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Analgesic effects of Tyr-W-MIF-1: a mixed μ_2-opioid receptor agonist/μ_1-opioid receptor antagonist

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Abstract

Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) is a naturally occurring neuropeptide that displays high selectivity for μ-opioid receptors. Recently, intrathecal (i.t.) Tyr-W-MIF-1 was shown to induce potent analgesia mediated through spinal μ_2-opioid receptors in mice. In the current study, we investigated the supraspinal analgesic effects of Tyr-W-MIF-1 using intracerebroventricular (i.c.v.) administration in mice. I.c.v. Tyr-W-MIF-1 induced a dose-dependent analgesic response with an ED_{50} of 31.4 μg that was antagonized by i.c.v. naloxone (ED_{50} = 4.46 nmol) and the μ-opioid receptor antagonist β-funaltrexamine but not by the μ_1-opioid receptor-selective antagonist naloxonazine. I.t. naloxone (ED_{50} = 0.12 nmol), however, was nearly 40-fold more potent than i.c.v. naloxone at antagonizing i.c.v. Tyr-W-MIF-1-induced analgesia. Tyr-W-MIF-1 also possesses antagonist activity at μ_1-opioid receptors in brain. Coadministration of i.c.v. Tyr-W-MIF-1 with i.c.v. morphine or i.c.v. [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO) significantly decreased the analgesic response to either drug administered alone. Thus, Tyr-W-MIF-1 functions as a mixed μ_2-opioid receptor agonist/μ_1-opioid receptor antagonist after i.c.v. administration in mice.

Keywords: Analgesia; μ-Opioid receptor; Tyr-W-MIF-1; Antinociception

1. Introduction

Systemic administration of morphine induces analgesia through an action at μ-opioid receptors located both supraspinally and spinally (Ling and Pasternak, 1983). Analgesia induced by microinjection of morphine into supraspinal sites such as the periaqueductal grey and rostral ventral medulla is mediated through μ_1-opioid receptors (Bodnar et al., 1988; Heyman et al., 1988; Paul et al., 1989a; Pick et al., 1991) and the activation of descending inhibitory monoamine pathways that terminate in the dorsal horn of the spinal cord (Yaksh, 1979; Basbaum and Fields, 1984; Jensen and Yaksh, 1986; Wigdor and Wilcox, 1987). Conversely, spinally administered μ-opioid agonists act directly in the dorsal horn where the activation of μ_2-opioid receptors (Heyman et al., 1988; Paul et al., 1989a; Pick et al., 1991) elicits analgesia modulated by the local release of inhibitory monoamines (Yaksh and Noueihed, 1985).

The supraspinal and spinal mechanisms of μ-opioid receptor-mediated analgesia can be distinguished anatomically by their sensitivity to intracerebroventricular (i.c.v.) versus intrathecal (i.t.) naloxone antagonism and pharmacologically by the μ-opioid receptor-selective antagonists β-funaltrexamine and naloxonazine which differ in their selectivity for the μ_1- and μ_2-subtypes of μ-opioid receptors (Recht and Pasternak, 1987). For these reasons, the induction of analgesia after intracerebroventricular (i.c.v.) or intrathecal (i.t.) administration of μ-opioid receptor agonists has been used as an in vivo functional assay of agonist activity at μ_1- and μ_2-opioid receptors respectively (Tive et al., 1992; Paul et al., 1989b).

Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) is an endogenous neuropeptide originally isolated from human cortex and named for its structural similarity to the melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1) family of brain peptides (Erchegyi et al., 1992). Tyr-W-MIF-1 displays high selectivity for μ-opioid receptors in

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binding assays (Zadina et al., 1994) and in the guinea pig ileum bioassay (Erchegyi et al., 1993) and it induces prolonged, naloxone-reversible analgesia after i.c.v. injection in rats (Zadina et al., 1993; Gergen et al., 1994).

