Korean J. of Pharmacology
Vol. 30, No. 1, pp. 11-18, 1994

The Changes of Cyclic AMP Content by Opiates in Chronic Haloperidol Treated Mouse Striatum

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ABSTRACT

Cyclic adenosine 3',5'-monophosphate (cyclic AMP) has been frequently accepted as an intracellular messenger for receptor-mediated action of opioids. In this experiment, it was designed to determine the interaction of dopaminergic and opioidergic system in the mouse striatum in normal and chronic haloperidol treated groups. Haloperidol 750ug/kg I.P. for 10 days was performed for dopamine denervation. The morphine, DAGO, DPDPE, and U50,488H inhibited the increase of haloperidol-induced cyclic AMP content in chronic haloperidol treated mouse striatum. The inhibition of DAGO and DPDPE showed significant increase compared to normal mouse striatum. Naloxone showed antagonistic effect on the morphine and U50,488H in chronic haloperidol treated group, and showed antagonistic effect on morphine, DAGO, DPDPE, and U50, 488H in normal mouse striatum. These findings support that there is a functional interrelationship of dopaminergic and opioidergic pathway in the striatum. This result provides an evidence that following destruction of striatal dopaminergic neuron, there are some changes of cAMP content on the μ, δ, and κ opioid receptor, but the κ opioid receptor still has its function.

Key Words: cAMP, Opiates, Haloperidol

INTRODUCTION

The concept that there are several types of opioid receptors was originally suggested by Martin (1984). Exogenous and endogenous opioids interact with at least three distinct subtypes of opioid receptors in peripheral and central nervous system, designated as μ, δ, and κ receptor(Wüster *et al.*, 1981; Paterson *et al.*, 1983; Martin, 1984). The multiple opioid receptors on the same neuron have been estabilished in the peripheral(Terry and North, 1981) and central nervous system (Fields *et al.*, 1980). However, the existence of such a multiple receptors might explain, at least partly, why the stimulation of one type of opioid receptors can affect another opioid receptor. There are evidences that exogenous opioids can influence the activity of mesolimbic dopaminergic neurons such as motivational(Wise, 1983; Herz and Shippenberg, 1989) and locomotor effect (Stinus *et al.*, 1980; Kalivas *et al.*, 1983) as well as the development of variable opiate dependence (Di Chiara and Imperato, 1988a; Wise and Rompre, 1989; Acquas *et al.*, 1991). Cyclic adenosine 3,5-monophosphate (cyclic AMP) has been frequently involved as an intracellular messenger for receptor mediated action of opioids (Schramm and Selinger, 1984; Worley *et al.*, 1987). In neuroblastoma X glioma hybrid cells, NG 108-15, δ-opiate receptors are coupled to adenylate cyclase by the inhibitory guanine nucleotide protein, termed Gi (Kurose *et al.*, 1983, Abood *et al.*, 1985). In the rat

*This study was supported by Keimyung University and Dong San Medical Center(1993)

striatum, which contains high numbers of both dopaminergic neuron (Dray, 1979) and enkephalin neuron (Cuello, 1983; Khachaturian *et al.*, 1985) as well as high density of μ and δ opioid (Atweh and Kuhar, 1983; Wamsley, 1983) receptors particularly. Some reports have shown that the activation of D-1 dopamine receptor results in a stimulation of cyclic AMP production, whereas simultaneous activation of D-2 dopamine receptors partly reverses above effect (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). There is a report that chronic dopaminergic receptor blockade with peripherally administered haloperidel increases enkephalin biosynthesis, particularly met-enkephalin level(Hong *et al.*, 1979). The high concentration of enkephalin is found in the striatum, and met-enkephalin is the predominant form among them. The mesolimbic dopaminergic system has been involved in mediating the motivational effects of opioids. However, the site of action of opioids within above system and the role of endogenous opioid peptides in modulating dopamine activity remain unknown. Regarding the modulation of opioidergic neurotransmission by haloperidol treatment, this study was designed to determine the effect of dopaminergic denervation by haloperidol administration on the change of cyclic AMP level related with μ, δ, and κ opioid receptor agonists.

MATERIALS AND METHODS

Animal treatment

Total animals were randomly divided into 3 groups. The first group was normal mouse treated with saline. The second group was treated with opioid agonists, such as morphine (20 mg/kg i.p.), DAGO: [D-Ala-Mephe-Gly-ol] enkephalin (50 ug /kg i.p.), DPDPE: [D-Pen, D-Pen] enkephalin (50 ug/kg i.p.), U50, 488H (500 ug/kg i.p.) and naloxone pretreatment to the above opioid agonists treated groups. The third group was chronic haloperidol (750 ug/kg i.p.) treatment group for 10 days and was treated with opioid agonists and naloxone.

