Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 1109–1112, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00250-9

BRIEF COMMUNICATION

# Opiate Withdrawal Intensity Correlates With the Presence of DSLET High-Affinity Binding

RUSTAM YU. YUKHANANOV,*[1] PYOTR M. KLODT,* ANNA D. IL'INA,†
SERGEY V. ZAITSEV† AND ALEXEY I. MAISKY*

*Institute of Pharmacology Russian Academy of Medical Science and
†Moscow State University, Moscow 125315, Russia

Received 11 February 1994

YUKHANANOV, R. YU., P. M. KLODT, A. D. IL'INA, S. V. ZAITSEV AND A. I. MAISKY. *Opiate withdrawal intensity correlates with the presence of DSLET high-affinity binding.* PHARMACOL BIOCHEM BEHAV **49**(4) 1109–1112, 1994.—The goal of this study was to compare the characteristics of $\mu$- and $\delta$-opioid receptors in the cortex of DBA/2 and C57BL/6 mice, which differ in sensitivity to the long- and short-term effects of morphine. The characteristics of $\mu$-opiate receptors were not different in the cortex of both strains. Both high- and low-affinity binding sites of DSLET, a specific ligand of $\delta$-opiate receptors, were present in the cortex of C57BL/6 mice, whereas the high-affinity binding sites were not found in the cortex of DBA/2 mice. The absence of high-affinity DSLET binding sites, which are similar to the $\delta_2$ type of opioid receptors, may explain the less intensive naloxone-precipitated withdrawal reaction of DBA/2 as compared with C57BL/6 mice.

Morphine dependence DBA/2 C57BL/6 DSLET $\delta$- and $\mu$-opioid receptors Naloxone withdrawal $\delta_1$ and $\delta_2$ opioid receptors

THE development of morphine dependence is controlled not only by $\mu$-, but also $\delta$- and $\kappa$-opioid receptors. These receptors appear to have opposite influences on the development of dependence. The selective $\kappa$-opioid antagonist, norbinaltorphimine, significantly increases the level of tolerance to morphine (16), whereas the $\kappa$-agonist, U-50,488, blocked the development of tolerance (19). The selective antagonists of $\delta$-receptors, naltrindole and naltrindole 5′-isothiocyanate, have been found to attenuate the level of tolerance to morphine and the ability of naloxone to precipitate withdrawal reaction (2,11).

Chronic morphine treatment increased the concentration of [D-Ala$^2$,D-Leu$^5$]-enkephalin-Tyr-D-Ala-Bly-Phe-D-Leu (DADLE) binding sites, corresponding to the $\delta$-opioid receptor, in the striatum of mice (1) and in the rat brain (4). However, DADLE has equal affinity for at least two subtypes of $\delta$-opioid receptor, $\delta_1$ and $\delta_2$, which have high affinity to [D-Pen$^{2,5}$]-enkephalin (DPDPE) and [D-Ser$^2$,Thr$^6$]-enkephalin (DSLET), respectively (9,17). The particular role of these subtypes of $\delta$-opioid receptors in the development of morphine dependence is unclear. The two subtypes of the receptors could be separated based on different affinities for DSLET (20). The high-affinity binding sites of DSLET correspond to $\delta_2$-opioid receptors, whereas low-affinity DSLET binding sites probably correspond to the $\delta_1$-opioid receptor. The correlation of the increasing affinity of high-affinity DSLET binding sites with the time course of the development of morphine dependence was found in the mouse cortex after morphine pellet implantation (20).

To clarify further the relationship of $\delta$-opioid receptors to the predisposition to develop opioid dependence, we studied the characteristics of $\mu$- and $\delta$-receptor binding in the cortex of two strains of mice, DBA/2 and C57BL/6, which differ in sensitivity to the short-term effects of morphine on pain perception, locomotor activity, and learning behavior (3,5), as well as intensity of morphine dependence (5,18).

