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Involvement of *Delta*$_2$ Opioid Receptors in the Development of Morphine Dependence in Mice[1]

Y. MIYAMOTO, P. S. PORTOGHESE and A. E. TAKEMORI

Department of Pharmacology, Medical School (Y.M., A.E.T.) and Department of Medicinal Chemistry, College of Pharmacy (P.S.P.), University of Minnesota, Minneapolis, Minnesota

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ABSTRACT

The possible involvement of δ_2 opioid receptors in the development of morphine dependence was investigated using selective δ_2 receptor antagonists, naltriben (NTB) and naltrindole 5′-isothiocyanate (5′-NTII). The degree of morphine dependence was estimated by the ED_{50} values of naloxone (s.c.) required to precipitate withdrawal jumping and diarrhea 72 hr after morphine pellet implantation. NTB administered s.c. as well as naloxone precipitated jumping and diarrhea in morphine-dependent mice. Chronic treatment with 5′-NTII (both i.c.v. and i.t. routes, 24 hr before, just before, 24 and 48 hr after morphine pellet implantation) increased the ED_{50} values of naloxone for jumping and diarrhea. These results suggest that both supraspinal and spinal δ_2 opioid receptors are involved in the development of physical dependence on systemically administered morphine. However, chronic treatment with NTB (s.c. route, 30 min before, 24 and 48 hr after morphine pellet implantation) failed to affect the ED_{50} values of naloxone for both withdrawal signs. These seemingly discrepant results suggest that continuous blockade of δ_2 opioid receptors (by a nonequilibrium and long-lasting antagonist, 5′-NTII) rather than intermittent blockade of δ_2 opioid receptors (by an equilibrium and relatively short-acting antagonist, NTB) is necessary to inhibit the development of morphine dependence.

The involvement of μ opioid receptors in the development of morphine dependence has been well recognized (DeLander *et al.*, 1984; Gmerek and Woods, 1985; Aceto *et al.*, 1986). However, the blockade of μ receptors inhibits not only morphine dependence, but also the antinociceptive action of morphine. Morphine is a μ-preferring agonist, but it also interacts with δ and κ opioid receptors both *in vitro* and *in vivo* (Takemori and Portoghese, 1987). Recently, the involvement of δ and κ opioid receptors in the development of morphine dependence has been reported (Abdelhamid *et al.*, 1991a; Yamamoto *et al.*, 1988; Suzuki *et al.*, 1992). With respect to the involvement of δ opioid receptors, it was demonstrated that selective blockade of δ receptors by i.c.v. administered naltrindole or 5′-NTII inhibited the development of morphine dependence without compromising the antinociceptive action of morphine. More recently, evidence for the presence of δ opioid receptor subtypes (*i.e.*, δ_1 and δ_2 receptors) has been reported (Sofuoglu *et al.*, 1991; Mattia *et al.*, 1991; Jiang *et al.*, 1991). NTB is an equilibrium and selective δ_2 opioid receptor antagonist and is much more potent in antagonizing DSLET-induced antinociception than naltrindole when administered systemically (Sofuoglu *et al.*, 1991; Takemori and Portoghese, 1992). 5′-NTII has been demonstrated to be a selective δ_2 opioid receptor antagonist (Jiang *et al.*, 1991; Mattia *et al.*, 1992). In the present study, we investigated the possible involvement of δ_2 opioid receptors in the development of morphine dependence, using NTB and 5′-NTII in mice.

Materials and Methods

Animals. Male Swiss-Webster mice (Sasco, Omaha, NE) weighing 20 to 25 g were used. They were housed at least 48 hr before the experiment in a temperature-controlled (23 °C) animal room. They were allowed free access to food and water. Each mouse was used only once.

Animal treatment. Mice were rendered dependent on morphine by s.c. implantation of one morphine pellet (containing 75 mg of morphine free base) for 72 hr. Mice were lightly anesthetized with methoxyflurane during the surgery for pellet implantation.

Antinociceptive assay. A radiant heat tail-flick assay of D'Amour and Smith (1941) was used as modified by Tulunay and Takemori (1974). A mouse was considered as positive for antinociception if the tail-flick latency was more than the latency plus 3 S.D. of the control tail-flick latency of the group. DSLET-induced antinociception was estimated 5 min after i.t. administration and morphine-induced antinociception was estimated 30 min after s.c. administration.

