# Differential expression of group Ⅱ mGluRs in rat brain and change of locomotor activity by blockade of these receptors in the expression of amphetamine-induced behavioral sensitization

Ju Kyong Jang

Department of Medical Science

The Graduate School, Yonsei University

# Differential expression of group Ⅱ mGluRs in rat brain and change of locomotor activity by blockade of these receptors in the expression of amphetamine-induced behavioral sensitization

Ju Kyong Jang

Department of Medical Science

The Graduate School, Yonsei University

# Differential expression of group Ⅱ mGluRs in rat brain and change of locomotor activity by blockade of these receptors in the expression of amphetamine-induced behavioral sensitization

Directed by Professor Jeong-Hoon Kim

The Master's Thesis submitted to the
Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Master of Medical Science

Ju Kyong Jang

June 2005

This certifies that the Master's
Thesis of Ju Kyong Jang
is approved.

Jeong-Hoon Kim

Dong Goo Kim

Jeong Won Jahng

The Graduate School
Yonsei University

June 2005

# Acknowledgements

First of all, I would especially like to thank my advisor, Dr. Jeong-Hoon Kim. He encouraged me to develop independent thinking and research skills. He continually stimulated my thinking and greatly assisted me with scientific writing. When I doubt about myself, he pushed me forward to overcome lots of difficulties that I faced. In fact, it would not have been possible to complete this work without his enthusiastic guidance. I would also like to thank my dissertation committee, Dr. Dong Goo Kim and Dr. Jeong Won Jahng, for their helpful guidance and comments throughout this project.

I owe many thanks to my colleagues in the Kim's laboratory, Seungwoo Kim, Wha Young Kim, Hyung Shin Yoon, Jeong Hoon Oh for their help, encouragement, and friendship. And I extend my thanks to all the people in the Department of Physiology, especially, Soo-Kyoung Choi and Hoo Hyung Kim.

There is no way to thank my family enough. They deserve my deep warm and special acknowledgement for their love and care. My parents have always been a constant supporter not only for materials but also for spiritual encouragement throughout my life.

And thanks God.

# Table of Contents

# List of figures

## Abstract

# Differential expression of group Ⅱ mGluR in rat brain and change of locomotor activity by blockade of this receptor in the expression of amphetamine-induced behavioral sensitization

Ju Kyong Jang

*Department of Medical Science*
*The Graduate School, Yonsei University*

(Directed by Professor Jeong-Hoon Kim)

Brain metabotropic glutamate receptors (mGluRs) have been implicated in the expression of sensitization by amphetamine (AMPH). It was previously shown that repeated exposure to AMPH develops cross-sensitization to the microinjection into the nucleus accumbens (NAcc) of the broad spectrum mGluR antagonist (RS)-MCPG, suggesting that mGluR mediated glutamate neurotransmission in this site is altered by AMPH pre-exposure. The present study further examined the contribution of mGluRs to AMPH-induced locomotor sensitization by measuring their expression levels in various brain regions and the contribution of group Ⅱ mGluRs to AMPH-induced locomotor sensitization both by systemic injection and by blocking these receptors directly in the NAcc. First, rats in different groups were administered five injections of saline or AMPH (1.5 mg/kg, i.p.), one injection every three to four days. Two weeks after the last injection, rats locomotor activity was assessed for 2h following a systemic injection of either saline or LY341495, a group Ⅱ mGluR antagonist.

When systemically injected, AMPH compared to saline pre-exposed rats showed an enhanced hyper-locomotor activity to LY341495 in a dose-dependent manner. Secondly, we examined the expression levels of mGluRs by using immunoblotting method. Interestingly, in both the NAcc and the striatum, expression level of group II (mGluR 2/3) mGluRs are increased in AMPH compared to saline pre-exposed rats. Furthermore, in the striatum , expression levels of group I mGluRs (mGluR1 and 5) are increased in AMPH compared to saline pre-exposed rats. However, in the VTA, expression level of all subtypes of mGluRs have not been changed. Lastly microinjection of LY341495 into the NAcc were made after AMPH sensitization development described as above. It increases locomotor activity equally in AMPH and saline pre-exposed rats. Interestingly, it was previously shown by others that microinjection of a relatively high dose of LY341495 (10 $\mu g$/0.5$\mu \ell$/side) into the NAcc produced a significantly different locomotor activity in AMPH compared saline pre-exposed rats. These results suggest that repeated AMPH alters glutamatergic neurotransmission mediated by group II mGluRs in the NAcc, but animals require relatively high amount of antagonist in the NAcc to produce hyper-locomotion effect.

