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Antinociceptive properties of oxymorphazole in the mouse

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Abstract

Oxymorphazole (17-methyl-6,7-dehydro-3,14-dihydroxy-4,5 α-epoxy-6,7:3′,4′-pyrazolomorphinan), a hydrophilic opioid, given intracerebroventricularly (2.5–50 nmol) or intrathecally (0.3–5 nmol) dose-dependently produced tail-flick inhibition in male CD-1 mice. However, oxymorphazole given subcutaneously even at high doses (10–80 mg/kg) produced weak tail-flick inhibition. Oxymorphazole given intraperitoneally (0.1 to 10 mg/kg) dose-dependently inhibited abdominal constriction response induced by intraperitoneally injection of 0.6% acetic acid. Oxymorphazole given intracerebroventricularly (25 nmol) or intrathecally (5 nmol) induced tail-flick inhibition was blocked by pretreatment with the μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH2, but not κ-opioid receptor antagonist nor-binaltrophimine. The δ-opioid receptor antagonist, naltrindole, blocked the tail-flick inhibition induced by oxymorphazole given intrathecally but not intracerebroventricularly. The inhibition of the abdominal constriction response by oxymorphazole given intraperitoneally was blocked by intraperitoneally pretreatment with naloxone, but not naltrindole or nor-binaltrophimine. Thus, oxymorphazole given systemically produces antinociception only with the abdominal constriction test, but not the tail-flick test, suggesting that it produces the antinociception at the peripheral sites when administered systemically. The oxymorphazole-induced antinociception is mainly mediated by the stimulation of μ-opioid receptors when given either centrally or systemically and also the δ-opioid receptors when given intrathecally. The lack of central antinociceptive effect of oxymorphazole given systemically may have interesting clinical implications.

Keywords: Antinociception; Opioid; Oxymorphazole; Mouse

1. Introduction

A new opioid ligand, oxymorphazole (17-methyl-6,7-dehydro-3,14-dihydroxy-4,5 α-epoxy-6,7:3′,4′-pyrazolomorphinan) has recently been reported (Xu et al., 1999). The rationale for its design was to introduce a weakly basic, strongly hydrogen bonding, hydrophilic group into the 6,7-position of the morphinan system so that a region of the 6-opioid receptor proposed to anchor 6-specific compounds could be probed (Strahs and Weinstein, 1997). In vitro study has shown oxymorphazole to be a potent μ- and δ-opioid receptor agonist, but a κ-opioid receptor antagonist (Xu et al., 1999). We report here that the oxymorphazole given centrally either by intracerebroventricular (i.c.v.) or intrathecal (i.t.) injection produces a strong antinociception with the tail-flick test. However, oxymorphazole given systemically by subcutaneous (s.c.) injection produces no or weak antinociception with the tail-flick test but produces a strong antinociception with the abdominal constriction test. The failure for this compound to induce central antinociception after systemic injection probably is due to its high hydrophilic property making it difficult to cross the blood–brain barrier. The types of opioid receptors involved in the oxymorphazole-induced antinociception were also characterized.

2. Materials and methods

2.1. Animals

Male CD-1 mice weighing 25–30 g (Charles River Breeding Laboratory, Wilmington, MA) were used for the

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studies. The animals were housed five per cage in a room maintained at 22 ± 0.5 °C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. All experiments were approved by and conformed to the guidelines of the Medical College of Wisconsin Animal Care Committee.

2.2. Assessment of antinociception and drug injection

2.2.1. Tail-flick test

Antinociceptive responses were determined using the tail-flick test (D'Amour and Smith, 1941). To measure the latency of the tail-flick response, mice were gently held with the tail put on the apparatus (Model TF6, EMDIE Instrument, Maidens, VA) for radiant heat stimuli. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the mouse-tail. The intensity of the heat stimulus was set to provide a predrug tail-flick response time of 3 to 4 s. The latency of the tail-flick response was measured before (T_0) and at various times after (T_1) i.c.v. or i.t. injection of oxymorphazole. The inhibition of the tail-flick responses to oxymorphazole was expressed as a "percentage of the maximum possible effect (% MPE)" which was calculated as $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where the cut-off time, T_2, was set at 10 s for the tail-flick response. To establish the dose–response curves, at least three drug doses were used with 8 to 10 mice at each dose.

