

Effects of Neuroleptics on the Opioid Receptor Binding in the Mouse Striatum

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ABSTRACT

Our purpose was to gain insight into a possible modulatory role for μ , δ , and κ opioid receptors by neuroleptics (chlorpromazine, thioridazine, haloperidol, sulpiride, and pimozone) in chronic morphine 5 mg/kg and 20 mg/kg treated mouse striatum. We attempted quantitative receptor assays using highly specific radioligands, [3 H] DAGO ([D-Ala², N-Mephe⁴, Glycol⁵] enkephalin), [3 H]DPDPE ([D-Pen², D-Pen⁵] enkephalin) and [3 H] DPN(diprenorphine) to measure the binding affinity in the experimental groups.

The decrease of [3 H]DAGO binding was potentiated by sulpiride and pimozone in the chronic morphine treatment (5 mg/kg and 20 mg/kg). The decrease of [3 H]DPDPE binding was inhibited by chlorpromazine, thioridazine, haloperidol, sulpiride, and pimozone in chronic morphine treatment (5 mg/kg and 20 mg/kg). The decrease of [3 H] DPN binding was significantly inhibited by chlorpromazine, thioridazine, sulpiride, and pimozone in chronic morphine 20 mg/kg treatment. [3 H] DPN binding on the neuroleptics was antagonized by naloxone pretreatment in chronic morphine 20 mg/kg treatment.

These findings suggest that neuroleptics influence opposing tonically active on the δ and κ opioid receptor compared with μ opioid receptor in the chronic morphine treated mouse striatum.

Key Words: Opioid binding, Neuroleptics

INTRODUCTION

Anatomical and functional relationships between the opioidergic and dopaminergic (DA) circuitry are well documented in dopaminergic pathway of the brain. Opioids are well known to influence a variety of behavioral responses in mammals (Iwamoto, 1981; Locke and Holtzman, 1986), possibly by interaction with brain dopamine systems in various area of brain. Opioid peptides are located within both the nigrostriatal and mesolimbic regions (Hokfelt *et al.*, 1977), and

opiate receptors have been also localized on the DA neurons within these regions (Pollard *et al.*, 1977a; Pollard *et al.*, 1997b). It is known that many of the properties of opiates, including analgesia, addiction, and respiratory depression, are mediated by selective receptors in the central nervous system. The definitive existence of multiple opioid receptors was first presented by Martin (1976) and was reviewed by Goldstein and James (1984).

In vivo, systemic administration of morphine increases striatal dopamine release and turnover (Chesselet *et al.*, 1981; Wood *et al.*, 1987) and the firing rate of mesencephalic dopamine neurons (Matthews and German, 1984). Studies using receptor-selective agonists have demonstrated that *in vivo* administration of both μ and δ agents stimu-

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lates dopamine release (Di Chiara and Imperato, 1988; Spanagel *et al.*, 1990; Pentney and Gratton, 1991) and dopamine cell firing (Latimer *et al.*, 1987). The μ agonists modulate dopamine release indirectly, whereas δ agonists may activate receptors that are localized presynaptically on dopamine terminals (Spanagel *et al.*, 1990; Arenas *et al.*, 1991; Dourmap *et al.*, 1992). In contrast, κ agonists inhibit the activity of mesencephalic dopaminergic neuron *in vivo* (Di Chiara and Imperato, 1988; Spanagel *et al.*, 1990; Manzanares *et al.*, 1991).

The above evidence indicates that the effects of the DA agonist may be altered by opioids (Swerdlow *et al.*, 1985; Swerdlow *et al.*, 1987). However, the precise mechanism underlying opioid modulation of dopamine function has yet to be established.

In the present study, the effects of chlorpromazine, thioridazine, haloperidol, sulpirides and pimozide on the [3 H] DAGO, [3 H] DPDPE, and [3 H] DPN binding in chronic morphine treatment were examined by using binding assay, which can explain the kinetic aspects of the binding on the each opioid receptor in morphine dependence.

MATERIALS AND METHODS

Animals

6 animals of ICR mice weighing 20~30 g were used in following groups. Animals were housed in a controlled environment of $25 \pm 1^\circ\text{C}$ and regular food throughout the experimental period.