In conclusion, the present study indicates that group II mGluR levels in the NAcc differentially expressed when AMPH sensitization developed and this change may contribute to hyper-locomotor activity produced by an antagonist to these receptors. All together, these results suggest that group II mGluRs may posit to play an important role in the NAcc to regulate the expression of AMPH-induced behavioral sensitization.

Key words : metabotropic glutamate receptor(mGluR), nucleus accumbens(NAcc), amphetamine, ventral tegmental area(VTA), behavioral sensitization

# Differential expression of group Ⅱ mGluR in rat brain and change of locomotor activity by blockade of this receptor in the expression of amphetamine-induced behavioral sensitization

Ju Kyong Jang

*Department of Medical Science*
*The Graduate School, Yonsei University*

(Directed by Professor Jeong-Hoon Kim)

## I. INTRODUCTION

Repeated intermittent exposure to psychomotor stimulant drugs such as amphetamine (AMPH) and cocaine leads to a long lasting enhancement in locomotor responding to these drugs, a phenomenon known as locomotor sensitization[1]. Exposure to sensitizing regimens of psychomotor stimulant drugs has also been suggested to enhance the rewarding or incentive motivational properties of these drugs[2] as evidenced by facilitation of their subsequent self-administration[3-5] and enhanced preferences for places associated with these drugs[6-7]. The nucleus accumbens (NAcc) is known to be an important site for the expression of locomotor sensitization by AMPH[8-9]. It receives extensive dopaminergic innervation from the ventral mesencephalon[10] and

glutamatergic projections directly from the prefrontal cortex (PFC) and limbic structures such as the hippocampal formation and amygdala[11-12]. These projections are known to contribute to the expression of locomotor sensitization.

Metabotropic glutamate receptors (mGluRs) are widely expressed in rat brain, especially in the NAcc[13-16]. The mGluRs constitute a diverse group and differ in several characteristics from a majority of other known G protein-coupled receptors. Until now 8 subtypes of mGluRs have been cloned and numbered successively beginning from those discovered the earliest (mGluR1 - mGluR8). Further studies based on intracellular effects of stimulation of these receptors, on their pharmacological profile and their mutual amino acid sequence homology, allowed for assignment of individual mGluR subtypes to three main groups[17-18]. Group I includes mGluR1 and mGluR5, group Ⅱ dose mGluR2 and mGluR3, and group Ⅲ dose mGluR4, mGluR6, mGluR7 and mGluR8.

The role of mGluRs in psychostimulant-induced behavioral sensitization has also been studied. While repeated co-infusion with AMPH into the VTA of the broad spectrum mGluR antagonist, (RS)-α-methyl-4-carboxyphenylglycine [(RS)-MCPG], has been shown to prevent the development of locomotor sensitization by AMPH[19], this antagonist also elicits a greater locomotor response in AMPH compared to saline pre-exposed rats when administered intracranially into the NAcc 2 weeks after the last drug pre-exposure injection[20]. It has been suggested that this latter finding may reflect a contribution by Group Ⅱ mGluRs in the NAcc to the expression of sensitization by psychomotor stimulant drugs[21]. Consistent with this view, it was recently reported that the Group Ⅱ mGluR-selective antagonist LY341495 also elicits a

greater locomotor response in AMPH compared to saline pre-exposed rats[22]. It remains to be directly determined, however, whether the expression of locomotor sensitization by drugs like AMPH is mediated by NAcc group II mGluRs. Therefore the present study examined the contribution of group II mGluRs to AMPH-induced locomotor sensitization by blocking these receptors both systemically and directly in the NAcc and measuring their expression levels in this site.

## II. MATERIALS AND METHODS

### 1. Subjects and surgery

Male Sprague-Dawley rats weighing 250-275 g on arrival from Samtako were used. They were housed individually in a 12 h light/12 h dark cycle room, with food and water available at all times. A week after arrival, rats were anesthetized with ketamine (10 ㎎/㎏ i.p.) followed by xylazine (6 ㎎/㎏ i.p.) and placed in a stereotaxic instrument with the incisor bar positioned 5.0 ㎜ above the interaural line (Pellegrino et al., 1979). They were then implanted with chronic bilateral guide cannulae (22 gauge, Plastics One, Roanoke, VA) aimed at the NAcc (A/P, + 3.4; L, ± 1.5; D/V, - 7.5 from bregma and skull). Cannulae were angled at 10° to the vertical and positioned 1 ㎜ above the final injection site. All cannulae were secured with dental acrylic cement anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge obturators were placed in the guide cannulae and rats were returned to their home cages for a 7-10 days recovery period.

### 2. Drugs

D-Amphetamine sulfate (U.S.P., USA) was dissolved in sterile 0.9% saline. LY341495 (Ely Lilly Research Laboratories, Indianapolis, IN, USA) was dissolved in 1.2 equivalent of NaOH solution and small aliquots were stored at -70℃. Immediately before use, frozen aliquots of the drugs were diluted in sterile 0.9% saline.