Preparation of homogenate from mouse striatum

Mouse weighing between 25 g and 30 g were decapitated and then the striatum was dissected carefully. Each tissue piece was placed immidiately in 2M ice-cold perchloric acid and hand homogenized with 20 volume of ice-cold 0.32 M sucrose containing 5 mM HEPES K (pH 7.4) buffer. The homogenate was centrifuged at 1, 000 g for 5 min. at 4℃ and clear supernatant fraction was taken, and the supernatant was recentrifuged at 10,000×g for 30min. The pellet was resuspended in 10 volume of 0.05 M Tris-HCl buffer, pH 7.4. The preparated membrane samples were stored at −70℃ until use.

Determination of cAMP content

Cyclic AMP was determined by cyclic AMP[^{125}I] RIA-kit (Dupont Co.).

Protein Assay

Protein content was measured by the method of Lowry *et al* (1951) using bovine serum albumin as standard.

Chemicals

The following drugs were obtained commercially. The morphine (SamSung Co.), naloxone (Sigma Chemical Co.), haloperidol (Sigma Chemical Co.) DAGO (Sigma Chemical Co.), DPDPE (Sigma Chemical Co.), U50, 488H (Sigma Chemical Co.) were used.

Statistical evaluation

The observations are stated as mean± S.E. The statistical significance of differences was determined by independent t-test. The P-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of morphine

The cyclic AMP content was 73.64±7.28 pmol/mg in normal mouse striatum and 152.98± 11.52 pmol/mg protein in haloperidol treated mouse striatum. The prototype μ-receptor agonist, mor-

Table 1. Effects of opiate agonists and naloxone on cAMP accumulation in normal mouse striatum

Treatment	Normal group (pmol/mg protein)	Naloxone pretreated group (pmol/mg protein)
Control	73.64±7.28	
Morphine	70.97±8.12	116.34±19.40
DAGO	65.05±5.92	181.05±17.80***
DPDPE	55.27±5.26	133.70±11.36***
U50,488H	58.32±4.82	168.47±15.92***

***means $p<0.05$.

Table 2. Effects of opiate agonists and naloxone on cAMP accumulation in chronic haloperidol treated mouse striatum

Treatment	Haloperidol treated group (pmol/mg protein)	Naloxone pretreated haloperidol group (pmol/mg protein)
Control	152.98±11.52	
Morphine	66.97± 5.29***	87.17± 9.24***
DAGO	101.95±12.88*	82.36± 8.43***
DPDPE	129.01±10.26	86.34± 8.78***
U50,488H	50.23± 4.18***	59.34± 5.49***

* and *** mean $p<0.05$ and $p<0.005$, respectively.

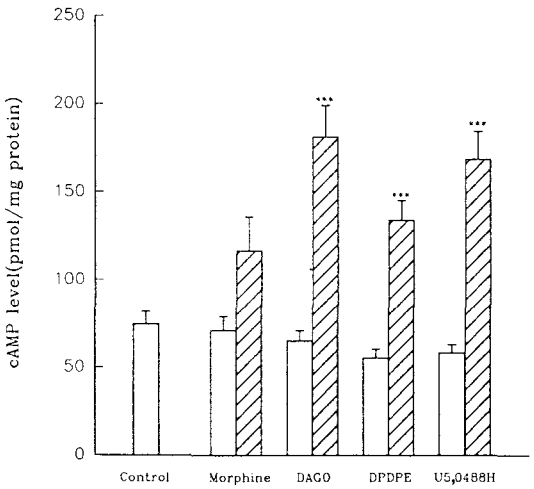

Fig. 1. Conparison of the effects of opiates(□) and naloxone (▨) on the cAMP accumulation in normal mouse striatum.
▨: naloxone pretreated group
***means $p<0.005$

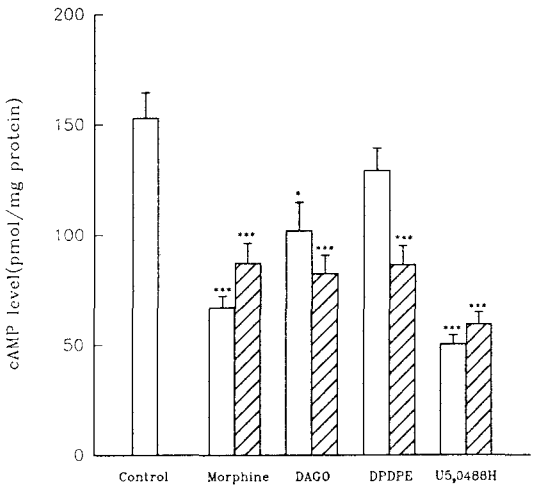

Fig. 2. Comparison of the effects of opiates (□) and naloxone (▨) on the cAMP accumulation in chronic haloperidol treated mice striatum.
▨: naloxone pretreated group
* and *** mean $p<0.05$ and $p<0.005$ respectively.

phine, showed mild decrease of cyclic AMP content in normal mouse striatum (Table 1, Fig. 1) and significant decrease of cyclic AMP content in haloperidol treated mouse striatum comparing to each control group (Table 2, Fig. 2).