Evaluation of morphine dependence. The degree of morphine dependence was estimated by the ED_{50} values of naloxone required to

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ABBREVIATIONS: NTB, naltriben; 5′-NTII, naltrindole 5′-isothiocyanate; C.I., confidence interval.

precipitate withdrawal jumping (Way *et al.*, 1969) and diarrhea. Mice were placed individually into 30 × 30 cm Plexiglas cylinders immediately after s.c. administration of naloxone. The numbers of vertical jumps were counted for 15 min and a response of more than four jumps was considered a positive jumping response (Yano and Takemori, 1977). After the 15-min observation period, the presence of diarrhea was checked.

Statistics. The ED_{50} values, ED_{50} ratios and their 95% C.I. were computed according to Litchfield and Wilcoxon (1949) using computer programs described by Tallarida and Murray (1987, Pharmacologic Calculation System, version 4.0, #47). At least three groups of 10 mice were used to generate dose-response curves.

Chemicals. Morphine sulfate was bought from Mallinckrodt Inc. (St. Louis, MO). Morphine pellets were supplied by the National Institute on Drug Abuse (Rockville, MD). Naloxone hydrochloride was a gift from Du Pont Pharmaceuticals (Wilmington, DE). DSLET was bought from Serva Biochemicals (Westbury, NY). NTB and 5′-NTII were synthesized as described previously (Portoghese *et al.*, 1988, 1990). Intracerebroventricular and i.t. injections were performed in a volume of 5 μl according to the methods of Haley and McCormick (1957) and Hylden and Wilcox (1980), respectively, and s.c. administration was given in a volume of 10 ml/kg. When two drugs were administered s.c. concomitantly, they were dissolved in the same solution and administered in a total volume of 10 ml/kg.

Results

Antagonism of DSLET-induced antinociception by NTB. Figure 1 shows the time course of inhibition by NTB of DSLET-induced antinociception. The peak time of NTB action was 30 min and the antagonism lasted at least 4 hr and dissipated in 8 hr after s.c. administration. NTB (s.c. 30 min before the tail-flick test) inhibited DSLET-induced antinociception in a dose-dependent manner without affecting morphine-induced antinociception (table 1). NTB itself did not affect the tail-flick latency (data not shown).

NTB-precipitated withdrawal signs. NTB administered s.c. precipitated withdrawal jumping and diarrhea in morphine-dependent mice (fig. 2). ED_{50} values and their 95% C.I. of NTB were 6.3 (4.7–8.4) μmol/kg for jumping and 5.8 (4.5–7.4) μmol/kg for diarrhea. In comparison, ED_{50} values and their 95% C.I. of naloxone were 0.06 (0.04–0.11) μmol/kg for jumping and 0.09 (0.05–0.14) μmol/kg for diarrhea. NTB was administered s.c. concomitantly with naloxone to investigate a possible interaction between these two antagonists. The dose ratio of NTB/naloxone (100:1) was used because ED_{50} ratios (NTB/naloxone)

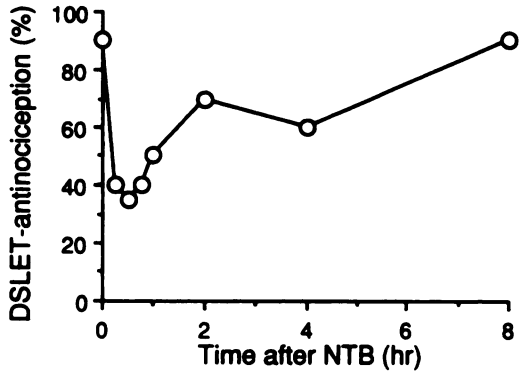

Fig. 1. Time course of the inhibition of DSLET-induced antinociception by NTB. NTB (1 μmol/kg) was administered s.c. at various times before the assessment of DSLET-induced antinociception. DSLET-induced antinociception was assessed 5 min after i.t. administration of 80 pmol (ED_{84} value) of DSLET.

TABLE 1

Selective inhibition of DSLET-induced antinociception by NTB

Treatment[a]	ED_{50} (95% C.I.)	ED_{50} ratio (95% C.I.)
	nmol, i.t.	
DSLET antinociception		
Saline	0.033 (0.020–0.055)	1.0
NTB 1 (μmol/kg)	0.088 (0.059–0.132)	2.7 (1.4–5.1)[b]
NTB 2	0.124 (0.077–0.198)	3.8 (1.8–7.5)[b]
NTB 10	0.511 (0.307–0.852)	15.5 (7.5–31.9)[b]
	μmol/kg, s.c.	
Morphine antinociception		
Saline	8.1 (5.1–13.1)	1.0
NTB 2 (μmol/kg)	8.5 (5.7–12.8)	1.1 (0.6–2.0)
NTB 10	12.8 (7.7–21.3)	1.6 (0.8–3.2)

[a] Saline or NTB was administered s.c. 30 min before the assessment of antinociception.
[b] Value is greater than 1.0 ($P < .05$).