Experimental group

The first experiment was designed to determine the μ , δ , and κ opioid receptor affinity after morphine (5 mg/kg, 20 mg/kg, I.P.) treatment for 10 days. The second experiment were designed to determine whether chlorpromazine (10 mg/kg), thioridazine (10 mg/kg), haloperidol (750 $\mu\text{g}/\text{kg}$), sulpiride (10 mg/kg), and pimozide (10 mg/kg) as neuroleptics would affect on the opioid receptor binding. The third experiment was designed to study the effect of naloxone pretreatment on the above neuroleptics to opioid receptor binding in the morphine (5 mg/kg, 20 mg/kg, I.P. for 10 days) treated mouse striatum.

Opiate receptor binding assay

Membrane preparation was carried out as previously described (Tempel *et al.*, 1985). Aliquots (100 μl , mg/ml of protein) of freshly prepared homogenate from striatal tissue were incubated with [3 H] [D-Ala², N-Mephe⁴, Glycol⁵] enkephalin : DAGO (for μ -specific opioid receptor assays), [3 H] [D-Pen², D-Pen⁵] enkephalin: DPDPE (for δ -specific opioid receptor assays), [3 H] diprenorphine: DPN (for κ -specific opioid receptor assays). Nonspecific binding was determined by the addition of 5 μM nonreactive DAGO, DPDPE, and DPN. The incubations were terminated by collecting the Whatman GF/B filter, and excess radioactivity were removed by washing the filters three times with 5 ml of 25 mM HEPES at 0°C . After incubation at 20°C overnight in 10 ml of Liquiscint (National Diagnostics, Somerville, N. J.), its radioactivity on the filters was determined by liquid scintillation counter.

Protein determination

The concentrations of protein in membrane preparations were measured by the method of Lowry *et al.*, (1951). Bovine serum albumin was used as the protein standard.

Materials

[3 H] DAGO (59Ci/mmol), [3 H] DPDPE (46Ci/mmol), and [3 H] DPN (31Ci/mmol) were obtained from Amersham Int. Chlorpromazine, thioridazine, haloperidol, sulpiride, and pimozide used in these studies were purchased from Sigma Chemical Company (St. Louis, MO).

Statistical analysis

To quantify the degree of each selective opioid receptor binding, data were expressed as a percentage with respect to the each nonspecific binding.

RESULTS

Effect of morphine to the opioid receptor binding

Specific binding to the [3 H] DAGO and [3 H] DPDPE after morphine 5 mg/kg I.P. for 10 days

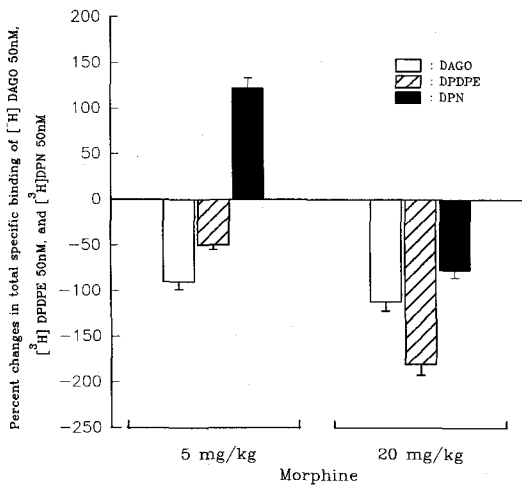


Fig. 1. Effect of morphine to the [³H] DAGO(□), [³H] DPDPE(▨), and [³H] DPN(■) binding in the mouse brain striatum.

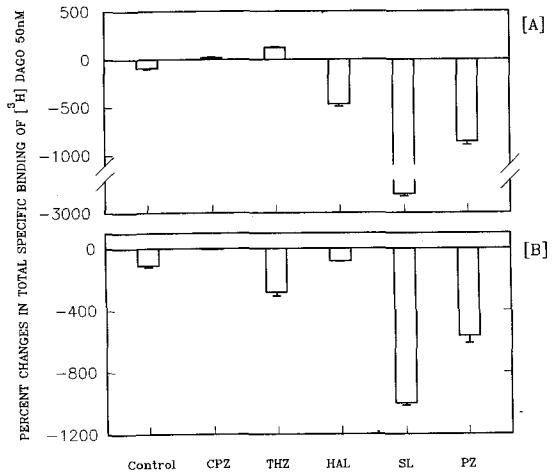


Fig. 2. Effects of several neuroleptics to the [³H] DAGO binding in the chronic morphine ([A] 5 mg/kg, [B] 20mg/kg) treated mouse brain striatum. CPZ: chlorpromazine, THZ: thioridazine, HAL: haloperidol, SL: sulphiride, PZ: pimozide