2.2.2. Abdominal constriction test

The abdominal constriction test was also used to determine antinociceptive response to systemically administered oxymorphazole (Koster et al., 1959). Mice were gently held and injected intraperitoneally (i.p.) with 0.6% acetic acid (0.1 ml/10 g of body weight). After acetic acid injection, mouse was observed for their abdominal constriction behavior in transparent Plexiglas chamber (12 × 12 × 25 cm). The number of writhes was counted for 20 min after injection. To establish the dose–response curves, four drug doses were used with 8 to 9 mice at each dose.

2.2.3. Drug injection

Intracerebroventricular injection (i.c.v.) was performed according to the method described by Haley and McCormick (1957), using a 25-μl Hamilton syringe with a 26-gauge needle. Intrathecal injection (i.t.) was performed according to the procedure of Hylden and Wilcox (1980), using a 25-μl Hamilton syringe with a 30-gauge needle. Injection volumes for i.c.v. and i.t. were 4 and 5 μl, respectively. Subcutaneous injection (s.c.) and intraperitoneal (i.p.) injection was injected to the mouse's back and peritoneum, respectively, with injection volume at 0.1 ml per 10 g of mouse's body weight. A corresponding volume of vehicle was also injected s.c. or i.p. served as control group.

2.3. Experimental protocols

Groups of mice were injected i.c.v., i.t. or s.c. with various doses of oxymorphazole and the tail-flick responses were measured at different times after the injection. In other experiments, groups of mice were pretreated i.c.v. or i.t. with D-Phe-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH2 (CTOP), naltrindole (NTI) 10 min or nor-binaltorphimine (nor-BNI) 24 h prior to i.c.v. or i.t. injection with oxymorphazole (25 nmol for i.c.v. and 5 nmol for i.t.) and the tail-flick response was then measured 30 min after oxymorphazole injection. The effectiveness of the doses of the antagonists used in blocking the respective opioid receptors has been previously verified in other experiments that the same doses of the antagonists significantly blocked the antinociception induced by its own opioid receptor agonist (Calcagnetti and Holzman, 1991; Tseng et al., 2000; Tseng and Collins, 1991; Wu et al., 2002; Narita et al., 2001).

Groups of mice were pretreated i.p. with various dose of oxymorphazole 15 min before i.p. injection of 0.6% acetic acid. In other experiments, groups of mice were co-administered oxymorphazole (10 mg/kg) with naloxone, NTI, or vehicle 15 min or nor-BNI 24h before i.p.-administration of oxymorphazole and 0.6% acetic acid was then injected i.p. All mice were observed for 20 min and number of writhes was counted.

2.4. Drugs

Oxymorphazole (17-methyl-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7:3′,4′-pyrazolomorphinan) (Fig. 1) was synthesized and converted to the dihydrochloride as reported (Xu et al., 1999). It was tested as the water-soluble salt. The following selective opioid antagonists were used to identify the types of opioid receptor mediating the antinociception of oxymorphazole: μ-opioid receptor antagonist D-Phe-Cys-Try-D-Try-Orn-Thr-Phe-Thr-NH2 (CTOP) (Gulya et al., 1988) purchased from Bachem Bioscience (King of Prussia, PA), naloxone, δ-opioid receptor antagonist naltrindole (NTI) (Calcagnetti and Holzman, 1991; Portoghese, 1991) and κ-opioid receptor antagonist nor-binaltrophimine (nor-BNI) (Spanagel et al., 1994) purchased from Sigma (St. Louis, MO). Acetic acid was also purchased from Sigma. Oxymorphazole, naloxone, NTI and nor-BNI were dissolved in 0.9% NaCl for i.c.v., i.t. and i.p. injections. CTOP was dissolved in 0.9% NaCl containing 0.01% Triton X-100 for i.c.v. and i.t. injections.

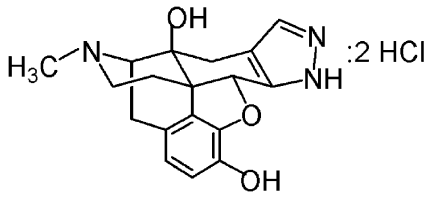

Fig. 1. Chemical structure of oxymorphazole.

