ELSEVIER

European Journal of Pharmacology 335 (1997) 161–166

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Effects of NMDA receptor antagonists on δ_1- and δ_2-opioid receptor agonists-induced changes in the mouse brain [^{3}H]DPDPE binding

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Received 21 April 1997; revised 22 July 1997; accepted 29 July 1997

Abstract

Male Swiss-Webster mice were injected intracerebroventricularly (i.c.v.) with [D-Pen^2,D-Pen^5]enkephalin (20 μg/mouse) twice a day for 2 days. This procedure resulted in down-regulation of binding sites for [^{3}H][D-Pen^2,D-Pen^5]enkephalin as evidenced by a 52% decrease in the B_{max} value. Twice daily injections of (+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine (MK-801) (0.1 mg/kg, i.p.) or [(−)3-*SR*,4a-*RS*,8a-*SR*-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid] (LY 235959) (2 mg/kg, i.p.), the noncompetitive and competitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, respectively, for 2 days did not alter the B_{max} or K_d value of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding to the mouse brain. Concurrent treatment of MK-801, but not of LY 235959 with [D-Pen^2,D-Pen^5]enkephalin, reversed the decreases in B_{max} value of [^{3}H][D-Pen^2,D-Pen^5]enkephalin. Twice daily injections of [D-Ala^2,Glu^4] deltorphin II (20 μg/mouse) for 2 days caused an increase in the K_d value, but not the B_{max} value of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to bind to brain membranes. Concurrent treatment of [D-Ala^2,Glu^4]deltorphin II with LY 235959 reversed the increase in K_d value of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding induced by multiple injections of [D-Ala^2,Glu^4]deltorphin II, but MK-801 had no effect. The results suggest that multiple injections of δ_1- and δ_2-opioid receptor agonists down-regulate δ_1-opioid receptors of the brain by modifying B_{max} and K_d values of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding, respectively. MK-801 and LY 235959 reverse δ_1- and δ_2-opioid receptor agonists-induced down-regulation of brain δ_1-opioid receptor, respectively, apparently by different mechanisms. It is concluded that short term treatment of mice with δ_1-opioid receptor agonist down-regulates brain δ_1-opioid receptors by decreasing B_{max} of the ligand which is partially reversed by concurrent treatment with MK-801 but not by LY 235959. On the other hand, short term treatment of mice with δ_2-opioid receptor agonist down-regulates brain δ_1-opioid receptors by increasing K_d of the ligand which is partially reversed by concurrent treatment with LY 235959 but not by MK-801.

Keywords: DPDPE ([D-Pen^2,D-Pen^5]enkephalin); [D-Ala^2,Glu^4]deltorphin II; δ_1-Opioid receptor agonist; δ_2-Opioid receptor agonist; NMDA receptor antagonist; MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine); LY 235959 ([(−)3-*SR*,4a-*RS*,8a-*SR*-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid])

1. Introduction

Considerable evidence suggests the possible existence of subtypes of δ-opioid receptors. They have been designated as δ_1- and δ_2-opioid receptors with their prototypical agonists, [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II, respectively (Mattia et al., 1991). Although both [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II produce analgesia by interacting with δ-opioid receptor and produce tolerance on chronic administration, they do not exhibit cross-tolerance (Mattia et al., 1991). Differential antagonism of [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II induced analgesia by the irreversible δ_1- and δ_2-opioid receptor antagonists, [D-Ala^2,Leu^5,Cys^6]enkephalin and naltrindole-5′-isothiocyanate, respectively, also suggested δ-opioid receptor heterogeneity (Jiang et al., 1991). Chronic administration of highly selective δ_1-opioid receptor antagonist, 7-benzylidenenaltrexone and its subsequent withdrawal enhanced the analgesic response to [D-Pen^2,D-Pen^5]enkephalin but not to [D-Ala^2,Glu^4]deltorphin II. On the other hand, chronic administration and subsequent withdrawal of naltriben, a highly selective δ_2-opioid receptor antagonist,

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enhanced the analgesic response to [D-Ala^2,Glu^4]deltorphin II but not to [D-Pen^2,D-Pen^5]enkephalin (Bhargava et al., 1996).