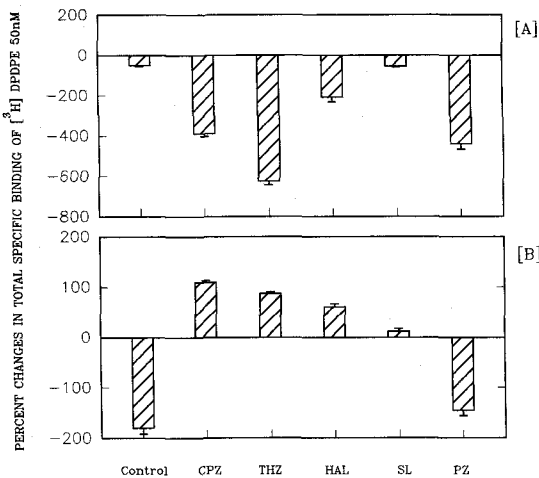


Fig. 3. Effects of several neuroleptics to the [³H] DPDPE binding in the chronic morphine ([A] 5 mg/kg, [B] 20 mg/kg) treated mouse brain striatum.

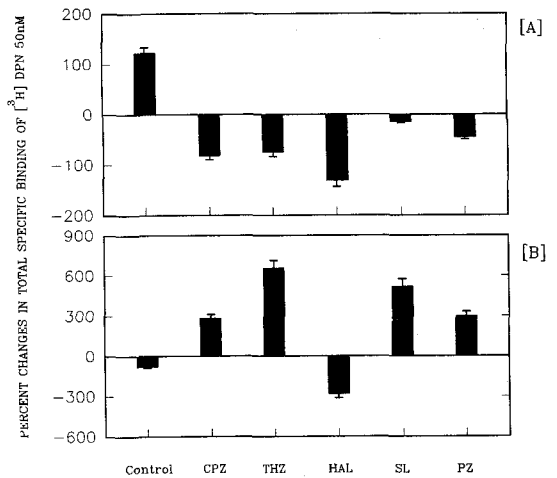


Fig. 4. Effects of several neuroleptics to the [³H] DPN binding in the chronic morphine ([A] 5 mg/kg, [B] 20mg/kg) treated mouse brain striatum.

was decreased, but binding to [³H] DPN was increased. In higher dose, 20 mg/kg of morphine, [³H] DAGO, [³H] DPDPE, and [³H] DPN binding showed lower affinity than morphine 5mg/kg treated group (Fig. 1).

Effects of chlorpromazine, thioridazine, haloperidol, sulphiride, and pimozide to the opioid receptor binding

The chlorpromazine and thioridazine showed

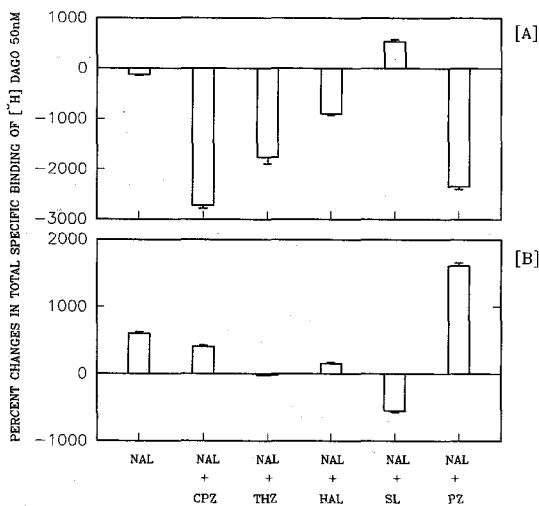


Fig. 5. Effects of naloxone on the [³H] DAGO binding by neuroleptics in the chronic morphine([A] 5 mg/kg, [B] 20 mg/kg) treated mouse brain striatum. NAL: naloxone

decrease of [³H] DAGO binding and increases of [³H] DPDPE and [³H] DPN binding in morphine 20 mg/kg treated group comparing with morphine 5 mg/kg treated group. Haloperidol showed increases to [³H] DAGO and [³H] DPDPE binding and decrease to [³H] DPN binding in morphine 20 mg/kg treated group comparing with morphine 5 mg/kg treated group. Sulpiride and pimozide showed increases of [³H] DAGO, [³H] DPDPE, and [³H] DPN binding in morphine 20 mg/kg treated group comparing with morphine 5 mg/kg treated group (Fig. 2-4).