Those cyclic AMP contents were inhibited by naloxone pretreatment in normal mouse striatum, but were slightly inhibited by naloxone pretreatment in haloperidel treated mouse striatum (Fig. 3).

Effect of DAGO

The selective μ opioid receptor agonist, DAGO, showed mild decrease of cyclic AMP content in normal mouse striatum (Table 1, Fig. 1) and significant decrease of cyclic AMP content in haloperidol treated mouse striatum comparing to each control group (Table 2, Fig. 2).

Those cyclic AMP contents were inhibited by naloxone pretreatment in normal mouse striatum,

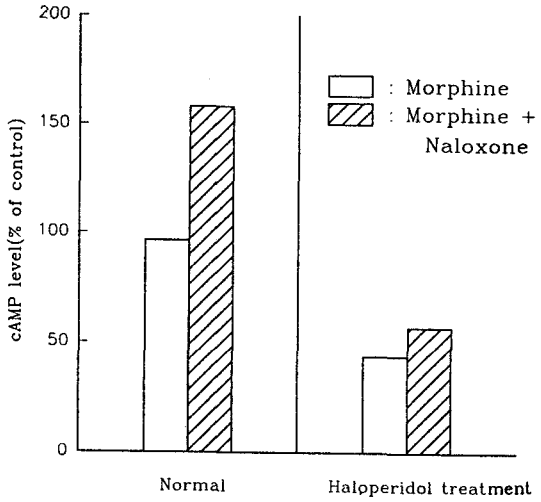

Fig. 3. Effect of naloxone on the changes of cAMP accumulation by morphine in normal and chronic haloperidol treated mouse striatum.

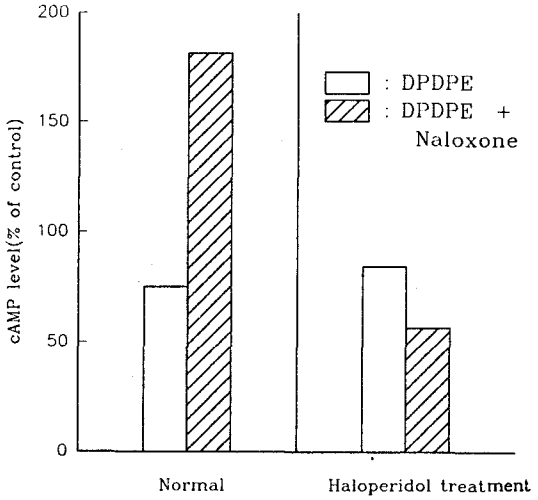

Fig. 6. Effect of naloxone on the changes of cAMP accumulation by DPDPE in normal and chronic haloperidol treated mouse striatum.

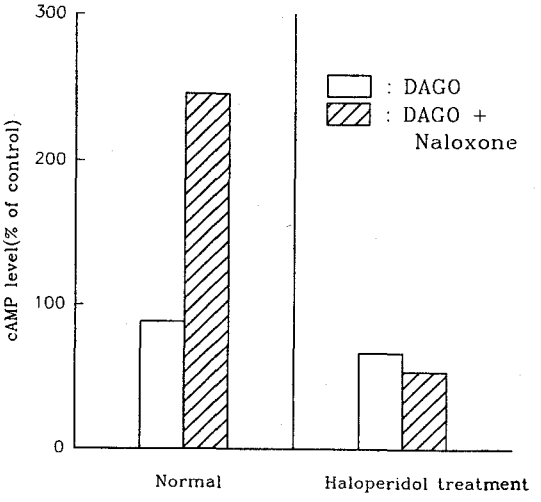

Fig. 4. Effect of naloxone on the changes of cAMP accumulation by DAGO in normal and chronic haloperidol treated mouse striatum.