Studies in our laboratory have provided immunological evidence for the subtypes of δ-opioid receptors. In general, [D-Pen^2,D-Pen^5]enkephalin produced greater immunostimulant activity than [D-Ser^2,Leu^5,Thr^6]enkephalin or other δ_2-opioid receptor agonists (Bhargava et al., 1995). On the other hand, 7-benzylidenenaltrexone produced immunosuppression whereas naltriben was devoid of any effect on cellular immune function (House et al., 1995).

There are, however, instances where δ_1- and δ_2-opioid receptors could not be distinguished. For example, tolerance to analgesic effect of [D-Pen^2,D-Pen^5] enkephalin and [D-Ala^2,Glu^4]deltorphin II could be blocked by NMDA receptors (Bhargava and Zhao, 1996a; Zhao and Bhargava, 1996a) but not by nitric oxide synthase inhibitors (Bhargava and Zhao, 1996b; Zhao and Bhargava, 1996b). Similarly, NMDA receptor antagonists dose-dependently attenuate δ_1- and δ_2-opioid receptor agonists-induced analgesia in mice (Bhargava and Zhao, 1996c).

Recently, we have demonstrated that chronic intracerebroventricular administration of [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II produced tolerance to their analgesic action and also down-regulated brain δ-opioid receptors labeled with [^{3}H][D-Pen^2,D-Pen^5]enkephalin (Zhao and Bhargava, 1997). Since the tolerance to the analgesic action of both [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II was antagonized by NMDA receptor antagonists (Bhargava and Zhao, 1996a; Zhao and Bhargava, 1996a), it was of interest to determine whether the down-regulation of brain δ-opioid receptors induced by chronic treatment with [D-Pen^2,D-Pen^5]enkephalin or [D-Ala^2,Glu^4]deltorphin II is modified by concurrent treatment with MK-801 and LY 235959, the noncompetitive and competitive antagonists of the NMDA receptor, respectively. The results indicate that MK-801 reverses the down-regulation of brain δ-opioid receptor induced by chronic administration of [D-Pen^2,D-Pen^5]enkephalin, whereas LY 235959 reverses the down-regulation of brain δ-opioid receptor induced by chronic administration of [D-Ala^2,Glu^4]deltorphin II in mice.

2. Materials and methods

2.1. Animals

Male Swiss-Webster mice weighing 25–30 g (Sasco King Animal, Oregon, WI, USA) were housed five to a cage in a room with controlled temperature (23 ± 1°C), humidity (50 ± 10%) and light (06.00–18.00 h) for at least 4 days before being used. Food and water were made available continuously.

2.2. Chemicals

[D-Pen^2,D-Pen^5]enkephalin, [D-Ala^2,Glu^4]deltorphin II and [^{3}H][D-Pen^2,D-Pen^5]enkephalin (specific activity 40 Ci/mmol) were obtained from the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD, USA), through the courtesy of Mr. Kevin Gormley. LY 235959 was generously donated by Eli Lilly (Indianapolis, IN, USA), courtesy of Dr. Dennis M. Zimmerman. MK-801 was purchased from Research Biochemicals International (Natick, MA, USA). The drugs were dissolved in physiological saline and were injected i.p. in a volume of 10 ml/kg of body weight.

2.3. Method for chronic treatment of mice with [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2, Glu^4]deltorphin II in mice

[D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II were dissolved in distilled water and 10% dimethylsulf-