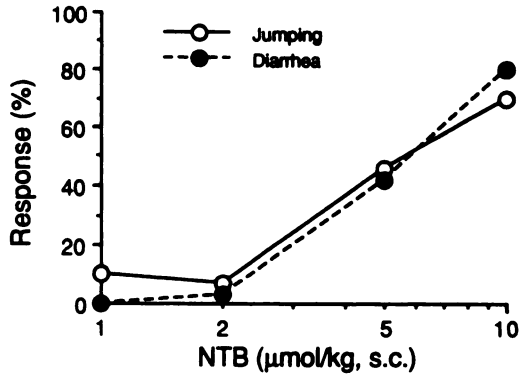

Fig. 2. NTB-precipitated withdrawal signs in morphine-dependent mice. NTB was administered s.c. 72 hr after the implantation of a morphine pellet.

were 100 for jumping and 67 for diarrhea as described above. Isobolographic analysis revealed an additive interaction between NTB and naloxone for inducing both withdrawal signs [fig. 3, ED_{50} (95% C.I.) values after concomitant administration were 2.66 (1.58–4.47) μmol/kg NTB + 0.03 (0.02–0.05) μmol/kg naloxone for withdrawal jumping and 3.60 (2.08–6.23) μmol/kg NTB + 0.04 (0.02–0.06) μmol/kg naloxone for diarrhea].

Effect of chronic treatment with NTB on naloxone-precipitated withdrawal. NTB, 2 or 10 μmol/kg, was administered s.c. chronically (at 30 min before, 24 and 48 hr after the implantation of a morphine pellet). ED_{50} (95% C.I.) values of naloxone for withdrawal jumping were 0.07 (0.04–0.12) μmol/kg in saline-treated mice, 0.08 (0.05–0.12) μmol/kg in NTB (2 μmol/kg)-treated mice and 0.08 (0.04–0.13) μmol/kg in NTB (10 μmol/kg)-treated mice. ED_{50} (95% C.I.) values of naloxone for diarrhea were 0.10 (0.06–0.16) μmol/kg in saline-treated mice, 0.11 (0.07–0.17) μmol/kg in NTB (2 μmol/kg)-treated mice and 0.12 (0.07–0.20) μmol/kg in NTB (10 μmol/kg)-treated mice. Accordingly, these chronic treatments with NTB did not affect the ED_{50} values of naloxone for jumping and diarrhea.

Inhibition of naloxone-precipitated withdrawal by i.c.v. administered 5′-NTII. Various doses of 5′-NTII (0.3–10 nmol) were administered i.c.v. chronically (at 24 hr before, just before, 24 and 48 hr after the implantation of a morphine pellet). ED_{50} (95% C.I.) values of naloxone for withdrawal jumping were 0.07 (0.04–0.11) μmol/kg in saline-treated mice, 0.08 (0.05–0.13) μmol/kg in 5′-NTII (0.3 nmol)-treated mice, 0.18 (0.10–0.32) μmol/kg in 5′-NTII (1 nmol)-treated mice,