We have recently reported (Gergen et al., 1996) that spinal administration of Tyr-W-MIF-1 in mice induces analgesia mediated by μ_2-opioid receptors with an unexpectedly high potency compared to its analgesic potency after i.c.v. injection in rats (Zadina et al., 1993) and compared to morphine (Paul et al., 1989b). Since supraspinal analgesia is mediated through μ_1-opioid receptors whereas spinal analgesia is mediated through μ_2-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989a; Pick et al., 1991), we postulated that Tyr-W-MIF-1 either lacked efficacy at supraspinal μ_1-opioid receptors compared to spinal μ_2-opioid receptors or, alternatively, that pharmacokinetic factors present in brain (Banks et al., 1993) but not in spinal cord prevented the binding of Tyr-W-MIF-1 to μ-opioid receptors for the induction of analgesia. Results obtained using the functional assay of μ_1-opioid receptor-mediated supraspinal analgesia indicate that Tyr-W-MIF-1 is a novel, endogenous mixed μ_2-opioid receptor agonist/μ_1-opioid receptor antagonist.

2. Materials and methods

Male CD-1 (25–35 g; Charles River Breeding Laboratories, Wilmington, MA, USA) or male ICR/CD-1 (25–35 g; Harlan Sprague Dawley, Houston, TX, USA) mice were used in all experiments and maintained on a 12 h light/dark cycle with ad libitum access to food and water. Intracerebroventricular (i.c.v.) or intrathecal (i.t.) injections were made under light halothane anesthesia with a 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 mm (Haley and McCormick, 1957) and i.t. injections were administered by lumbar puncture (Hylden and Wilcox, 1980). The injection volumes were 1 μl for i.c.v. and i.t. injections and 1 ml/kg for subcutaneous (s.c.) injections.

Antinociception was determined using the radiant heat tail-flick technique (D'Amour and Smith, 1941). A photocell was used to measure the latency to withdraw the tail from a focused light stimulus. Baseline latencies (2.0–4.0 s) were determined before experimental treatment as the mean of two trials. A maximum latency of 10 s was imposed to minimize tissue damage. Post-treatment latencies were determined 15 min after i.c.v. or i.t. injections. Analgesia was assessed quantally as the percentage of mice in the treatment group at least doubling their individual baseline tail-flick latencies.

Morphine sulfate (M.W. 668.76) and β-funaltrexamine (M.W. 490.99) were obtained from the Research Technologies Branch of NIDA. Naloxone hydrochloride (M.W. 363.82) was purchased from Sigma (St. Louis, MO, USA), and [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO; M.W. 513.66) from Peninsula Laboratories (Belmont, CA, USA). Tyr-W-MIF-1 (M.W. 520.57) was a gift from Dr Abba Kastin and naloxonazine (M.W. 650.78) was a gift from Dr Gavril Pasternak. All drugs were dissolved in saline, except β-funaltrexamine and naloxonazine, which were dissolved in water.

β-Funaltrexamine (40 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were injected 24 h before treatment. Under these conditions, β-funaltrexamine irreversibly antagonizes both μ_1- and μ_2-opioid receptors (Recht and Pasternak, 1987) and inhibits both supraspinal and spinal analgesia while naloxonazine selectively antagonizes μ_1-opioid receptors and supraspinal analgesia but does not antagonize spinal analgesia mediated through μ_2-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989a; Pick et al., 1991). I.c.v. injections of naloxone were administered simultaneously with morphine or Tyr-W-MIF-1 while i.t. injections of naloxone were immediately followed by i.c.v. injections of Tyr-W-MIF-1 or morphine.

Dose-response curves were analyzed using a modification of the BLISS-20 computer program (Department of Statistics, Edinburgh University). 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 with the level of significance set at $P < 0.05$.