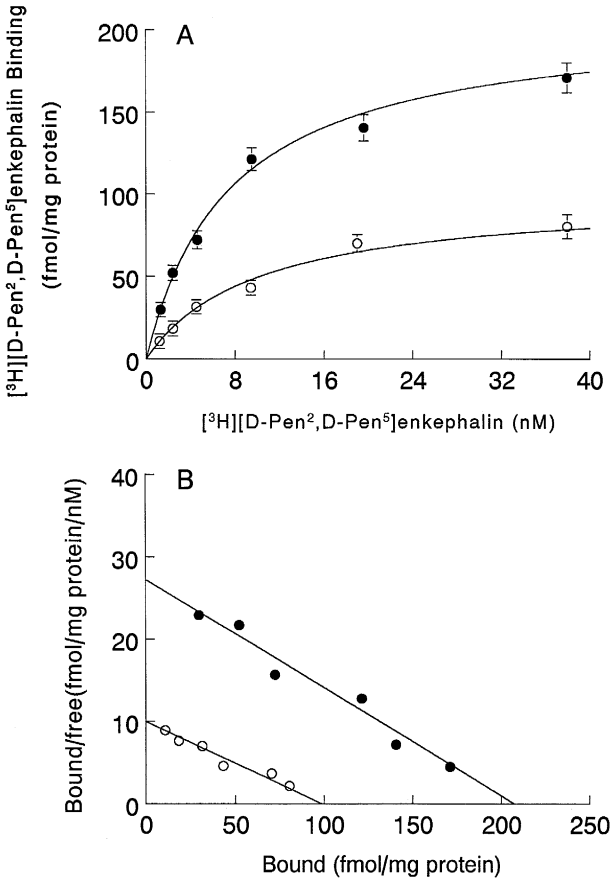

Fig. 1. Saturation curves (A) and the Scatchard plots (B) of the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes of [D-Pen^2,D-Pen^5] enkephalin-tolerant and non-tolerant mice. Mice were injected i.c.v. with [D-Pen^2,D-Pen^5]enkephalin (20 μg/mouse) (○) or its vehicle (●) twice a day for 2 days. Points in saturation isotherms represent the means ± S.E.M. of the values from independent experiments carried out on three different preparations.

oxide in water. The drugs were injected intracerebroventricularly (i.c.v.) (20 μg/mouse) for 2 days. Under light ether anesthesia, bregma was exposed. An injection volume of 5 μl was delivered 2 mm lateral and caudal to bregma at a depth of 3 mm by using a 10 μl Hamilton syringe with a 27 gauge needle according to the method of Haley and McCormick (1957). This procedure has previously been shown to induce tolerance to the analgesic effect of the two peptides (Zhao and Bhargava, 1997).

2.4. Determination of the effect of MK-801 or LY 235959 on the changes in the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin by multiple injections of [D-Pen^2,D-Pen^5]enkephalin or [D-Ala^2,Glu^4]deltorphin II in mice

Mice were divided into two groups and were injected with MK-801 (0.1 mg/kg, i.p.) or its vehicle before each injection of [D-Pen^2,D-Pen^5]enkephalin or [D-Ala^2,Glu^4]deltorphin II as described above. Similarly, the effect of LY 235959 (2.0 mg/kg, i.p.) was determined analogous to that described for MK-801.

2.5. Determination of binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes

2.5.1. Membrane preparation

Mice treated with [D-Pen^2,D-Pen^5]enkephalin or [D-Ala^2,Glu^4]deltorphin II as described above were sacrificed on the morning of day 3 and the brain was quickly excised into an ice-cold Petri dish. The cerebellum was removed and the remainder of the brain was homogenized in 60 volumes of ice-cold Tris–HCl buffer (0.05 M, pH 7.4) using a Brinkman polytron homogenizer (setting 5 for 20 s). The homogenate was centrifuged at 49 000 × g for 15 min in a refrigerated Sorvall RC-5B centrifuge. The process was repeated twice. After the third centrifugation, the pellet was stored at −80°C. For the binding assay, the pellet was suspended in 25 volumes of Tris–HCl buffer by homogenizing for 15 s as described above.