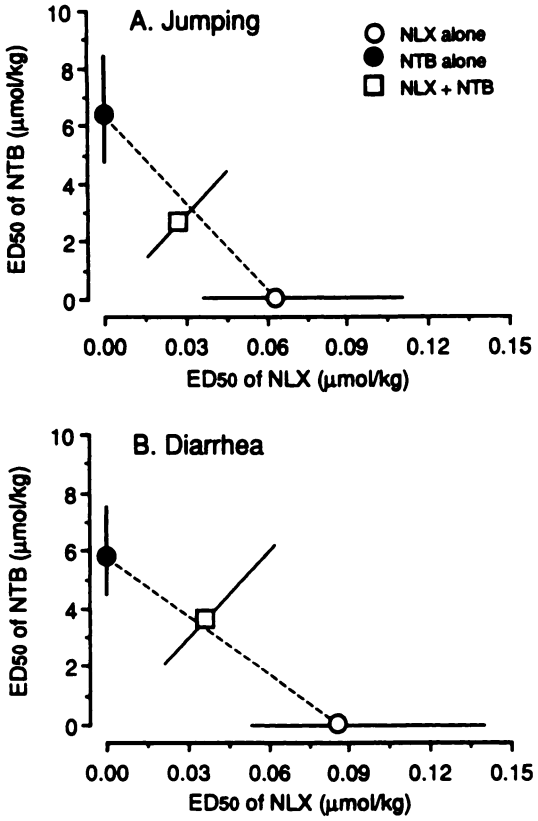

Fig. 3. Isobologram of the additive interaction between NTB and naloxone in precipitating withdrawal signs in morphine-dependent mice. Each point and bar represents the ED_{50} value and its 95% C.I., respectively. When NTB and naloxone were administered s.c. concomitantly, a dose ratio of 100:1 was used.

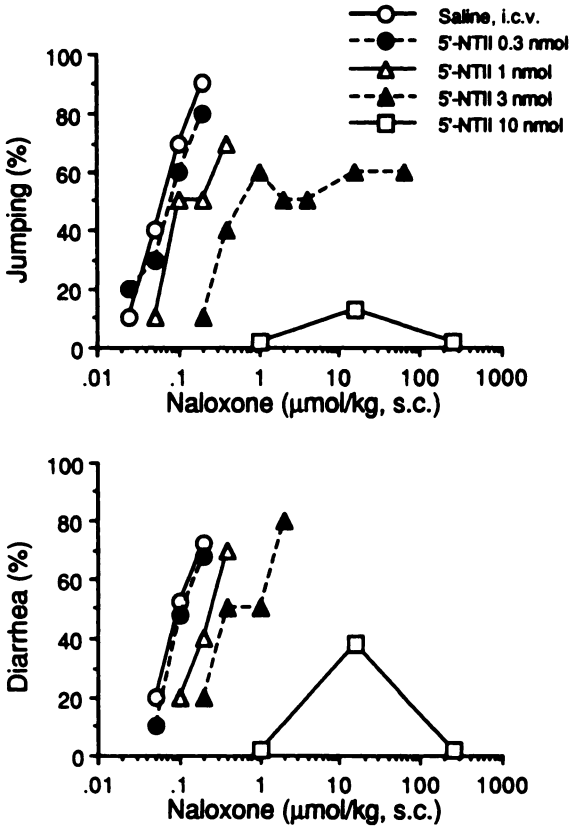

Fig. 4. Inhibition of the development of morphine dependence by chronic treatment with i.c.v. administered 5'-NTII. Saline or 5'-NTII (0.3, 1, 3 or 10 nmol) was administered i.c.v. four times at 24 hr before, just before, 24 and 48 hr after the implantation of a morphine pellet.

0.68 (0.35–1.32) μmol/kg in 5'-NTII (3 nmol)-treated mice and more than 256 μmol/kg in 5'-NTII (10 nmol)-treated mice (fig. 4, upper frame). ED_{50} ratios (95% C.I.) of naloxone *vs.* saline-treated mice were 1.1 (0.6–2.4), 2.7 (1.2–5.8), 10.2 (4.4–23.4) and more than 3800 after chronic treatments with 0.3, 1, 3 and 10 nmol of i.c.v. administered 5'-NTII, respectively.

ED_{50} (95% C.I.) values of naloxone for diarrhea were 0.11 (0.07–0.19) μmol/kg in saline-treated mice, 0.12 (0.08–0.20) μmol/kg in 5'-NTII (0.3 nmol)-treated mice, 0.24 (0.15–0.40) μmol/kg in 5'-NTII (1 nmol)-treated mice, 0.63 (0.32–1.26) μmol/kg in 5'-NTII (3 nmol)-treated mice and more than 256 μmol/kg in 5'-NTII (10 nmol)-treated mice (fig. 4, lower frame). ED_{50} ratios (95% C.I.) of naloxone *vs.* saline-treated mice were 1.1 (0.5–2.2), 2.2 (1.1–4.5), 5.7 (2.4–13.4) and more than 2300 after chronic treatments with 0.3, 1, 3 and 10 nmol of i.c.v. administered 5'-NTII, respectively. Accordingly, chronic treatments with i.c.v. administered 5'-NTII (at doses of 1 nmol and higher) significantly increased the ED_{50} values of naloxone for both withdrawal signs.