3. Results

3.1. Supraspinal potency of Tyr-W-MIF-1

The analgesic dose-response curves for i.c.v. morphine and i.c.v. Tyr-W-MIF-1 are shown in Fig. 1. Groups of mice ($n = 7–10$) were tested for analgesia 15 min after i.c.v. injection using the tail-flick method. Analgesia was assessed quantally as the doubling or greater of the average baseline tail-flick latency of each animal in the treatment group. I.c.v. Tyr-W-MIF-1 dose dependently induced analgesia with an ED_{50} value of 31.2 μg ($CI_{95} = 30.8–31.6$) compared to 1.92 μg ($CI_{95} = 1.63–2.21$) for i.c.v. morphine. The apparent lack of supraspinal potency for Tyr-W-MIF-1 is striking when compared to its potent analgesic effects in the spinal cord where the ED_{50} is 0.41 μg (Gergen et al., 1996). The supraspinal/spinal potency ratio (i.c.v. ED_{50}/i.t. ED_{50}) of Tyr-W-MIF-1 (76.1) is over 100-fold higher than the supraspinal/spinal potency ratios of traditional μ-opioid agonists like morphine (0.71; Paul et al., 1989b) and DAMGO (0.51; Paul et al., 1989a).

3.2. Differential μ-opioid receptor antagonism

The effects of β-funaltrexamine and naloxonazine on analgesia induced by equipotent doses of i.c.v. Tyr-W-

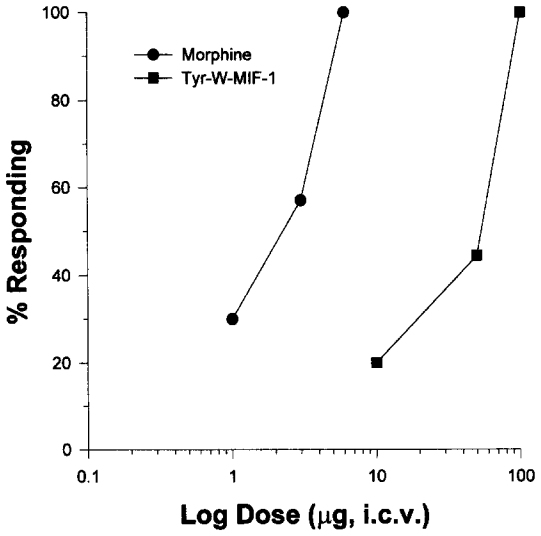

Fig. 1. Log dose-response curves comparing the analgesic potency of Tyr-W-MIF-1 and morphine after i.c.v. administration in mice. Groups of mice ($n = 7–10$) were tested for analgesia 15 min after i.c.v. injection using the tail-flick method. Analgesia was assessed quantally as the doubling or greater of the average baseline tail-flick latency of each animal. Tyr-W-MIF-1 produced a dose-dependent analgesic response ($ED_{50} = 31.2$ μg) that was about 15-fold less potent than that observed for morphine ($ED_{50} = 1.92$ μg).

MIF-1 and i.c.v. DAMGO are shown in Fig. 2. Groups of mice ($n = 18–20$) received either β-funaltrexamine (40 mg/kg, s.c.), naloxonazine (35 mg/kg, s.c.) or water