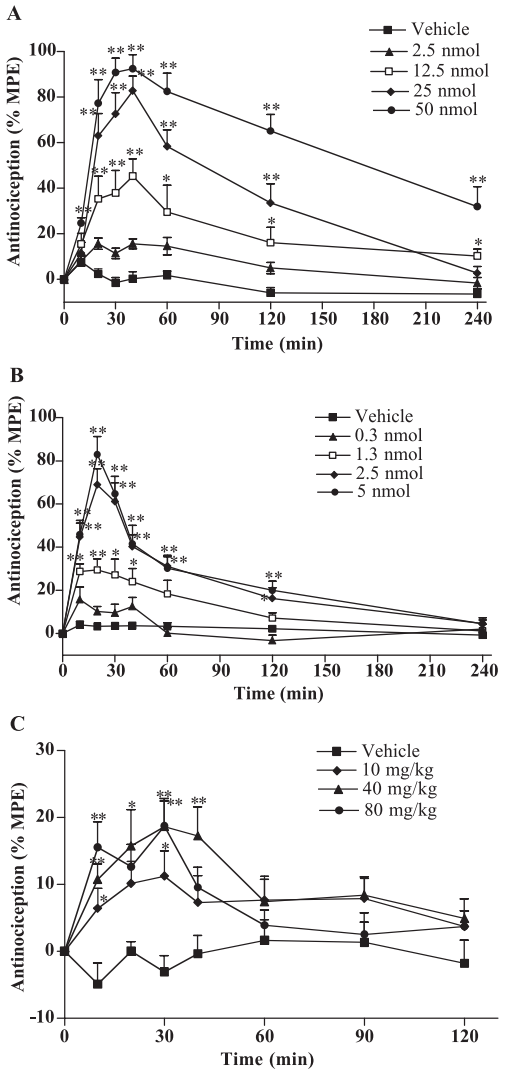

Fig. 2. Time courses of the tail-flick responses after i.c.v., i.t., or s.c. injection of various doses of oxymorphazole. (A) Groups of mice were injected i.c.v. with 2.5, 12.5, 25, or 50 nmol of oxymorphazole, or vehicle; $n=7$ to 10 in each group. (B) Groups of mice were injected i.t. with 0.3, 1.3, 2.5 or 5 nmol of oxymorphazole, or vehicle; $n=8$ to 10 in each group. (C) Groups of mice were injected 10, 40, or 80 mg/kg of oxymorphazole, or vehicle; $n=8$ to 10 in each group. The tail-flick responses were measured at different time after injection. Each point represents the mean and the vertical bar represents the S.E.M. One-way ANOVA followed by Dunnett's post-test was used to test the difference between groups. $^{*}P<0.05$, $^{**}P<0.001$ when compared with the vehicle group.

2.5. Statistical analysis

The antinociceptive responses, percent maximum possible effect (% MPE), were presented as the mean ± S.E.M. The one-way analysis of variance (ANOVA) followed by Dunnett's post-test was used to test the difference between groups compared with vehicle group and the Student's *t*-test was used to test the difference between selective opioid antagonist and vehicle pretreatment group. In order to construct the dose–response curves, at least three doses were used with 8 to10 mice at each dose. The dose–response curves, ED_{50} values and their 95% confidence intervals were determined by using GraphPad Prism software (version 3.0, GraphPad Software, San Diego, CA).

3. Results

3.1. Time course and dose–response curve of the tail-flick inhibition induced by i.c.v., i.t., or s.c. administration of oxymorphazole

Intracerebroventricular injection of oxymorphazole at 2.5, 12.5, 25 and 50 nmol caused a dose-dependent increase of the inhibition of the tail-flick response. The tail-flick inhibition developed in 5 to 15 min, reached its peak 40 min after injection, declined rather slowly and returned to the pre-injection level 2 to 4 h after injection (Fig. 2A). The ED_{50} for oxymorphazole-induced tail-flick inhibition given i.c.v. was estimated to be 11.42 nmol (Fig. 3).

Similarly, oxymorphazole at 0.3, 1.3, 2.5 and 5 nmol given i.t. also dose-dependently produced inhibition of the tail-flick responses. The tail-flick inhibition developed in 5–10 min, reached its peak at 20 min, and returned to control in 1 to 2 h after injection. Thus, the duration of the tail-flick inhibition after i.t. injection of oxymorphazole was shorter than that after i.c.v. injection (Fig. 2). The ED_{50} for oxymorphazole-induced tail-flick inhibition given i.t. was estimated to be 1.82 nmol. Thus, oxymorphazole given i.t. was 6.3-fold more potent than that of i.c.v. to produce the tail-flick inhibition (Fig. 3).