Effect of naloxone on the chlorpromazine, thioridazine, haloperidol, sulpiride, and pimozide to the opioid receptor binding

Naloxone caused antagonistic effect on [³H] DAGO, [³H] DPDPE and [³H] DPN binding by chlorpromazine and thioridazine in morphine 5 mg/kg treated mouse group. There was an antagonistic effect of naloxone on the [³H] DPN binding by haloperidol in morphine 20 mg/kg treated mouse group. We also found antagonistic effect of naloxone on [³H] DAGO binding by sulpiride in morphine 5 mg/kg treated group and [³H] DPDPE binding by sulpiride in morphine 20 mg/kg treated group. Naloxone caused significant an-

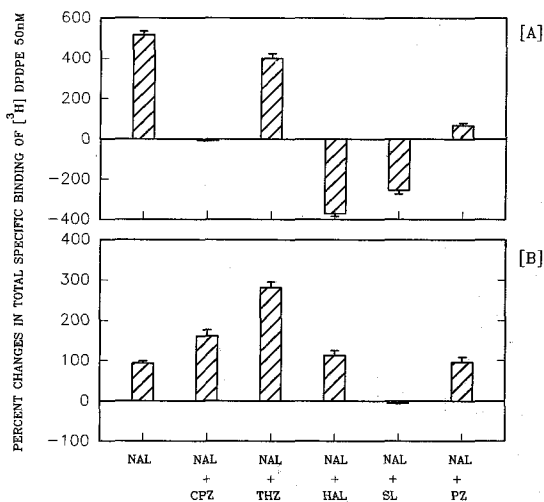


Fig. 6. Effects of naloxone on the [³H] DPDPE binding by neuroleptics in the chronic morphine ([A] 5 mg/kg, [B] 20mg/kg) treated mouse brain striatum.

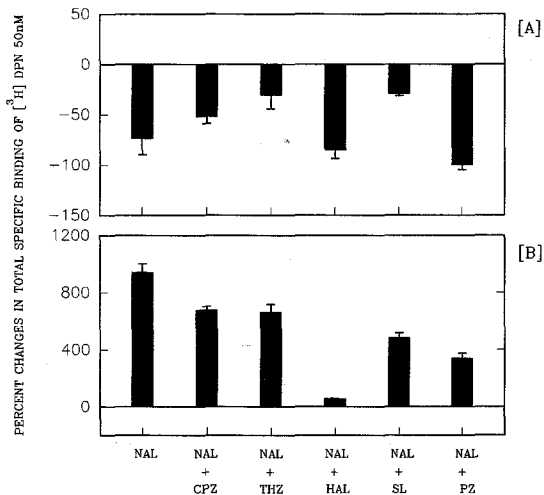


Fig. 7. Effects of naloxone on the [³H] DPN binding by neuroleptics in the chronic morphine ([A] 5 mg/kg, [B] 20 mg/kg) treated mouse brain striatum.

tagonistic effect on [³H] DAGO binding by pimozide in morphine 20 mg/kg treated group and [³H] DPDPE binding in morphine 5 mg/kg and 20 mg/kg treated group (Fig. 5-7).

DISCUSSION

Morphine and other opiates are known to influence the function of striatal dopaminergic system by stimulation of the synthesis, metabolism, and turnover of dopamine (Chesselet *et al.*, 1981; Wood *et al.*, 1987) and the firing rate of dopaminergic neurons (Mattews and German, 1984; Di Chiara and Imperato, 1998). On the other hand, there are confliction data concerning the effects of opioids on the release of DA in rats, cats, and guinea pigs. Some authors suggest that opioids stimulate striatal DA release, others conclude that they do not influence DA release or actually inhibit DA release (Werling *et al.*, 1988; Illes, 1989; Illes and Jackisch, 1991). Indeed, the occupancy of multiple opioid receptors on the same neuron has been established in the peripheral and central nervous system (Fields *et al.*, 1980; Egan and North, 1981).