[1] Requests for reprints should be addressed to Dr. Rustam Yu. Yukhananov, Department of Cell Biology, Neurobiology, and Anatomy, Loyola University Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153.

TABLE 1

THE CHARACTERISTICS OF $\mu$- AND $\delta$-OPIATE RECEPTORS IN THE MOUSE BRAIN

| Ligand | Strain | High Affinity | | Low Affinity | |
|---|---|---|---|---|---|
| | | Kd (nM) | B (fmol/mg of protein) | Kd (nM) | B (fmol/mg of protein) |
| DAMGO | DBA/2 | 0.30 ± 0.09 | 1.81 ± 0.44 | 10.5 ± 2.1 | 87.5 ± 12.1 |
| | C57BL/6 | 0.29 ± 0.11 | 1.80 ± 032 | 12 ± 3.6 | 96.1 ± 9.6 |
| DSLET | DBA/2 | | | 3.29 ± 0.8 | 78.3 ± 10.4 |
| | C57BL/6 | 0.41 ± 0.09 | 5.5 ± 1.23 | 6.8 ± 1.6 | 66.3 ± 14.1 |

The receptor binding was performed as described in METHODS. The receptor characteristics were calculated using the difference method. Data represent means ± SEM of three independent experiments in duplicate.

## METHODS

DBA/2 and C57BL/6 male mice weighing 21–24 g (Svetljie Gory Farm, Moscow Region, Russia) were housed seven per cage with food and water available ad lib, and were allowed to acclimate for 1 week before the beginning of the experiments.

Mice were rendered dependent on morphine by SC implantation of one morphine pellet. The pellet was implanted under light ether anesthesia. The withdrawal reaction was induced 96 h after pellet implantation by naloxone injection (1 mg/kg, IP) in six animals of each strain. Five minutes after injection withdrawal, jumping was counted for a 10-min period.

To determine the characteristics of opiate receptors, 30 naive animals of each strain were decapitated, and the brains were immediately removed and dissected on ice. Radioligand binding was performed as described elsewhere (15,20), with some modifications.

The cortex was homogenized by glass-Teflon homogenizer in 50 mM Tris-HCl (pH 7.4) and centrifuged at 18,000 × $g$ for 20 min. The pellet was rehomogenized in buffer B (pH 7.4, 5 mM $N$-2-hydroxyethylpiperazine-$N'$-ethane sulfonic acid, 14 mM NaCl, 111 mM KCl, 0.5 mM $Na_2HPO_4$, and 5 mM $MgCl_2$) and incubated for 30 min in this buffer at 37°C to remove all endogenous peptides. Then the homogenate was centrifuged at 18,000 × $g$ and washed twice with buffer B. The final suspension in buffer B was placed in liquid nitrogen until the binding assay.

Aliquots of the membrane suspension were incubated in buffer B with different concentrations (0.05–20 nM) of labeled peptide for 20 min at 37°C and then filtered under vacuum through GF/B filters (Whatman, Maidstone, UK). The nonspecific binding of [$^3$H]-DAMGO was determined in the presence of 0.5 $\mu$M of unlabeled DAMGO, and the nonspecific binding of [$^3$H]-DSLET in the presence of 0.5 $\mu$M of DSLET. The binding of [$^3$H]-DAMGO was performed in the presence of 40 nM unlabeled DSLET, and the binding of [$^3$H]-DSLET in the presence of 40 nM unlabeled DAMGO to exclude binding with $\delta$- or $\mu$-receptors, respectively.

### *Peptide and Chemicals*

Morphine pellets contained 75 mg base and were composed according the method of Gibson and Tingstad (7). Placebo pellets contained microcellulose instead of morphine. Unlabeled peptides were purchased from Sigma (St. Louis, MO); [$^3$H]-DAMGO (Tyr-D-Ala-Gly-MePhe-Gly-ol, 48 Ci/mmol) and [$^3$H]-DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr-OH, 32 Ci/mmol) were purchased from Amersham (UK).

