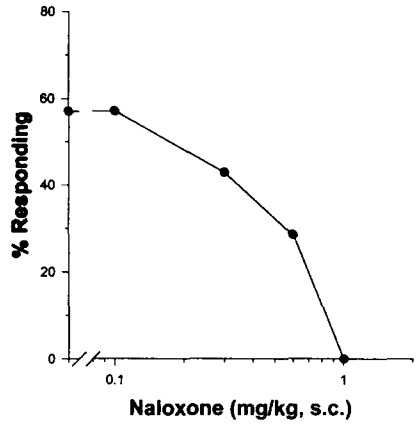

Fig. 2. Dose-dependent reversal by naloxone of the analgesic effect of 0.75 μg of Tyr-W-MIF-1 (i.t.). Groups of mice ($n = 7$) received naloxone (0.10, 0.30, 0.60 and 1.0 mg/kg, s.c.) 15 min before i.t. injection of Tyr-W-MIF-1. Animals were tested for analgesia before naloxone injection and 15 min after injection of Tyr-W-MIF-1. Naloxone dose dependently reversed the analgesic effect of Tyr-W-MIF-1 with an ED_{50} value of 0.56 mg/kg ($P < 0.05$). Control mice ($n = 7$) administered naloxone (1 mg/kg, s.c) 15 min before i.t. injection of saline showed no analgesic response.

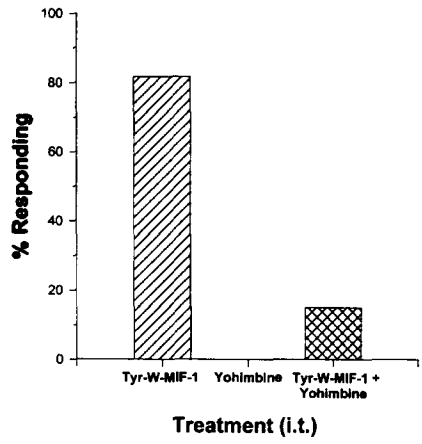

Fig. 4. Antagonism of Tyr-W-MIF-1 analgesia by yohimbine. Groups of mice ($n = 20$–22) were administered either Tyr-W-MIF-1 (0.85 μg), yohimbine (1 μg) or Tyr-W-MIF-1 and yohimbine simultaneously by i.t. injection. All animals were tested for analgesia before and 15 min after i.t. injection. Yohimbine significantly decreased tail-flick latencies compared with control values for Tyr-W-MIF-1 ($P < 0.05$). Yohimbine alone had no effect on tail-flick latencies.

analgesic effect [$ED_{50} = 0.56$ mg/kg, $CI_{95} = 0.53$–0.61]. Control animals ($n = 7$) given i.t. saline after pretreatment with 1.0 mg/kg naloxone showed no effect.

The analgesic effect of 0.75 μg of i.t. Tyr-W-MIF-1 was also blocked by the μ-opioid receptor-selective antagonist β-funaltrexamine which irreversibly antagonizes both μ_1- and μ_2-opioid receptors, but not by the μ_1-opioid receptor-selective antagonist naloxonazine (Fig. 3). β-Funaltrexamine (40 mg/kg, s.c.) injected 24 h before i.t. Tyr-W-MIF-1 completely abolished the response ($n = 19$; $P < 0.05$), indicating that the analgesic effect is mediated through μ-opioid receptors. The analgesic response of mice pretreated with the μ_1-opioid receptor-selective antagonist naloxonazine (35 mg/kg, s.c., $n = 18$) 24 h before i.t. injection of Tyr-W-MIF-1 did not significantly differ from control animals pretreated with water ($n = 23$; $P > 0.05$). That the analgesic response of i.t. Tyr-W-MIF-1 was completely reversed by β-funaltrexamine, but not significantly attenuated by naloxonazine, indicates that the actions of Tyr-W-MIF-1 are mediated by spinal μ_2-opioid receptors.

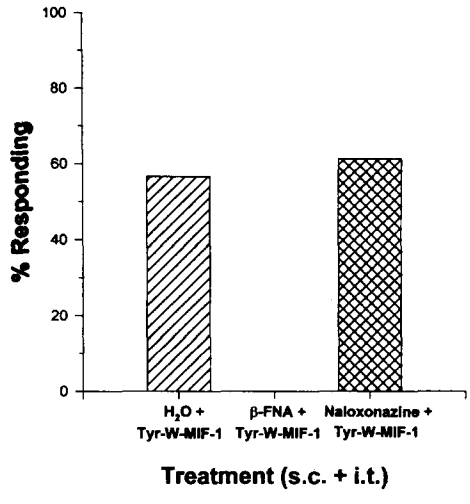

Fig. 3. Effects of β-funaltrexamine and naloxonazine on Tyr-W-MIF-1 analgesia. Groups of mice ($n = 18$–23) received either β-funaltrexamine (40 mg/kg, s.c.), naloxonazine (35 mg/kg, s.c) or water 24 h before i.t. administration of 0.75 μg of Tyr-W-MIF-1. Tail-flick latencies were determined before and 15 min after Tyr-W-MIF-1 injection. β-Funaltrexamine significantly decreased tail-flick latencies compared with controls ($P < 0.05$). However, pretreatment with naloxonazine had no statistically significant effect upon Tyr-W-MIF-1-induced analgesia.