2.5.2. Binding assays

The binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin was performed as described previously (Bhargava et al., 1991; Zhao and Bhargava, 1997). Binding was carried out in a total volume of 0.25 ml which contained 0.1 ml of homogenate (200–250 μg protein) and 0.05 M Tris–HCl buffer. In saturation experiments, the [^{3}H][D-Pen^2,D-Pen^5]enkephalin concentration range was 1.0–45.0 nM. All binding assays were done in duplicate at 37°C for 60 min. Binding was terminated by rapidly filtering the contents of the incubation tubes through Whatman GF/B glass fiber filter under reduced pressure using a Brandell cell harvester (model M-24R). The filters were washed twice with 5 ml of the ice-cold 0.05 M Tris–HCl buffer. The filters were transferred to liquid scintillation vials containing 5 ml of SCINT-AXF scintillation fluid (Packard Instruments, Meriden, CT, USA). After an overnight equilibration period, the radioactivity in the samples was determined using a Packard liquid scintillation counter (model 4640) with a 60% counting efficiency. Specific binding was defined as the difference in binding observed in the absence and presence of 3.5 μM unlabeled [D-Pen^2,D-Pen^5]enkephalin. The concentration of protein in the samples was determined by employing the method of Lowry et al. (1951).

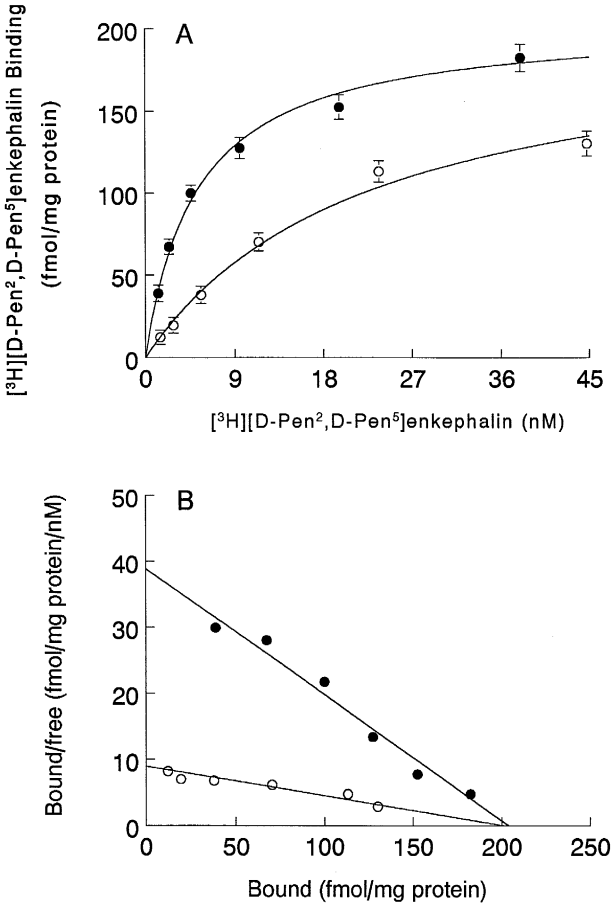

Fig. 2. Saturation curves (A) and the Scatchard plots (B) of the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes of deltorphin II-tolerant and non-tolerant mice. Mice were injected i.c.v. with deltorphin II (20 μg/mouse) (○) or its vehicle (●) twice a day for 2 days. Points in saturation isotherms represent the means ± S.E.M. of the values from independent experiments carried out on three different preparations.

Receptor density (B_{max}) and apparent dissociation constant (K_d) for the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes were determined from the saturation curves and the Scatchard plots using the LIGAND program (Munson and Rodbard, 1980). The results were expressed as means ± S.E.M. Four to six mice were used to determine the binding constants. Data were analyzed by the Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of multiple injections of [D-Pen^2,D-Pen^5]enkephalin or [D-Ala^2, Glu^4]deltorphin II on the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes

The saturation curves and the Scatchard plots for the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain of mice treated with vehicle and [D-Pen^2,D-Pen^5]enkephalin are given in Fig. 1A and B. [^{3}H][D-Pen^2,D-Pen^5]enkephalin bound to brain membranes of vehicle-injected mice at a single high affinity site with a B_{max} of 207.6 fmol per mg protein and a K_d of 7.6 nM. Multiple i.c.v. injections of [D-Pen^2,D-Pen^5]enkephalin for 2 days decreased the B_{max} of [^{3}H][D-Pen^2,D-Pen^5]enkephalin by 52% but the K_d value was not altered. The saturation curves and the Scatchard plots for the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain of mice treated with vehicle and [D-Ala^2,Glu^4]deltorphin II are given in Fig. 2A and B. Multiple i.c.v. injections of [D-Ala^2,Glu^4]deltorphin II did not alter the B_{max} of [^{3}H][D-Pen^2,D-Pen^5]enkephalin but increased the K_d value by 333%.