The characteristics of the receptors were determined by the difference method using the "Delta" program (10). Statistical significance was determined by two-tailed Student's $t$-test and $F$-test using the Logstat program (Medstat, Moscow, Russia).

## RESULTS

The binding of DAMGO, a selective ligand of $\mu$-receptors, could be divided into high- and low-affinity components in both DBA/2 ($F_{8,9}$ = 10.8, $p < 0.01$) and C57BL/6 ($F_{9,10}$ = 9.2, $p < 0.01$) strains of mice (Table 1). The affinity and concentration of high- and low-affinity binding sites were not different in both strains of mice.

The binding of [$^3$H]-DSLET to the cortical membranes of the C57BL/6 mice also better fit a model with high- and low-affinity binding sites ($F_{12,14}$ = 9.8, $p < 0.01$) (Table 1, Fig. 1). Both the low- and high-affinity binding sites corresponded to $\delta$-receptors because the binding assay was performed in the

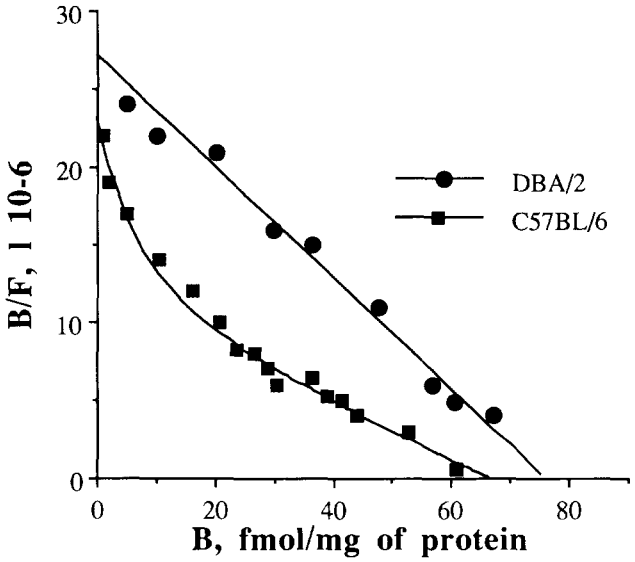


FIG. 1. The binding of [$^3$H]-DSLET to mouse cortex membranes. The membranes were prepared as described in METHODS. Each point represents the average value of duplicate determination from a single representative experiment. High- and low-affinity binding sites of the membrane from C57BL/6 mice have $K_d$ of 0.35 and 6 nM, and $B_{max}$ of 4.5 and 62 fmol/mg of protein, respectively. For the DBA/2 mice membranes $K_d$ and $B_{max}$ were of 2.79 nM and 76.3 fmol/mg of protein. The data were analyzed by the difference method using "Delta" program. The choice between the one- or two-binding-site model was made based on $F$-distribution.

presence of 40 nM of unlabeled DAMGO to block $\mu$-sites. The binding of DSLET in DBA/2 mice, however, revealed only low-affinity binding sites ($F_{10,12} = 1.4$, $p > 0.05$) (Table 1, Fig. 1).

The injection of naloxone (1 mg/kg, IP) 96 h after pellet implantation induced jumping in both strains of mice. The number of jumps per 10 min was significantly lower ($p < 0.05$) in DBA/2 (24 ± 4.5) than in C57BL/6 (58 ± 7.1) mice.