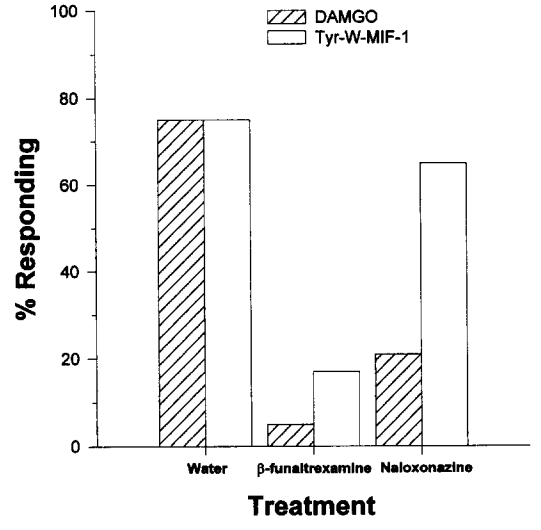

Fig. 2. Comparison of the effects of β-funaltrexamine and naloxonazine on analgesia induced by i.c.v. Tyr-W-MIF-1 and DAMGO. Groups of mice ($n = 18–20$) received either β-funaltrexamine (40 μg/kg, s.c.), naloxonazine (35 μg/kg, s.c.) or water 24 h before i.c.v. injection of Tyr-W-MIF-1 or DAMGO. Tail-flick latencies were determined before and 15 min after i.c.v. injections. β-funaltrexamine significantly decreased the analgesic response in both the DAMGO and Tyr-W-MIF-1 treatment groups compared to control ($P < 0.05$). However, while naloxonazine significantly attenuated analgesia in mice receiving i.c.v. DAMGO ($P < 0.05$), it had no statistically significant effect on analgesia induced by i.c.v. Tyr-W-MIF-1.

(s.c.) 24 h before i.c.v. injection of Tyr-W-MIF-1 (ED_{80} = 80 μg) or DAMGO ($ED_{80} = 8$ ng). Pretreatment with β-funaltrexamine significantly decreased the analgesic response in both the Tyr-W-MIF-1 and DAMGO treatment groups compared to control ($P < 0.05$), confirming the involvement of μ-opioid receptors in both responses. Naloxonazine (35 mg/kg), however, was ineffective at attenuating the Tyr-W-MIF-1 response while significantly decreasing the DAMGO response compared to control ($P < 0.05$). The naloxonazine insensitivity of the analgesia induced by i.c.v. Tyr-W-MIF-1 compared to i.c.v. DAMGO suggests that the Tyr-W-MIF-1 response is mediated through μ_2-opioid receptors in contrast to the supraspinal DAMGO response that is mediated through μ_1-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989b; Pick et al., 1991).

3.3. Localization of the analgesic effect

The antagonism of i.c.v. Tyr-W-MIF-1-induced analgesia by i.c.v. and i.t. naloxone is shown in Fig. 3. Naloxone was administered to groups of mice ($n = 8–10$) by i.c.v. injection with the test compound or by i.t. injection immediately followed by i.c.v. injection of the test compound. Both i.c.v. (0.3, 5.0, 10.0 and 30 nmol) and i.t. naloxone (0.05, 0.3, 4.0 and 10 nmol) significantly antagonized the