Fig. 4 illustrates the attenuation of Tyr-W-MIF-1 analgesia by yohimbine, an α_2-adrenoceptor-selective antagonist. The simultaneous i.t. injection of 1.0 μg yohimbine and 0.85 μg of Tyr-W-MIF-1 ($n = 22$) produced an 82% inhibition of the analgesic response compared with control animals given i.t. Tyr-W-MIF-1 alone ($n = 20$; $P < 0.05$). The baseline tail-flick latencies of control animals ($n = 20$) given i.t. injections of 1.0 μg of yohimbine were unchanged after treatment.

4. Discussion

Tyr-W-MIF-1 produced a dose-dependent elevation in tail-flick latencies with an unexpectedly high potency. When molar conversions are made to account for differences in molecular weight, morphine is approximately 120 times more potent than Tyr-W-MIF-1 at producing analgesia when administered i.c.v. in rats (Zadina et al., 1993, 1994b). In the present study, however, the ED_{50} value for i.t. Tyr-W-MIF-1 was found to be 0.41 μg (0.78 nmol) whereas the ED_{50} value for i.t. morphine has been reported

as 0.27 μg (0.81 nmol) using the same technique in mice (Wigdor and Wilcox, 1987). Therefore, i.t. Tyr-W-MIF-1 induced analgesia with a substantially greater potency than would have been predicted from its potency after i.c.v. administration.

The analgesic effect of i.t. Tyr-W-MIF-1 was dose dependently reversed by naloxone. Maximal inhibition of an opiate response by 1 mg/kg of naloxone indicates that the response is likely mediated through μ-opioid receptors (Ward and Takemori, 1983). The antagonism of Tyr-W-MIF-1 analgesia by β-funaltrexamine confirmed a μ-opioid analgesic mechanism (Ward et al., 1982; Pick et al., 1991) and the failure of naloxonazine, a μ_1-opioid receptor-selective antagonist, to block the analgesic response indicates that the effect is mediated through spinal μ_2-opioid receptors (Heyman et al., 1988; Paul et al., 1989; Pick et al., 1991). These results are consistent with both the selectivity of Tyr-W-MIF-1 for μ/δ- and κ-opioid receptors in binding assays (Zadina et al., 1994a) and the reversal by the μ-opioid receptor antagonist, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), but not by the κ-opioid receptor-selective antagonist norbinaltorphimine, of the Tyr-W-MIF-1-induced inhibition of guinea-pig ileum contractions (Erchegyi et al., 1992). The presence of μ- and κ- but not δ- (Lord et al., 1977) or μ_1-opioid receptors (Ginzler and Pasternak, 1983) in the guinea pig ileum is also consistent with a μ_2-opioid site of action for Tyr-W-MIF-1.

Pretreatment with systemic desipramine, an inhibitor of norepinephrine reuptake, shifted the dose-response curve for Tyr-W-MIF-1 3.1-fold to the left. The same dose of desipramine (5 mg/kg, s.c.) has been shown to produce a similar 5.3-fold leftward shift in the dose-response curve for i.t. DAMGO antinociception (Paul and Hornby, 1995). Desipramine alone had no analgesic effect in either study. The potentiation of the i.t. effect of Tyr-W-MIF-1 by desipramine is also consistent with the finding that i.t. norepinephrine potentiates i.t. morphine analgesia (Hylden and Wilcox, 1983).

Finally, i.t. coadministration of the α_2-adrenoceptor-selective antagonist yohimbine and Tyr-W-MIF-1 attenuated the effect of Tyr-W-MIF-1. The i.t. dose of yohimbine used in this study has been shown to produce 75% antagonism of the antinociceptive response of 2 μg of morphine given i.c.v. (Wigdor and Wilcox, 1987). Similarly, we report an 82% antagonism of 0.85 μg of Tyr-W-MIF-1 after i.t. yohimbine.

In summary, Tyr-W-MIF-1 produces potent, naloxone-reversible analgesia after i.t. administration with an ED_{50} (0.78 nmol) comparable to that reported for morphine [0.81 nmol (Wigdor and Wilcox, 1987)]. Blockade of the analgesic effect of i.t. Tyr-W-MIF-1 by naloxone and β-funaltrexamine shows that the actions of Tyr-W-MIF-1 in the spinal cord are mediated through μ-opioid receptors. Because naloxonazine selectively blocks μ_1-opioid receptors and supraspinal analgesia, but does not block spinal analgesia mediated through μ_2-opioid receptors (Heyman et al., 1988; Paul et al., 1989; Pick et al., 1991), the failure of naloxonazine to block the analgesic effect of i.t. Tyr-W-MIF-1 indicates that the effect is mediated through spinal μ_2-opioid receptors. Desipramine, a specific inhibitor of norepinephrine reuptake, potentiated the analgesic effect of i.t. Tyr-W-MIF-1 whereas yohimbine, an α_2-adrenoceptor antagonist, attenuated the effect. These results indicate that spinally administered Tyr-W-MIF-1 produces potent antinociception mediated by spinal μ_2-opioid receptors and modulated by norepinephrine.