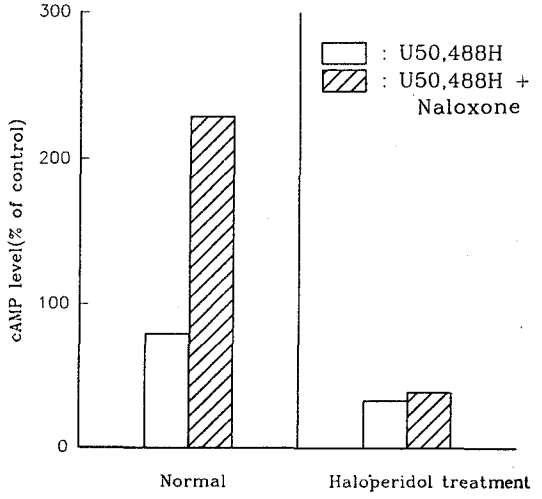

Fig. 5. Effect of maloxone on the changes of cAMP accumulation by DPDPE in normal and chronic haloperidol treated mouse striatum.

but were not inhibited by naloxone pretreatment in haloperidol treated mouse striatum (Fig. 4).

Effect of DPDPE

The selective δ opioid receptor agonist, DPDPE, showed mild decrease of cyclic AMP content in normal (Table 1, Fig. 1) and haloperidol treated mouse striatum comparing to each control group (Table 2, Fig. 2). Those cyclic AMP contents were inhibited by naloxone pretreatment in normal

mouse striatum, but were not inhibited by naloxone pretreatment in haloperidol treated mouse striatum (Fig. 5).

Effect of U50, 488H

The selective κ opioid receptor agonist, U50, 488H, showed mild decrease of cyclic AMP content in normal mouse striatum (Table 1, Fig. 1) and significant decrease in haloperidol treated mouse striatum coparing to each control group (Table 2, Fig. 2).

Those cyclic AMP contents were significantly inhibited by naloxone prereatment in normal mouse striatum, but were slightly inhibited by naloxone pretreatment in haloperidol treated mouse striatum (Fig. 6).

DISCUSSION

The opiate agonists exert ther action by decreasing the intracellular cyclic AMP levels. This hypothesis has been based primarily on biochemical experiment that opioids inhibit adenylate cyclase activity or decrease the levels of cyclic AMP in neuroblastoma-glioma hybrid cell and variable sites in central nervous system, primarily the rat striatum (Law *et al.*, 1983, Schramm and Selinger, 1984; Worley *et al.*, 1987, Attali *et al.*, 1989, De Vries *et al.*, 1991). Few investigations have attempted to estabilish whether the decrease of cyclic AMP is important for the inhibitory actions of opioids on neurotransmission, suggesting the effects of opioids are mediated through the inhibitory guanine nucleotide binding protein Gi (Kurose *et al.*, 1983, Abood *et al.*, 1985, Eriksson *et al.*, 1992). However, the role of cyclic AMP in opioid-mediated inhibition remains to be estabilished although another intracellular mechanisms, such as the phosphatidylinositol-phospholipase C pathway are suspicious (Illes, 1986; Schffelmeer *et al.*, 1986). There are now some evidences to support the hypothesis that endogenous opioids paly an important role in the regulation of dopaminergic neurons in brain (Gudelsky and Porter, 1979; Spanagel *et al.*, 1992 a). However the precise mechanism underlying opioid modulation of dopamine function has not yet estabilished. In vivo, systemic administration of morphine increases striatal dopamine release and turnover and the firing rate of mesencephalic dopamine neuron (Matthew *et al.*, 1984; Wood *et al.*, 1987). Studies using receptor selective agonists have demonstrated that in vivo administrations of μ and δ agonists stimulate dopamine release indirectly (Chesselet *et al.*, 1981; Di Chiara and Imperato, 1988 b), whereas δ agonists may activate directly receptors that are localized presynaptically on dopamine terminal (Spanagel *et al.*, 1990 b; Arenas *et al.*, 1991). In contrast, κ agonists inhibit the activity of mesencephalic dopaminergic neuron in vivo. However, direct administration of κ agonists onto the substantia nigra pars compacta does not produce any influence on the cell firing. Therefore it has been suggested that κ ogonists can indirectly control the activity of nigral dopamine (Lavin and Garcia-Munoz, 1985; Lacey *et al.*, 1989). In morphine tolerant rat, Dafny *et al.* (1979) report that morphine produces profound enhancement of dopamine-sensitive adeny-late cyclase activity in striatal slice. The increased adenylate cyclase activity was also observed in the striatum eventhough the presence of naloxone. Therefore they suggested that this hyperactivity of dopamine D_1-stimulated adenylate cyclase was caused by a desensitization of opioid-mediated inhibition of adenylate cyclase activity. Our data showed that the inhibitory effect of the highly selective κ agonist, U50, 488H on dopamine-sensitive adenylate cyclase was marked following long-term administration with halope-ridol in rat striatal slices. After 6-hydroxydopa-mine lesion of rat substantia nigra, the density of μ and δ binding sites in the caudate-putamen is decreased, but after lesion of ventral tegmental area, only μ binding site density is reduced in the nucleus accumbens (Unterward *et al.*, 1989). In contrast, it has been reported that the density of κ-opioid binding sites in the caudate-putamen and nucleus accumbens is unchanged (Dilts and Kalivas, 1990). Recently, it has been proposed that the lesion-induced binding changes of μ and δ sites are the result of transsynaptic effects (Trovero *et al.*, 1990, Dilts and Kalivas, 1990). The behavioral effects of opioids were abolished after 6-hydroxydopamine lesions of mesolimbic system, suggesting that mesolimbic dopaminergic neurons