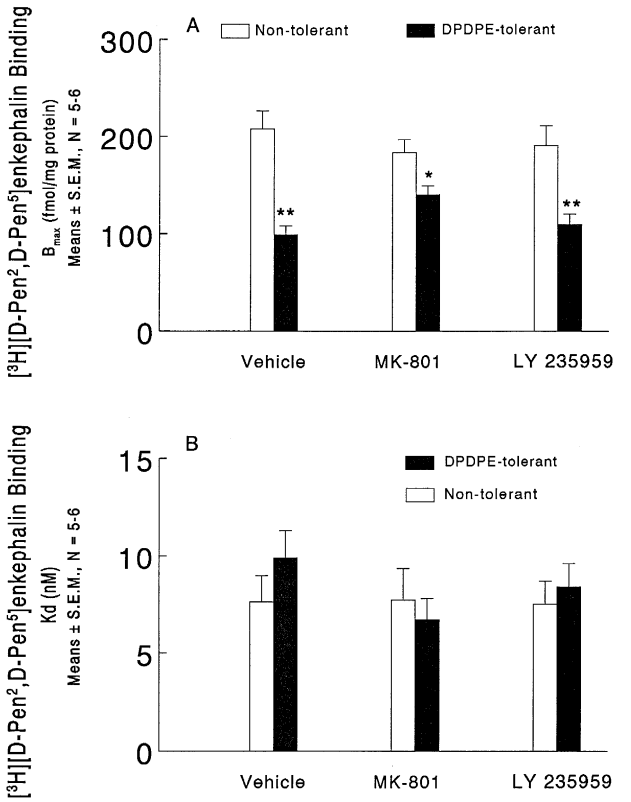

Fig. 3. Effect of MK-801 and LY 235959 on the binding constants of [^{3}H][D-Pen^2,D-Pen^5]enkephalin in the brain membranes of [D-Pen^2,D-Pen^5]enkephalin-tolerant mice. Mice were injected i.p. with MK-801 (0.1 mg/kg), LY 235959 (2 mg/kg) or the vehicle followed 20 min later by the i.c.v. injection of [D-Pen^2,D-Pen^5]enkephalin (20 μg/mouse) twice a day for 2 days. The B_{max} (A) and K_d (B) values for the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes was then determined. * $P < 0.05$ vs. its non-tolerant control or vehicle-injected [D-Pen^2,D-Pen^5] enkephalin-tolerant group; ** $P < 0.01$ vs. its non-tolerant control.