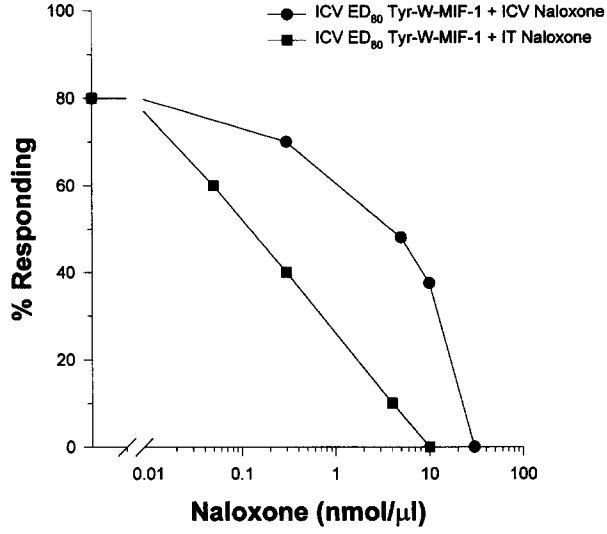

Fig. 3. Differential antagonism of i.c.v. Tyr-W-MIF-1 analgesia by i.c.v. and i.t. naloxone. Naloxone was administered to groups of mice ($n = 8–10$) either by i.t. injection immediately followed by i.c.v. Tyr-W-MIF-1 (80 μg) or by simultaneous injection with i.c.v. Tyr-W-MIF-1 (80 μg). Tail-flick latencies were determined before and 15 min after i.c.v. injections. Supraspinal naloxone (0.3, 5.0, 10 and 30 nmol) dose-dependently inhibited the analgesic effect of i.c.v. Tyr-W-MIF-1 with an ED_{50} value of 4.46 nmol. Spinal administration of naloxone (0.05, 0.3, 4 and 10 nmol), however, was nearly 40-fold more potent at antagonizing the effect of i.c.v. Tyr-W-MIF-1 ($ED_{50} = 0.12$ nmol) compared to supraspinal naloxone ($P < 0.05$). The baseline tail-flick latencies of mice administered 10 nmol naloxone i.t. followed by i.c.v. saline ($n = 10$) were unchanged after treatment.

analgesia induced by i.c.v. Tyr-W-MIF-1 (80 μg) compared to control ($P < 0.05$). However, i.t. naloxone (ED_{50} = 0.12 nmol, CI_{95} = 0.01–0.38) was nearly 40-fold more potent than i.c.v. naloxone (ED_{50} = 4.46 nmol, CI_{95} = 3.08–5.84) in antagonizing the analgesic effect of i.c.v. Tyr-W-MIF-1 (80 μg; $P < 0.001$). The baseline tail-flick latencies of control animals ($n = 10$) administered i.c.v. saline ($n = 10$; 1 μl) after i.t. naloxone (10 nmol) were unchanged after treatment.

I.c.v. naloxone (2.0, 5.0, 7.5 and 10.0 nmol) also dose dependently antagonized the analgesia induced by an equipotent dose of i.c.v. morphine (5 μg; ED_{50} = 2.82 nmol, CI_{95} = 2.51–3.13; data not shown). However, in contrast to the results obtained with i.c.v. Tyr-W-MIF-1, i.c.v. naloxone potently antagonized the analgesic response of i.c.v. morphine whereas the maximally effective dose of i.c.v. naloxone (10 nmol) had no significant effect on i.c.v. morphine-induced analgesia when injected i.t. These results indicate that the analgesia induced by supraspinal administration of Tyr-W-MIF-1, but not morphine, is predominantly mediated through opioid receptors located in the spinal cord. Moreover, since the analgesia induced by i.c.v. Tyr-W-MIF-1 is also antagonized by β-funaltrexamine but not naloxonazine, these results strongly suggest that supraspinal Tyr-W-MIF-1 induces analgesia via caudal diffusion to the spinal cord and the subsequent activation of μ_2-opioid receptors.