## DISCUSSION

There are two subtypes of $\delta$-opioid receptors in the brain. One has high affinity for DSLET, whereas the selective ligand for the other is DPDPE (9,17). However, both ligands have only relative selectivity for each subtype of receptor. By using DSLET, which is a relatively selective ligand for $\delta_2$-opioid receptors, we determined the characteristics of both $\delta_2$ and $\delta_1$ subtypes, which were the high- and low-affinity binding sites of DSLET. A similar approach was used to show that the affinity of the high-affinity binding sites of DSLET in the mouse cortex, which correspond to $\delta_2$-opioid receptors, was increased after implantation of the morphine pellet, and that the kinetics of the receptor upregulation correlated well with the development of morphine dependence (20). Upregulation of the $\delta$-opioid receptor was also observed in the striatum of mice (1) and in the rat brain (4) after morphine pellet implantation. In this study, we have found that DBA/2 mice have significantly less intense naloxone-precipitated withdrawal and have no, or very low, concentration of high-affinity binding sites for DSLET, which probably corresponds to the $\delta_2$-opioid receptors. These findings indirectly corroborate previous observations that antagonists of $\delta_2$-opioid receptor decreased dependence to morphine in mice (11).

Anatomic sites involved in the development of naloxone-precipitated withdrawal reaction are not fully understood (8). The interaction among different brain areas may regulate the intensity of withdrawal reaction. Within the brain, the most intensive withdrawal reaction can be precipitated after the injection of methylnaloxonium in the locus coeruleus (8). The naloxone injection activates a noradrenaline (NA) turnover in the NAergic projection from the locus coeruleus to mice cortex (6) and NA release in the rat prefrontal cortex (12). The $\delta$-opioid receptors in the cortex regulate the activity of NAergic terminals and may thus modulate the withdrawal reaction. The attenuated activation of $\delta_2$-opioid receptors in the cortex by either the injection of an antagonist or the genetically controlled diminished expression that appears in DBA/2 mice may result in a less-intensive withdrawal reaction after naloxone injection.

It is not clear how the $\delta$-opioid receptors are involved in the development of morphine dependence. Subtypes of $\delta$-opioid receptors have been shown to form a complex with the $\mu$-opioid receptor, and may regulate each other's affinity (13,14). The $\delta$-opioid receptor linked to the $\mu$-opioid receptor ($\delta_{complexed}$ receptor) probably corresponds to the $\delta_2$-opioid receptor, because DSLET is a high-affinity agonist of this receptor, whereas $\delta_{noncomplexed}$ receptors correspond to $\delta_1$-opioid receptors (13). Perhaps, the development of dependence requires the presence and activation of a complex of $\mu$- and $\delta_2$-opioid receptors. This would suggest that the DBA/2 mice, which have no, or a very low, concentration of $\delta_2$-opioid receptors, should express a significantly decreased naloxone-precipitated withdrawal reaction. Thus, the genetic regulation of the expression of the $\delta_2$-opioid receptors may be a mechanism to control the intensity of opioid dependence.

## ACKNOWLEDGEMENTS

The authors thank Drs. Alan Nagahara and Robert Handa for their editorial assistance in the preparation of the manuscript.