As outlined in the introduction, concerning the type of opioid receptors on the modulation of striatal DA release are contradictory. There is direct evidence for the regulation of striatal met-enkephalin neuron by nigrostriatal dopaminergic neurons. Therefore, binding of ligand selective for subtype of opioid receptors may change the binding for other type of receptors in same neurons. This data show the effect of antipsychotics on the changes of binding affinity on the distinct subtypes of opioid receptor in the mouse striatum.

The [³H] DAGO and [³H] DPDPE binding were decreased by chronic morphine treatment (5 mg/kg and 20 mg/kg) (Fig. 1). But the [³H] DPN binding was increased in 5 mg/kg chronic morphine treated group and decreased in 20 mg/kg morphine treated group (Fig. 1). This tendency on the opioid receptor binding may provide a basic framework for understanding the mechanism of the opiate dependence.

Recent study (Acquas *et al.*, 1991) demonstrated a long-lasting reduction of DA release in the mesolimbic system after morphine withdrawal. Thus, after prolonged μ agonist administration, there was a compensatory increase in the activity of the functionally opposing dynorphin system. Spanagel *et al.*, (1992) had reported that highest

dose of κ agonist U-69593 was less effective in modifying DA release than lower doses. Interestingly U-69593 as well as other agonist produced biphasic response of dose (Mucha and Herz, 1985; Bals-Kubik *et al.*, 1989)

The dopamine D₂ antagonist, sulpiride and pimozide showed the significant potentiation of decrease of the μ opioid receptor binding in 5 mg/kg and 20 mg/kg morphine treated group (Fig. 2). Chlorpromazine and thioridazine showed the significant potentiation of the decrease on the δ opioid receptor binding in 5 mg/kg morphine treated group (Fig. 3). But, in morphine 20 mg/kg treated group, [³H] DPN binding were changed oppositely comparing with μ selective agonist, DAGO binding (Fig. 4).

We could not find significant differences about each receptor binding affinity within D₁ and D₂ selective or atypical and typical neuroleptics. But δ and κ opioid receptor exhibited a property opposing tonically active by neuroleptics in chronic morphine treated group. In summary, in view of the present results, it is tempting to speculate that neuroleptics in sensitive to the reinforcing effects of morphine may result, at least in part, from either a decrease in binding affinity of μ opioid receptor or increased activity of δ and κ opioidergic system.

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=국문초록=

마우스 선조체에서 Opioid 수용체 결합에 대한 Neuroleptics의 영향

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이 연구에서는 선조체에서 opioid 신경계와 dopamine 신경계의 상호 관계를 알아보기 위해서 morphine을 5mg/kg, 20 mg/kg로 10일간 복강내 투여한 후 chlorpromazine, thioridazine, haloperidol, sulpiride, pimozide를 투여하였다. Opioid μ , δ , κ 수용체의 binding의 변화를 관찰하고자 [^3H] DAGO, [^3H] DPDPE, 및 [^3H] DPN binding assay를 하였으며, 그 결과 morphine (20 mg/kg) 장기 투여된 실험군에서 [^3H] DAGO, [^3H] DPDPE, 및 [^3H] DPN 결합이 감소되었다. Morphine 20 mg/kg 장기 투여군에 chlorpromazine, thioridazine 주사시에는 morphine 5mg/kg 투여군에 비하여 [^3H] DAGO 결합의 감소와, [^3H] DPDPE, 및 [^3H] DPN 결합의 증가를 나타내었고, haloperidol 주사군은 [^3H] DAGO, [^3H] DPN 결합의 감소, 및 [^3H] DPDPE 결합의 증가를 나타내었다. Sulpiride, pimozide 주사군은 morphine 5 mg/kg 투여군에 비하여 20 mg/kg 투여군에서 [^3H] DAGO, [^3H] DPDPE, 및 [^3H] DPN 결합의 증가를 나타내었다.

이상의 결과로 보아 각 약물간의 opioid 결합에 대한 차이점은 있었으나, morphine 5mg/kg 투여군보다 20 mg/kg 투여군에서 [^3H] DPDPE 및 [^3H] DPN의 결합이 증가의 경향을 보임으로써, 다량의 morphine을 투여했을 때 μ opioid 수용체에 비하여 δ 와 κ opioid 수용체가 더 활성화되는 것을 알 수 있었다.