Subcutaneous administration of oxymorphazole even at high doses 40 or 80 mg/kg produced only a small degree (18% MPE) of the tail-flick inhibition (Fig. 2C). Com-

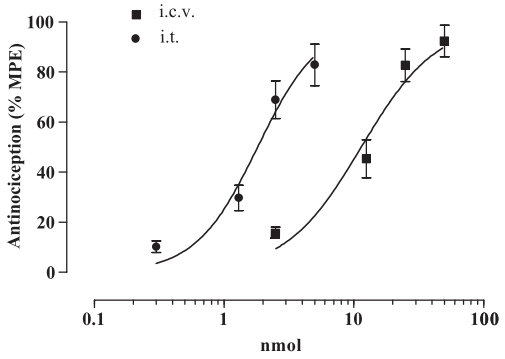

Fig. 3. Dose–response curve for the inhibition of the tail-flick response induced by i.c.v.- or i.t.-administered oxymorphazole. Based on the data in Fig. 2, the tail-flick responses at 40 and 20 min after i.c.v. and i.t. injection, respectively, were used to calculate the ED_{50} of i.c.v.- or i.t.-administered oxymorphazole. Each point represents the mean and the vertical bar represents the S.E.M. with 7 to 10 mice in each group. Nonlinear regression model was used to fit the dose–response curve. Log scale was used as horizontal axis.

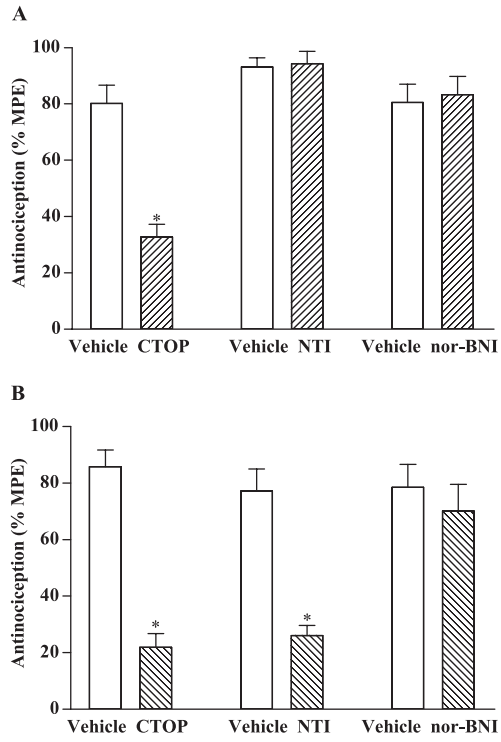

Fig. 4. Effects of i.c.v. (A) or i.t. (B) pretreatment with an μ-opioid antagonist CTOP, δ-opioid antagonist NTI, or κ-opioid receptor antagonist nor-BNI on the tail-flick inhibition induced by i.c.v.- or i.t.-administered oxymorphazole. Groups of mice were injected i.c.v. or i.t. with CTOP 100 pmol, NTI 11.1 nmol 10 min, or nor-BNI 6.6 nmol 24 h prior to i.c.v. and i.t. challenge with oxymorphazole 25 and 5 nmol, respectively. The tail-flick response was measured 40 min (i.c.v.) or 30 min (i.t.) after oxymorphazole injection. Each column represents the mean and the vertical bar represents the S.E.M. and the Student's *t*-test was used to test the difference between groups. $*P<0.001$ when compared with the vehicle group; $n=8$ to 11 in each group.

pared with that of mice injected by saline, mice injected with oxymorphazole did not exhibit increased locomotor activity.

3.2. Effects of i.c.v. and i.t. pretreatment with CTOP, NTI or nor-BNI on inhibition of the tail-flick response induced by the i.c.v.- and i.t.-administered oxymorphazole, respectively

Intracerebroventricular pretreatment with CTOP (100 pmol), but not NTI (11.1 nmol) or nor-BNI (6.6 nmol), effectively blocked the tail-flick inhibition induced by i.c.v.-administered oxymorphazole (25 nmol). Intrathecal pretreatment with CTOP (100 pmol) or NTI (11.1 nmol), but not nor-BNI (6.6 nmol), effectively attenuated the tail-flick inhibition induced by i.t.-administered oxymorphazole (5 nmol) (Fig. 4).