References

Camarata, P.J. and T.L. Yaksh, 1985, Characterization of the spinal adrenergic receptors mediating the spinal effects produced by microinjection of morphine into the periaqueductal gray, Brain Res. 336, 133.

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

Erchegyi, J., A.J. Kastin and J.E. Zadina, 1992, Isolation of a novel tetrapeptide with opiate and antiopiate activity from human cortex: Tyr-Pro-Trp-Gly-NH_2 (Tyr-W-MIF-1), Peptides 13, 623.

Gergen, K.A., S.L. Chang, Y.-F. Niu, A.J. Kastin and J.E. Zadina, 1994, Expression of the FOS proto-oncogene protein in brain after ICV administration of Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2), Peptides 15, 1505.

Ginzler, A.R. and G.W. Pasternak, 1983, Multiple mu receptors; evidence for μ_2 sites in the guinea pig ileum, Neurosci. Lett. 39, 51.

Hackler, L., A.J. Kastin, J. Erchegyi and J.E. Zadina, 1993, Isolation of Tyr-W-MIF-1 from bovine hypothalami, Neuropeptides 24, 159.

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

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

Hylden, J.L.K. and G.L. Wilcox, 1983, Pharmacological characterization of substance P-induced nociception in mice: modulation by opioid and noradrenergic agonists at the spinal level, J. Pharmacol. Exp. Ther. 226, 398.

Jensen, T.S. and T.L. Yaksh, 1986, Examination of spinal monoamine receptors through which brainstem opiate-sensitive systems act in the rat, Brain Res. 363, 114.

Larsen, J.-J. and J. Arnt, 1984, Spinal 5-HT or NA uptake inhibition potentiates supraspinal morphine antinociception in rats, Acta Pharmacol. Toxicol. 54, 72.

Ling, G.S.F., R. Simantov, J.A. Clark and G.W. Pasternak, 1986, Naloxonazine actions in vivo, Eur. J. Pharmacol. 129, 33.

Lord, J.A.H., A.A. Waterfield, J. Hughes and H.W. Kosterlitz, 1977, Endogenous opioid peptides: multiple agonists and receptors, Nature 267, 495.

Paul, D. and P.J. Hornby, 1995, Potentiation of intrathecal DAMGO antinociception, but not gastrointestinal transit inhibition, by 5-hydroxytryptamine and norepinephrine uptake blockade, Life Sci. 56, PL 83.

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

Pick, C.G., D. Paul and G.W. Pasternak, 1991, Comparison of naloxonazine and β-funaltrexamine antagonism of μ_1 and μ_2 opioid actions, Life Sci. 48, 2005.

Recht, L.D. and G.W. Pasternak, 1987, Effects of β-funaltrexamine on radiolabeled opioid binding, Eur. J. Pharmacol. 230, 341.

Reed, G.W., G.A. Olson and R.D. Olson, 1994, The Tyr-MIF-1 family of peptides, Neurosci. Biobehav. Rev. 18, 519.

Sullivan, A.F., M.R. Dashwood and A.H. Dickenson, 1987, α_2-Adrenoceptor modulation of nociception in rat spinal cord: Location, effects and interactions with morphine, Eur. J. Pharmacol. 138, 169.

Ward, S.J. and A.E. Takemori, 1983, Relative involvement of mu, kappa and delta receptor mechanisms in opiate-mediated antinociception in mice, J. Pharmacol. Exp. Ther. 224, 525.

Ward, S.J., P.S. Portoghese and A.E. Takemori, 1982, Pharmacological characterization in vivo of the novel opiate, β-funaltrexamine, J. Pharmacol. Exp. Ther. 220, 494.

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

Zadina, J.E., A.J. Kastin, L.-J. Ge and V. Brantl, 1990, Hemorphins, cytochrophins and human beta-casomorphins bind to antiopiate (Tyr-MIF-1) as well as opiate binding sites in the rat brain, Life Sci. 47, PL 25.

Zadina, J.E., A.J. Kastin, V. Kenigs, C. Bruno and L. Hackler, 1993, Prolonged analgesia after intracerebroventricular Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2), Neurosci. Lett. 155, 220.

Zadina, J.E., A.J. Kastin, L.-J. Ge and L. Hackler, 1994a, Mu, delta and kappa opiate receptor binding of Tyr-MIF-1 and Tyr-W-MIF-1, its active fragments and two potent analogs, Life Sci. 55, PL 461.

Zadina, J.E., A.J. Kastin, L. Hackler and S.L. Chang, 1994b, Cyclic analogues of Tyr-W-MIF-1 with prolonged analgesic activity and potency comparable to DAMGO and morphine, Peptides 15, 1567.