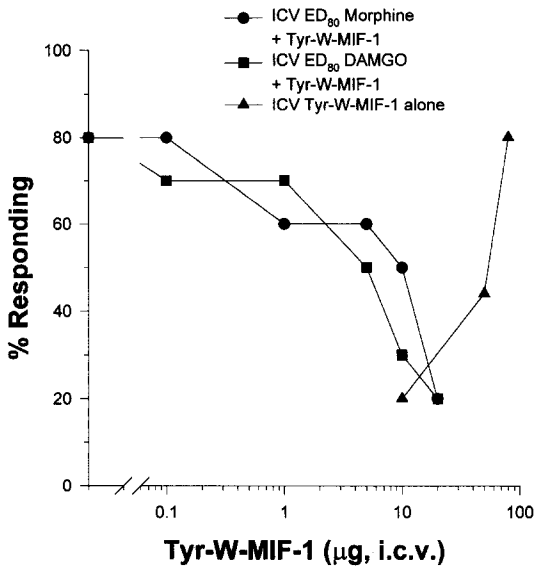

Fig. 4. Reversal of supraspinal morphine- and DAMGO-induced analgesia by Tyr-W-MIF-1. Coadministration of Tyr-W-MIF-1 (0.1, 1.0, 5.0, 10 and 20 μg) with i.c.v. morphine (ED_{80} = 5 μg) resulted in a dose-dependent inhibition of the analgesic response compared to mice administered i.c.v. morphine (5 μg) alone (ED_{50} = 5.15 μg; $P < 0.05$). Coadministration of Tyr-W-MIF-1 (0.1, 1.0, 5.0, 10 and 20 μg) with i.c.v. DAMGO (ED_{80} = 8 ng) dose-dependently inhibited the analgesic response compared to mice administered i.c.v. DAMGO (8 ng) alone (ED_{50} = 4.45 μg; $P < 0.05$). The agonist dose-response curve for i.c.v. Tyr-W-MIF-1 alone (ED_{50} = 31.2 μg) is superimposed from Fig. 1 for comparison with the antagonist potency of Tyr-W-MIF-1 after i.c.v. injection. Analgesia was assessed quantally 15 min after i.c.v. injection using the tail-flick method and ten mice per dose.

3.4. μ_1-Opioid receptor antagonism by Tyr-W-MIF-1

The dose-dependent antagonism of supraspinal morphine- and DAMGO-induced analgesia by i.c.v. Tyr-W-MIF-1 is shown in Fig. 4. In groups of mice ($n = 10$) coadministered i.c.v. Tyr-W-MIF-1 (0.1, 1.0, 5.0, 10.0 and 20 μg) and i.c.v. morphine (5 μg), Tyr-W-MIF-1 significantly antagonized the analgesic effect of i.c.v. morphine compared to control (ED_{50} = 5.15 μg, CI_{95} = 4.33–5.97; $P < 0.05$). In groups of mice ($n = 10$) coadministered i.c.v. Tyr-W-MIF-1 (0.1, 1.0, 5.0, 10.0 and 20 μg) and i.c.v. DAMGO (8 ng), Tyr-W-MIF-1 significantly antagonized the analgesic effect of i.c.v. DAMGO compared to control (ED_{50} = 4.45 μg, CI_{95} = 3.12–5.78; $P < 0.05$). The dose-dependent antagonism of supraspinal, μ_1-opioid receptor-mediated analgesia by i.c.v. Tyr-W-MIF-1 indicates that Tyr-W-MIF-1 is distributed in brain and that it binds to μ_1-opioid receptors but lacks sufficient efficacy at μ_1-opioid receptors to induce an analgesic response.

4. Discussion

Supraspinal administration of Tyr-W-MIF-1 induced a dose-dependent analgesic response in mice that was about 75-fold less potent than we have recently reported after spinal administration of Tyr-W-MIF-1 in mice (Gergen et al., 1996). The lack of analgesic potency of Tyr-W-MIF-1 at supraspinal μ_1-opioid receptors compared to spinal μ_2-opioid receptors could not be attributed to either a lack of affinity at μ_1-opioid receptors or a novel selectivity for μ_2- over μ_1-opioid receptors since we have recently shown that Tyr-W-MIF-1 binds to both the μ_1- and μ_2-subtypes of opioid receptors with a 3-fold higher affinity at μ_1- compared to μ_2-opioid receptors (Zadina et al., 1996). Similarly, the 200-fold higher selectivity of Tyr-W-MIF-1 at μ- compared to δ- and κ-opioid receptors (Zadina et al., 1994) seemed to preclude the involvement of a separate type of opioid receptor.

We used the μ-opioid receptor selective antagonists β-funaltrexamine and naloxonazine to determine the subtype of μ-opioid receptor mediating the analgesic response. Under the conditions used in this study, β-funaltrexamine antagonizes both supraspinal and spinal analgesia mediated through μ_1- and μ_2-opioid receptors whereas naloxonazine selectively inhibits only supraspinal analgesia mediated through μ_1-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989b; Pick et al., 1991). Analgesia induced by i.c.v. Tyr-W-MIF-1 was antagonized by β-funaltrexamine but not by naloxonazine, indicating the response is mediated through μ_2-opioid receptors. Although supraspinal analgesia induced through μ_2-opioid receptors has been reported in a brainstem model