3.3. Inhibition of the acetic acid-induced abdominal constriction by i.p.-administered oxymorphazole

Intraperitoneal injection of oxymorphazole at 0.1, 1, 5 and 10 mg/kg inhibited dose-dependently the acetic acid-induced abdominal constriction (Fig. 5A). Oxymorphazole at 10 mg/kg given i.p. completely inhibited the abdominal constriction response. The data in Fig. 5A was expressed as the percent inhibition of the saline vehicle control and presented in Fig. 5B. The ED_{50} value for oxymorphazole-induced inhibition of the abdominal constriction was estimated to be 0.61 mg/kg.

3.4. Effects of i.p. pretreatment with naloxone, NTI or nor-BNI on inhibition of the writhing response induced by the i.p.-administered oxymorphazole

The inhibition of the acetic acid-induced abdominal constriction by oxymorphazole (10 mg/kg, i.p.) was significantly reversed by naloxone (1 mg/kg, i.p.). However, i.p. pretreatment with NTI (1 mg/kg) or nor-BNI (3 mg/kg) did not affect the oxymorphazole-produced inhi-

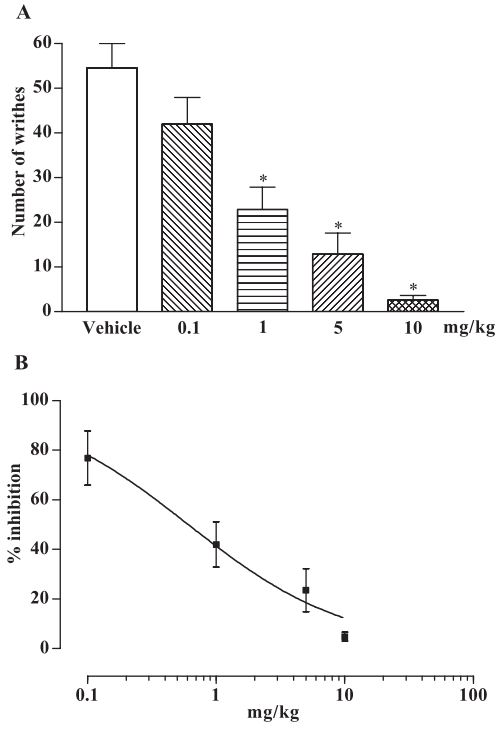

Fig. 5. Dose–response curves of i.p.-administered oxymorphazole on the inhibition of the writhing response induced by i.p.-administered acetic acid. (A) Groups of mice were i.p. injected with 0.1, 1, 5, or 10 mg/kg of oxymorphazole or vehicle (0.1 ml/10 g) prior to i.p. injection of 0.6% acetic acid (0.1 ml/10 g). The number of writhes was then counted for 20 min after acetic acid injection. Each column represents the mean and the vertical bar represents the S.E.M. with 8 to 9 mice in each group. One-way ANOVA followed by Dunnett's post-test was used to test the difference between groups. $*P<0.001$ when compared with the vehicle group. (B) Based on the data from (A), the dose–response curve and the ED_{50} of the oxymorphazole on the inhibition of acetic acid was obtained. The % inhibition was calculated as (number of writhes/54.5) × 100, in which 54.5 was the mean number of writhes of the vehicle-injected group. Nonlinear regression model was used to fit the dose–response curve. Log scale was used as horizontal axis.

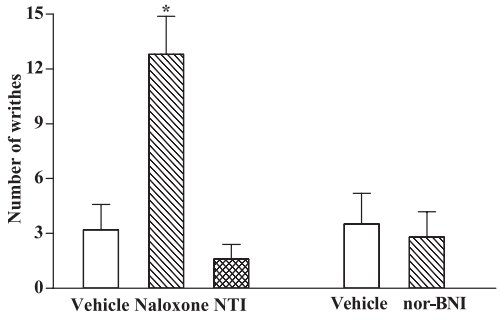

Fig. 6. Effects of i.p.-administered with non-selective μ-opioid antagonist naloxone, δ-opioid antagonist NTI, or κ-opioid receptor antagonist nor-BNI on the writhing inhibition induced by i.p.-administered oxymorphazole. Groups of mice were i.p. co-administered oxymorphazole (10 mg/kg) with naloxone (1 mg/kg), NTI (1 mg/kg), or vehicle 15 min prior to 0.6% acetic acid i.p. injection. Other groups of mice were i.p. pretreated with nor-BNI (3 mg/kg) or vehicle 24 h prior to i.p. injected with oxymorphazole (10 mg/kg) and then i.p. challenged with 0.6% acetic acid 15 min after oxymorphazole injection. Each column represents the mean and the vertical bar represents the S.E.M. with 8 to 9 mice in each group. One-way ANOVA followed by Dunnett's post-test and Student's *t*-test was used to test the difference between groups. $*P<0.001$when compared with the vehicle group; $n=10$.

bition of the acetic acid-induced abdominal constriction (Fig. 6).