Inhibition of naloxone-precipitated withdrawal by i.t. administered 5'-NTII. Various doses of 5'-NTII (0.3–10 nmol) were administered i.t. chronically (at 24 hr before, just before, 24 and 48 hr after the implantation of a morphine pellet). ED_{50} (95% C.I.) values of naloxone for withdrawal jumping were 0.05 (0.03–0.08) μmol/kg in saline-treated mice, 0.06 (0.03–0.12) μmol/kg in 5'-NTII (0.3 nmol)-treated mice, 0.28 (0.15–0.52) μmol/kg in 5'-NTII (1 nmol)-treated mice, 1.38 (0.44–4.31) μmol/kg in 5'-NTII (3 nmol)-treated mice and 7.16 (2.52–20.4) μmol/kg in 5'-NTII (10 nmol)-treated mice (fig. 5, upper frame). ED_{50} ratios (95% C.I.) of naloxone *vs.* saline-treated mice were 1.1 (0.5–2.5), 5.7 (2.7–11.8), 27.5 (8.2–92.7) and 143 (46.5–442) after chronic treatments with 0.3, 1, 3 and 10 nmol of i.t. administered 5'-NTII, respectively.

ED_{50} (95% C.I.) values of naloxone for diarrhea were 0.07 (0.04–0.10) μmol/kg in saline-treated mice, 0.14 (0.09–0.22) μmol/kg in 5'-NTII (0.3 nmol)-treated mice, 0.28 (0.16–0.50) μmol/kg in 5'-NTII (1 nmol)-treated mice, 0.52 (0.20–1.36) μmol/kg in 5'-NTII (3 nmol)-treated mice and 0.50 (0.27–0.92) μmol/kg in 5'-NTII (10 nmol)-treated mice (fig. 5, lower frame). ED_{50} ratios (95% C.I.) of naloxone *vs.* saline-treated mice were 2.1 (1.1–4.2), 4.4 (2.1–9.2), 8.1 (2.8–23.5) and 7.7 (3.5–16.7) after chronic treatments with 0.3, 1, 3 and 10 nmol of i.t. administered 5'-NTII, respectively. Accordingly, chronic treatments with i.t. administered 5'-NTII significantly increased the ED_{50} values of naloxone for jumping (at doses of 1 nmol and higher) and for diarrhea (at doses of 0.3 nmol and higher).

Discussion

NTB inhibited DSLET-induced antinociception at doses that did not affect morphine-induced antinociception, which corroborates previous observations (Sofuoglu *et al.*, 1991). In the same dose range, NTB as well as naloxone precipitated withdrawal signs in morphine-dependent mice, but naloxone was much more potent than NTB. These results suggested the involvement of δ_2 opioid receptors in morphine dependence. In the present study, jumping and diarrhea were used as withdrawal signs because naloxone precipitated these signs consistently in a dose-dependent manner in morphine-dependent mice

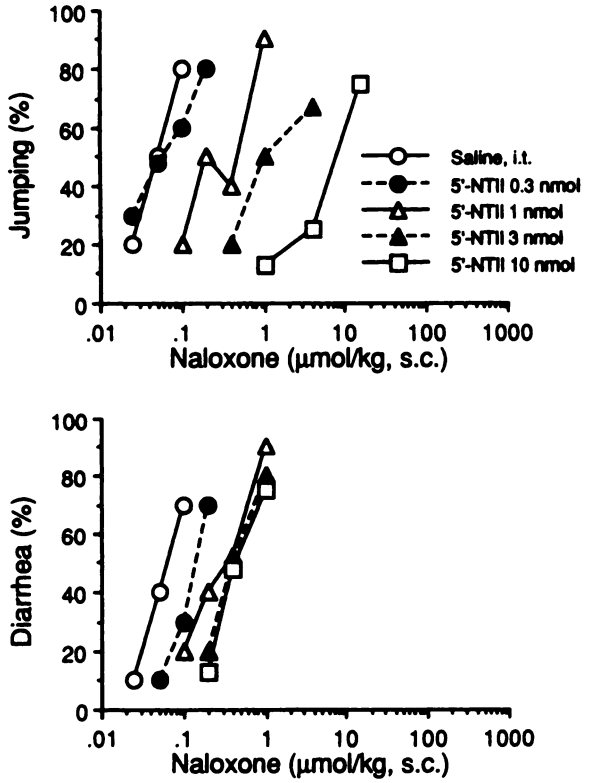

Fig. 5. Inhibition of the development of morphine dependence by chronic treatment with i.t. administered 5′-NTII. Saline or 5′-NTII (0.3, 1, 3 or 10 nmol) was administered i.t. four times at 24 hr before, just before, 24 and 48 hr after the implantation of a morphine pellet.

and also because jumping and diarrhea may reflect different aspects of the withdrawal syndrome (*i.e.*, an excitation of the central nervous system and a disorder of the autonomic nervous system, respectively). NTB had an additive interaction with naloxone in precipitating withdrawal jumping and diarrhea. These results support the interaction between δ and μ opioid receptors (Holaday *et al.*, 1982; Vaught *et al.*, 1982; Abdelhamid and Takemori, 1991b) and may suggest that δ_2 and μ receptors are coupled.