## REFERENCES

1. Abdelhamid, E. E.; Takemori, A. E. Characteristics of $\mu$ and $\delta$ opioid binding sites in striatal slices of morphine-tolerant and -dependent mice, Eur. J. Pharmacol. 198:157–163; 1991.
2. Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. Selective blockade of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. J. Pharmacol. Exp. Ther. 258:299–303; 1991.
3. Belknap, J. K.; Noordewier, B.; Lame M. Genetic dissociation of multiple morphine effects among C57BL/6J, DBA/2J and C$^3$H/HeJ inbred mouse strains. Physiol. Behav. 46:69–74; 1989.
4. Danks, J. A.; Tortella, F. C.; Long, J. B.; Bykov, V.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W.; Rothman, R. R. Chronic administration of morphine and naltrexone upregulate [$^3$H-D-Ala$^2$, D-Leu$^5$]-enkephalin binding sites by different mechanisms. Neuropharmacology 27:965–974; 1988.
5. Frischknecht, H. R.; Siegfried, B.; Waser, P. G. Opioid and behavior: Genetic aspects. Experientia 44:473–481; 1988.
6. Funada, M.; Narita, M.; Suzuki, T.; Misawa, M. Effect of pretreatment with pertussis toxin on the development of physical dependence to morphine. Naunyn Schmiedebergs Arch. Pharmacol. 348:88–95; 1993.
7. Gibson, R. D.; Tingstad, J. E. Formulation of a morphine implantation pellet suitable for tolerance–Physical dependence studies in mice. J. Pharmacol. Sci. 59:426–427; 1970.
8. Koob, G. F.; Maldonado, R.; Stinus, L. Neural substrates of opiate withdrawal. Trends Neurosci. 15:186–192; 1992.
9. Mattia, A.; Vanderah, T.; Mosberg, H. I.; Porreca, F. Lack of antinociceptive cross-tolerance between [D-Pen$^2$,D-Pen$^5$]-enkephalin and [D-Ala$^2$]-deltorphin II in mice: Evidence for delta receptor subtypes. J. Pharmacol Exp. Ther. 258:583–587; 1991.
10. Melichova, E. M.; Kurochkin, I. N.; Zaitsev, S. V.; Varfolomeev, S. D. Analysis of ligand-receptor binding by the difference method. Anal. Biochem. 175:507–515; 1988.
11. Miyamoto, Y.; Portoghese, P. S.; Takemori, A. E. Involvement of delta2 opioid receptors in the development of morphine dependence in mice. J. Pharmacol. Exp. Ther. 264: 1141–1145; 1993.
12. Rossetti, Z. L.; Longu, G.; Mercuro, G.; Gessa, G. L. Extraneuronal noradrenaline in the prefrontal cortex of morphine-dependent rats: Tolerance and withdrawal mechanisms. Brain Res. 609:316–320; 1993.
13. Rothman, R. R.; Danks, J. A.; Jacobson, A. E.; Burke, T. R., Jr.; Rice, K. C.; Tortella, F. C.; Holaday, J. W. Morphine tolerance increases $\mu$-noncompetitive $\delta$ binding sites. Eur. J. Pharmacol. 124:113–119; 1986.
14. Rothman, R. R.; Mahboubi, A.; Bykov, V.; Kim, C.-H.; DeCosta, B. R.; Jacobson, A. E.; Rice, K. C. Probing the opioid receptor complex with (±)-trans-superfit: ii. Evidence that $\mu$ ligands are noncompetitive inhibitors of the $\delta_{cx}$ opioid peptide binding site. Peptides 13:1137–1143; 1992.
15. Skliankina, O. A.; Kurochkin, I. N.; Varfolomeeva, A. D.; Krekhnov, B. V.; Zaitsev, S. V.; Varfolomeev, S. D. Kinetic characteristics of the effect of GTP on the properties of opioid receptors. Biokhimiia 55:1032–1042; 1990.
16. Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Maintenance of acute morphine tolerance in mice by selective blockage of $\kappa$ opioid receptors with norbinaltorphimine. Eur. J. Pharmacol. 210:159–162; 1992.

17. Sofuoglu, M.; Portoghese, P. S.; Takemory, A. E. Different antagonism of delta opioid agonist by naltrindole and its benzofuran analog (NTB) in mice: Evidence for delta opioid subtypes. J. Pharmacol. Exp. Ther. 257:676–680; 1991.
18. Suzuki, T.; Otani, K.; Misawa, M. Differential sensitivity to physical dependence on morphine and codeine in three inbred strains of mice. Jpn. J. Pharmacol. 57:455–462; 1991.
19. Yamamoto, T.; Ohno, M.; Ueki, S. A selective κ-opioid agonist U-50,488H, blocks the development of tolerance to morphine analgesia in rats. Eur. J. Pharmacol. 156:173–176; 1988.
20. Zaitsev, S. V.; Il'ina, A. D.; Klodt, P. M.; Varfolomeev, S. D.; Yukhananov, R. Yu. Heterogeneity of μ- and δ-opioid binding sites in the mouse cerebral cortex. Selective increase in the affinity of a high affinity delta-site during long-term administration of morphine. Biokhimiia 57:1242–1247; 1992.