are necessary for the expression of behavior response by opiates (Kalivas *et al.*, 1983; Spyraki *et al.*, 1983). Together, there are some reports that all three types of opioid binding site are present on nondopaminergic elememts in the caudate-putamen and nucleus accumbens. In this study, the mild decreases of cyclic AMP content were demonstrated by morphine, DAGO, DPDPE, and U50, 488H administration in mouse striatum (Table 1) and these decreases were antagonized by naloxone pretreatment. The nigrostriatal lesion is similar condition to chronic haloperidol treatment because haloperidol blocks dopamine receptors, and thereby produces a pharmacological equivalent of dopaminergic denervation. The content of cyclic AMP was increased in haloperidol treated group. However, in that group, the content of cyclic AMP showed variable decreases by each agonist. The morphine and U50, 488H showed significant decrease comparing to control level of normal and haloperidol treated group. The effect of DAGO showed mild decrease, and DPDPE showed tendency of decrease in cyclic AMP content. The decreases of morphine and U50, 488H were attenuated by naloxone pretreatment, but the decrease of DAGO and DPDPE were enhanced by naloxone pretreatment in haloperidol treatment group. North and Vitek(1980) reported presynaptic μ-, δ-, and κ-opioid receptors may exert as independent functional entities in rat brain. However, the μ and δ receptors appear to be associated at the level of adenylate cyclase stimulated by activation of postsynaptic D_1-dopamine receptors in the neostriatum. In conclusion, the present study has demonstrated net κ- μ- or δ-opioid receptor modulation on the dopaminergic pathway in mouse striatum. The κ agonist produces the inhibition of the cyclic AMP content both in normal and haloperidol treated mouse striatum. Therefore we could find κ opioid receptor has been still its activity in dopaminergic denervated condition.

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=국문초록=

Haloperidol 장기 투여된 Mouse Striatum에서 cAMP양에 미치는 Opiates의 영향

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김 수 경

Opioid수용체는 adenylate cyclase의 활성을 억제하므로써 cyclic AMP의 양을 감소시킨다. 본 연구에서는 striatum에서 dopamine과 opioid 신경전달계의 상호관계를 알아보고자 haloperidol (750ug/kg)을 10일간 복강내 투여하여 dopaminergic pathway를 차단시킨후 mouse striatum에서 선택적 opioid μ, δ, κ 수용체 agonist들에 의해 축적되는 cAMP양을 측정하여 본 결과, haloperidol 단독투여에 의해서 cAMP는 유의한 증가를 나타내었으며, haloperidol 장기투여된 mouse striatum에서 morphine(20 mg/kg), DAGO(50 ug/kg), DPDPE(50 ug/kg), U50,488H (500 ug/kg) 투여에 의해서 haloperidol에 의한 cAMP 증가는 억제되었으며, 정상 mouse에 투여된 morphine, DAGO, DPDPE, U50, 488H에 비해서는 DAGO, DPDPE 투여군에서 증가를 나타내었다. Haloperidol 장기투여로 인한 morphine, DAGO, DPDPE, U50, 488H의 영향은 naloxone에 의해서 morphine과 U50, 488H투여군에서 길항되었으며 정상 mouse에 투여된 morphine, DAGO, DPDPE, U50, 488H에 의한 cAMP의 감소는 naloxone에 의하여 모든 실험군에서 길항되었다.

이상의 결과로 보아 dopaminergic denervation시 mouse striatum에서 μ, δ, κ 효현제에 의하여 축적되는 cAMP양은 κ수용체 효현제인 U50, 488H에서 가장 현저한 감소를 보여 각 수용체의 활성화정도는 변화되며, 그 중에서 κ 수용체는 그 기능이 가장 보존되고 있음을 알 수 있었다.