Naloxone-precipitated withdrawal jumping was suppressed by either i.c.v. or i.t. chronic treatments with 1 nmol and higher doses of 5′-NTII, whereas i.c.v. administered 5′-NTII was more potent at the highest dose (10 nmol) than i.t. administered 5′-NTII in inhibiting the jumping. Similarly, naloxone-precipitated diarrhea was suppressed by either i.c.v. or i.t. treatment with 5′-NTII, whereas i.c.v. administered 5′-NTII was less potent at lower doses (0.3–1 nmol) and more potent at the highest dose (10 nmol) than i.t. administered 5′-NTII in inhibiting naloxone-precipitated diarrhea. Interestingly, no further increase in the ED_{50} value of naloxone was observed after i.t. treatment with higher than 1 nmol of 5′-NTII. These results suggested that both supraspinal and spinal δ_2 opioid receptors are involved in morphine dependence and that there was a difference in their contributions of supraspinal and spinal δ_2 opioid receptors in the development of morphine dependence. Also, different mechanisms underlying withdrawal jumping and diarrhea were suggested.

In contrast to results with 5′-NTII, chronic treatment with s.c. administered NTB did not affect the ED_{50} values of naloxone for jumping and diarrhea. It has been shown previously that NTB administered s.c. has the same selective antagonist profile as NTB administered i.c.v. or i.t. (*i.e.*, a δ_2-selective opioid receptor antagonist) (Sofuoglu *et al.*, 1991). In the present study, the NTB dose of 10 μmol/kg was high enough to antagonize DSLET-induced antinociception by 15-fold without affecting morphine-induced antinociception and high enough to precipitate withdrawal signs in 70 to 80% of morphine-dependent mice. A possible explanation for these seemingly discrepant results may be the difference in the time course of action between 5′-NTII and NTB. 5′-NTII is a nonequilibrium antagonist and has been reported to have a blocking action on δ_2 opioid receptors that peaked at 24 hr and lasted for at least 48 hr after i.c.v. administration (Portoghese *et al.*, 1990; Jiang *et al.*, 1991). On the other hand, the δ_2-blocking action of NTB peaked at 30 min and dissipated in 8 hr after s.c. administration. Thus, the present results suggested that continuous blockade rather than intermittent blockade of δ_2 opioid receptors was necessary to inhibit the development of morphine dependence. This is reminiscent of previous findings in which continuous and complete blockade of opioid receptors by naloxone is necessary to block the development of morphine tolerance and dependence (Yano and Takemori, 1977). Incomplete or intermittent blockade of receptors leads to some degree of tolerance and dependence.

It is well established that μ opioid receptors play a major role in the development of opiate tolerance and dependence (DeLander *et al.*, 1984; Gmerek and Woods, 1985; Aceto *et al.*, 1986). The present and previous results (Abdelhamid *et al.*, 1991a) together with the recent finding that selective blockade of κ opioid receptors with norbinaltorphimine prolongs and potentiates morphine tolerance and dependence (Sofuoglu *et al.*, 1992; Suzuki *et al.*, 1992) may suggest modulatory roles for κ and δ_2 opioid receptors on the μ opioid receptor system. Because the inhibitory and potentiating effects on the development of opiate dependence were observed with selective δ_2 and κ opioid receptor antagonists, respectively, δ_2 and κ opioid receptors may serve as positive and negative tonic influences, respectively, on the development of opiate tolerance and dependence that is mediated by μ opioid receptors.

In conclusion, the present results suggest that both supraspinal and spinal δ_2 opioid receptors are involved in the development of morphine dependence and that continuous blockade rather than intermittent blockade of δ_2 receptors is necessary to inhibit the development of morphine dependence.

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Send reprint requests to: Dr. A. E. Takemori, Department of Pharmacology, Medical School, University of Minnesota, 3-249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455.