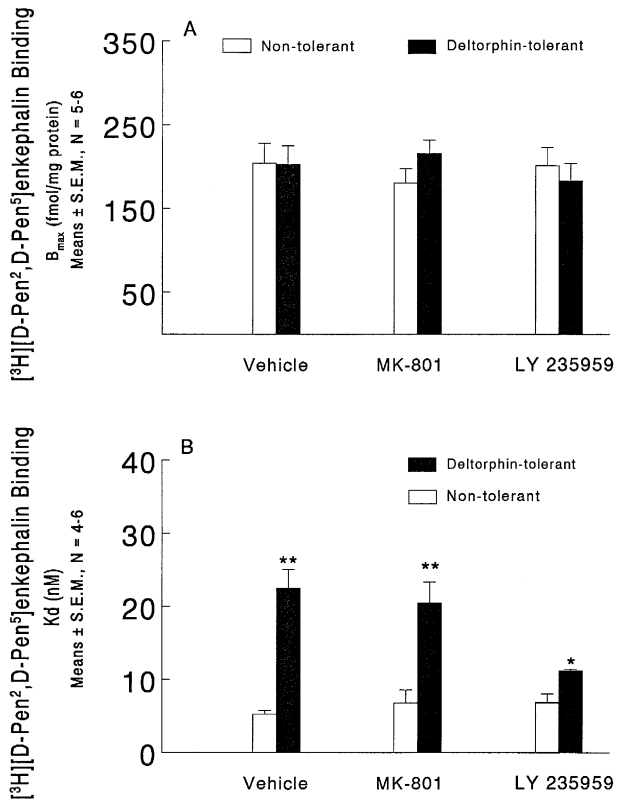

Fig. 4. Effect of MK-801 and LY 235959 on the binding constants of [^{3}H][D-Pen^2,D-Pen^5]enkephalin in the brain membranes of deltorphin II-tolerant mice. Mice were injected i.p. with MK-801 (0.1 mg/kg), LY 235959 (2 mg/kg) or the vehicle followed 20 min later by the i.c.v. injection of deltorphin II (20 μg/mouse) twice a day for 2 days. The B_{max} (A) and K_d (B) values for the binding of [^{3}H][D-Pen^2,D-Pen^5]enkephalin to brain membranes was then determined. * $P < 0.05$ vs. its non-tolerant control or vehicle-injected deltorphin II-tolerant group; ** $P < 0.01$ vs. its non-tolerant control.

3.2. Effect of MK-801 and LY 235959 on the changes in [^{3}H][D-Pen^2,D-Pen^5] enkephalin binding to mouse brain membranes induced by multiple injections of [D-Pen^2,D-Pen^5]enkephalin

Multiple i.p. injections of MK-801 or LY 235959 (2 mg/kg) for 2 days did not modify the B_{max} (Fig. 3A) or K_d (Fig. 3B) of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding to mouse brain membranes. Multiple injections of [D-Pen^2,D-Pen^5]enkephalin for 2 days significantly decreased the B_{max} of [^{3}H][D-Pen^2,D-Pen^5]enkephalin which was reversed significantly by concurrent treatment with MK-801 but not by LY 235959 treatment (Fig. 3A). The K_d value was unaffected by multiple injections of [D-Pen^2,D-Pen^5]enkephalin, MK-801 or LY 235959 alone or in combination (Fig. 3B).

3.3. Effect of MK-801 and LY 235959 on the changes in [^{3}H][D-Pen^2,D-Pen^5] enkephalin binding to mouse brain membranes induced by multiple injections of [D-Ala^2,Glu^4]deltorphin II

Multiple i.c.v. injections of [D-Ala^2,Glu^4]deltorphin II did not modify the B_{max} value (Fig. 4A) of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding to brain membranes but increased the K_d value by 333% (Fig. 4B). Concurrent injections of MK-801 did not affect the increases in K_d value but LY 235959 reversed it significantly (Fig. 4B).

4. Discussion

Considerable efforts are being made to develop drugs that are agonists and antagonists at the δ_1- and δ_2-opioid receptors. The agonists at these receptors produce antinociception (Mattia et al., 1991; Bhargava et al., 1996; Bhargava and Zhao, 1996a,b,c; Zhao and Bhargava, 1996a,b, 1997) and immunostimulation (Bhargava et al., 1995; House et al., 1995) whereas, antagonists at these receptors produce immunosuppression (Arakawa et al., 1992, 1993; House et al., 1995). Most of the currently known agonists at the δ_1- and δ_2-opioid receptors are peptides and are given i.c.v. but nonpeptidic agonists like (±)-4-((α-R^*)-α-((2S^*,5R^*)-4-allyl-2, 5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-*N,N*-diethylbenzamide (BW 373U86) are being examined for their pharmacological actions (Chang et al., 1993).