4. Discussion

We found in the present study that oxymorphazole given i.c.v. or i.t. dose-dependently produced antinociception with the tail-flick test. On the other hand, oxymorphazole even at high doses 40 or 80 mg/kg given s.c. only produced a small degree of antinociception and a lower dose 10 mg/kg did not produce any antinociception. Lack of antinociception of oxymorphazole with the tail-flick test is due to the high lipophilicity of this compound. The 1-octanol/water log *P* value was −0.33, which indicates that it has a slightly higher affinity for water than a lipid biophase. This makes oxymorphazole more hydrophilic than morphine, which has an estimated 1-octanol/water log *P* value of 1.53. Thus, the hydrophilic oxymorphazole is not likely to pass through the blood–brain barrier when it is applied peripherally such as by s.c. injection. Also, oxymorphazole given s.c. did not produce any morphine-like locomotor stimulation, suggesting the lack of central effect of this compound when it is given systemically.

It is known that opioid receptors also are present on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers (Coggeshall and Carlton, 1997). It is reasonable to speculate that opioids given systemically may also activate these peripheral opioid receptors to contribute to the opioid-induced antinociception. Another pain model the acetic acid-induced abdominal constriction test was then used for the study. We found that oxymorphazole even at doses much smaller than the doses used for the tail-flick experiments produced a potent and strong inhibition of the acetic acid-induced abdominal constriction. The tail-flick response is centrally organized task and the tail-flick inhibition induced by systemic opioids is mainly mediated by the activation of opioid receptors at the central sites. On the other hand, opioids given systemically may inhibit abdominal constriction by stimulation of the opioid receptors both at peripheral and central sites. Thus, it appears that the weak antinociceptive tail-flick inhibition after systemic administration of oxymorphazole appears to be due to lack of bioavailability to pass across the blood–brain brain and the remaining small degree of the tail-flick inhibition after systemic oxymorphazole may be due to the activation of peripheral opioid receptors outside the central nervous system. It is most likely that the oxymorphazole-produced inhibition of the acetic acid-induced abdominal constriction response is mainly mediated by the stimulation of peripheral opioid receptors.

Opioid lacking lipophilicity may be expected to be devoid of undesirable side effects such as respiratory depression, nausea, sedation or dependence liability. Based on such consideration, several compounds such as μ-opioid selective agonist [D-Arg^2,Lys^4]-demorphin-(1–4)-amide (DALDA), δ-opioid ligand H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2 (BW443C), or κ-opioid agonist GR94839, which all show antinociceptive activity attributable to peripheral sites of action (Schiller et al., 1990; Follenfant et al., 1988; Roger et al., 1992). Oxymorphazole lacking lipophilicity and thus, with very limited access to the central nervous system, may be expected to be devoid of the undesirable side effects of morphine.

The antinociception induced by oxymophazole given centrally are mediated by the stimulation of μ-opioid receptors. This is evidenced by the finding that the tail-flick inhibition induced by oxymorphazole given i.c.v. or i.t. is blocked by the pretreatment with μ-opioid receptor antagonist CTOP, but not κ-opioid receptor antagonist nor-BNI. This is consistent with the in vitro report that oxymorphazole is a μ-opioid agonist and κ-opioid antagonist (Xu et al., 1999). However, only the tail-flick inhibition induced by oxymorphazole given i.t. but not i.c.v. was blocked by δ-opioid receptor antagonist NTI. The discrepancy between the effects of i.c.v. and i.t. is unclear at this point.

The systemic oxymorphazole-produced antinociception with the acetic acid-induced abdominal constriction test is mediated by stimulation of peripheral μ-opioid receptor, but not δ- or κ-opioid receptor. This view is supported by the finding that the inhibition was blocked by μ-opioid receptor antagonist naloxone, but not δ-opioid receptor antagonist NTI or κ-opioid receptor antagonist nor-BNI. The same dose of naloxone (1 mg/kg) used in the present study has been found previously to block the tail-flick inhibition induced by subcutaneous injection of morphine (Kolesnikow et al., 1996).