of supraspinal/spinal synergy (Pick et al., 1992) and in μ_1-opioid receptor-deficient CXBK mice (Pick et al., 1993), selective induction of supraspinal analgesia mediated by μ_2-opioid receptors is not known to occur. A more plausible explanation for the naloxonazine-insensitivity of the i.c.v. Tyr-W-MIF-1 response is that Tyr-W-MIF-1 undergoes caudal distribution to the spinal cord where the activation of spinal μ_2-opioid receptors mediates the analgesic response. To investigate this possibility, we compared the potency of i.c.v. and i.t. naloxone at antagonizing analgesia induced by equipotent doses of i.c.v. morphine and i.c.v. Tyr-W-MIF-1.

Naloxone potently antagonized supraspinal morphine-induced analgesia when injected i.c.v. whereas the maximal effective dose of i.c.v. naloxone injected spinally had no effect alone or on supraspinal morphine-induced analgesia. These results are consistent with the indirect actions of supraspinal morphine on the inhibition of nociceptive transmission in the dorsal horn and with other reports in the literature (Jensen and Yaksh, 1986; Suh et al., 1989). In direct contrast to the results obtained with i.c.v. morphine, i.t. naloxone was nearly 40-fold more potent than i.c.v. naloxone at antagonizing supraspinal Tyr-W-MIF-1-induced analgesia. The greater potency of i.t. naloxone compared to i.c.v. naloxone at antagonizing the analgesia induced by i.c.v. Tyr-W-MIF-1 strongly suggests that i.c.v. Tyr-W-MIF-1 undergoes caudal distribution to the spinal cord where the activation of μ_2-opioid receptors mediates the analgesic response.

Although the naloxonazine-insensitive analgesia induced by i.c.v. Tyr-W-MIF-1 is predominantly mediated by spinal μ_2-opioid receptors, the involvement of supraspinal μ_2-opioid receptors cannot be precluded. Indeed, the ability of i.c.v. naloxone to antagonize i.c.v. Tyr-W-MIF-1-induced analgesia, albeit considerably less potently than i.c.v. morphine-induced analgesia, suggests that some portion of the analgesic response may be mediated supraspinally. Further studies utilizing pharmacological (Pick et al., 1992) or genetic models (Pick et al., 1993) of supraspinal/spinal synergism are needed to address the possible role of supraspinal/spinal synergism and supraspinal μ_2-opioid receptors in the analgesic effects of i.c.v. Tyr-W-MIF-1.

The dose-dependent inhibition of the analgesic effects of i.c.v. morphine and i.c.v. DAMGO by coadministration of i.c.v. Tyr-W-MIF-1 indicates that Tyr-W-MIF-1 is distributed in brain and that it binds competitively to μ_1-opioid receptors in vivo. This is consistent with the competitive binding of Tyr-W-MIF-1 to μ_1- and μ_2-opioid receptors demonstrated in vitro (Zadina et al., 1996). The functional antagonism of μ_1-opioid receptor mediated analgesia by low doses of Tyr-W-MIF-1 suggests that the reduced efficacy of Tyr-W-MIF-1 at higher affinity μ_1-opioid receptors contributes to the reduced potency of supraspinal compared to spinal Tyr-W-MIF-1.

In summary, Tyr-W-MIF-1 is an endogenous brain peptide with dual agonist and antagonist actions at μ opioid receptors in vivo. Supraspinal and spinal (Gergen et al., 1996) administration of Tyr-W-MIF-1 induces naloxonazine-insensitive analgesia through an agonist action at μ_2-opioid receptors located in the spinal cord. Conversely, supraspinal administration of Tyr-W-MIF-1 effectively antagonizes analgesia mediated by supraspinal μ_1-opioid receptors. Thus, Tyr-W-MIF-1 is a mixed μ_2-opioid receptor agonist/μ_1-opioid receptor antagonist.

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