Previous studies have shown that chronic administration of δ_1- and δ_2-opioid receptor agonists produce tolerance to their analgesic action (Mattia et al., 1991; Bhargava and Zhao, 1996a,b,c; Zhao and Bhargava, 1996a,b, 1997) and is associated with down-regulation of δ-opioid receptors in the brain (Zhao and Bhargava, 1997). The degree of down-regulation is dependent on the duration of treatment. The present studies demonstrate that short term (2 day) treatment with [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II down-regulated brain δ-opioid receptors labeled with [^{3}H][D-Pen^2,D-Pen^5]enkephalin which is purported to be δ_1-specific agonists, however, the nature of down-regulation appears to be different in the two cases. Down-regulation of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding sites by multiple injections of [D-Pen^2,D-Pen^5]enkephalin involved reduction of B_{max} value whereas that induced by [D-Ala^2,Glu^4]deltorphin II involved increases in the K_d values. Thus, it is clear that multiple i.c.v. injections of δ_1- and δ_2-opioid receptor agonists can down-regulate δ_1-opioid receptors in the brain. Although binding studies show down-regulation of brain δ-opioid receptors following chronic treatment with δ_1- and δ_2-opioid receptor agonists (the present study), brain levels of mRNA for the δ-opioid receptors were unaffected (Jenab et al., 1995). Similarly, studies in our laboratory have demonstrated that chronic administration δ_1- or δ_2-opioid receptor antagonists up-regulate brain δ-opioid receptors (Bhargava et al., 1996) but the brain levels of mRNA for δ-opioid receptors were unchanged (Kest et al., 1995).

We have earlier provided evidence that both noncompetitive and competitive antagonists of the NMDA receptor such as MK-801 and LY 235959, respectively, can block the development of tolerance to the analgesic action of δ_1- or δ_2-opioid receptor agonists (Bhargava and Zhao, 1996a; Zhao and Bhargava, 1996a). In addition, NMDA receptor antagonists can also inhibit the analgesic action of δ_1- or δ_2-opioid receptor agonists without any direct interaction with the [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding sites in the brain (Bhargava and Zhao, 1996b).

The present studies demonstrate differential actions of the competitive and noncompetitive NMDA receptor antagonists on the changes in brain δ-opioid receptors induced by multiple administration of δ_1- or δ_2-opioid receptor agonists in mice. Multiple injections of MK-801 or LY 235959 did not modify the B_{max} or the K_d values of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding to the brain. MK-801 reversed the increases in B_{max} of [^{3}H][D-Pen^2,D-Pen^5]enkephalin binding induced by multiple injections of [D-Pen^2,D-Pen^5]enkephalin but LY 235959 had no effect. On the other hand, [D-Ala^2, Glu^4]deltorphin II-induced increase in K_d of [^{3}H][D-Pen^2,D-Pen^5] enkephalin binding were reversed by LY 235959 but was unaffected by MK-801. Thus, the blockade of tolerance to [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2,Glu^4]deltorphin II by MK-801 and LY 235959 may partially be due to reversal of the down-regulation of brain δ-opioid receptors.

Another possibility is that NMDA receptor may be involved in the regulation of enkephalinergic tone in the brain. MK-801 has been shown to up-regulate the mRNA levels for preproenkephalin (Angulo et al., 1993, 1995). Thus, even though the δ-opioid receptors of the brain are down-regulated after multiple injections of δ_1- or δ_2-opioid receptor agonists, the increases in the levels of endogenous enkephalin will produce an enhancement in the analgesic response in tolerant mice.

In summary, the present studies indicated that both δ_1- and δ_2-opioid receptor agonists on short term treatment produce down-regulation of brain δ-opioid receptor but the mechanisms in the two systems appear to be different. Although both MK-801 and LY 235959 have been shown to block tolerance to δ_1- and δ_2-opioid receptor agonists, MK-801 reversed the down-regulation of δ_1-opioid receptor induced by δ_1-opioid receptor agonists but LY 235959 was inactive. On the other hand, LY 235959 reversed the down-regulation of δ_1-opioid receptor induced by δ_2-opioid receptor agonists but MK-801 was inactive.

Acknowledgements

These studies were supported by a grant DA-08867 and a Research Scientist Development Award, K02-DA-00130

to Dr. Hemendra N. Bhargava from the National Institute on Drug Abuse. The authors thank Mrs. Celina Tejada for secretarial assistance.

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