It is concluded that oxymorphazole given central, but not systemically, produces a marked tail-flick inhibition. However, oxymorphazole given systemically markedly inhibits the acetic acid-induced abdominal constriction response. These antinociceptive effects produced by oxymorphazole given centrally or systemically are mainly mediated by the stimulation of μ-opioid receptors. δ-Opioid receptors in the spinal cord also contribute to the antinociception produced by oxymorphazole given spinally.

Acknowledgements

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References

Calcagnetti, D.J., Holzman, S.G., 1991. Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine. Pharmacol. Biochem. Behav. 38, 185–190.

Coggeshall, R.E., Carlton, S.M., 1997. Receptor localization in the mammalian dorsal horn and primary neurons. Brain Res. Rev. 24, 28–66.

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

Follenfant, F.L., Hardy, G.W., Lowe, L.A., Schneider, C., Smith, T.W., 1988. Antinociceptive effects of the novel opioid peptide BW443C compared with classical opiates; peripheral versus central actions. Br. J. Pharmacol. 93, 85–92.

Gulya, K., Krivan, M., Nyolczas, N., Sarnyai, Z., Kovacs, G.L., 1988. Central effects of the potent and highly selective μ opioid antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) in mice. Eur. J. Pharmacol. 150, 355–360.

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

Hylden, J.L.K., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 167, 313–316.

Kolesnikow, Y.A., Jain, S., Wilson, R., Pasternak, G.W., 1996. Peripheral morphine analgesia: synergy with central sites and a target of morphine tolerance. J. Pharmacol. Exp. Ther. 279, 502–506.

Koster, R., Anderson, M., DeBeer, E.J., 1959. Acetic acid for analgesic screening. Fed. Proc. 18, 412.

Narita, M., Ozaki, S., Ioka, M., Mizoguchi, H., Nagase, H., Tseng, L.F., Suzuki, T., 2001. Different motivational effects induced by the endogenous μ-opioid receptor ligands endomorphin-1 and -2 in the mouse. Neuroscience 105, 213–218.

Portoghese, P.S., 1991. An approach to the design of receptor-type-selective non-peptide antagonists of peptidergic receptors: δ opioid antagonists. J. Med. Chem. 34, 1757–1762.

Roger, H., Birch, P.J., Harrison, S.M., Palmer, E., Manchee, G.R., Judd, D.B., Naylor, A., Scopes, D.I., Hayes, A.G., 1992. GR94839, a kappa-opioid agonist with limited access to the central nervous system, has antinociceptive activity. Br. J. Pharmacol. 106, 783–789.

Schiller, P.W., Nuyen, T.M., Chung, N.N., Dionne, G., Martel, R., 1990. Peripheral antinociceptive effect of an extremely mu-selective polar dermorphin analog (DALDA). Prog. Clin. Biol. Res. 328, 53–56.

Spanagel, R., Almeida, O.F., Shippenberg, T.S., 1994. Evidence that norbinaltrophimine can function as an antagonist at multiple opioid receptor subtypes. Eur. J. Pharmacol. 264, 157–162.

Strahs, D., Weinstein, H., 1997. Comparative modeling and molecular dynamics studies of the delta, kappa and mu opioid receptors. Protein Eng. 10, 1019–1038.

Tseng, L.F., Collins, K.A., 1991. Involvement of epsilon and kappa opioid receptors in inhibition of the tail-flick response induced by bremazocine in the mouse. J. Pharmacol. Exp. Ther. 259, 330–336.

Tseng, L.F., Narita, M., Suganuma, C., Mizoguchi, M., Ohsawa, M., Nagase, H., Kampine, J.P., 2000. Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse. J. Pharmacol. Exp. Ther. 292, 576–583.

Wu, H.E., Hung, K.C., Mizoguchi, M., Nagase, H., Tseng, L.F., 2002. Roles of endogenous opioid peptides in modulation of nocifensive responses to formalin. J. Pharmacol. Exp. Ther. 300, 647–654.

Xu, W., Huang, L.-F., Bauer, L., Bhargava, H., Dunn III, W.J., 1999. Synthesis and opiate receptor binding properties of 17-methyl-6,7-dehydro-3,14-dihydroxy-4,5 α-epoxy-6,7:3′,4′-pyrimidinomorphinans. Bioorg. Med. Chem. Lett. 9, 33075–33380.