### 3. Inracranial microinjections

Bilateral intracranial microinjections into the NAcc were made in the

freely moving rat. Injection cannulae (28 gauge) connected to 1 ㎕ syringes (Hamilton, USA) via PE-20 tubing were inserted to a depth 1 ㎜ below the guide cannula tips. Injections were made in a volume of 0.5 ㎕/side during 30s. After 60s the injection cannulae were withdrawn, the obturators replaced and testing begun immediately by placing rats in the activity boxes.

**4. Locomotor activity**

A bank of 12 activity boxes were used to measure locomotor activity. Each box (36 × 40 × 26 ㎝) was constructed of opaque plastic walls and a tubular stainless-steel floor. Two photocells, positioned 3.5 ㎝ above the floor and spaced evenly along the longitudinal axis of each box, estimated horizontal locomotion. Two additional photocells, positioned on the side walls 13.5 ㎝ above the floor estimated rearing. Separate interruptions of photocell beams were detected and recorded via an electrical interface by a computer situated in an adjacent room.

**5. Immunoblotting**

Two weeks after the last pre-exposure injection of saline or AMPH, rats were decapitated, and the brains were rapidly removed and dissected into coronal sections on ice. The brain regions were dissected on an ice-cooled Plexiglas plate, including the ventral tegmental area, striatum, and nucleus accumbens. Brain tissues were immediately frozen on dry ice and stored at -70 ℃. They were homogenized with a hand-held tissue grinder in homogenization medium (0.32 M sucrose, 2 mM EDTA, 1% SDS), subjected to low-speed centrifugation (2000 g, to remove insoluble material), and stored at -20 ℃. Protein concentrations were determined by the Folin-Lowry assay[23]. Samples (5 - 20 mg) of

protein were subjected to sodium dodecyl sulfate - polyacrylamide gel (6 - 8 %) electrophoresis, transferred to nitrocellulose membranes electrophoretically, and probed for mGluR subtype levels. Primary antibodies (Upstate Biotechnology, Lake Placid, NY, USA) against each mGluR subtypes (mGluR1, mGluR2/3, mGluR4 and mGluR5) were diluted 1 : 1000, 1 : 1300, 1 : 1000, and 1 : 5000, respectively. Labeled proteins were detected using a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody diluted 1 : 5000 (Sigma). They were visualized with enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). Bands were visualized using Fujifilm image gauge and quantified based on densitometric values using Fujifilm Science Lab 99 L-Process v1.96 software[24], and all bands were expressed as percentage of the saline control values, after normalization with β-actin.

**6. Design and procedure**

The experiments consisted of a pre-exposure phase and a test phase for sensitization. For pre-exposure, rats were administered either saline or AMPH (1.5 ㎎/㎏, i.p.) on five occasions, one injection every 2-3 days. This regimen of AMPH injections is known to produce enduring sensitization of the locomotor response to AMPH (Robinson and Becker, 1986; Vezina and Stewart, 1989). Immediately after the first and fifth injections, rats were placed in the activity boxes and their locomotor activity was measured for 2 h. During other injections, locomotor activity was not measured and animals following these injections were returned to their home cages. Test for sensitization was made two weeks after the last exposure injection. Rats were first habituated to the activity boxes for 1 h and then injected AMPH (1.5 ㎎/㎏, i.p.) and immediately their locomotor activity were measured for an additional 2h.

### 7. Histology

After completion of the experiments, rats were anesthetized and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and postfixed in 10% formalin. Coronal sections (40 ㎛) were subsequently stained with cresyl violet for verification of cannulae tip placements.

### 8. Data analysis

Immunoblotting data were analyzed with paired student t-test. Total locomotor activity scores (means ± s.e.m.) were analyzed by using two-way ANOVA with injections and groups as factors of variation. Post-hoc tests were performed by using Student-Newman-Keuls.

## III. Results

### 1. Repeated AMPH produces cross-sensitization to a group Ⅱ mGluR antagonist LY341495 in a dose-dependent manner.

Figure 1 shows locomotor activity counts obtained in rats pre-exposed 2 weeks earlier to AMPH or saline and tested after systemic injection of the selective group Ⅱ mGluR antagonist LY341495. When systemically injected, LY341495 produced a significant increase of locomotion in a dose-dependent manner. The ANOVA conducted on the locomotor counts obtained in the first hour post-injection revealed significant effects of injection [$F(1,51)=35.08$, $p<0.001$], group [$F(2,51)=40.52$, $p<0.001$], and injection x group [$F(2,51)=5.60$, $p<0.01$]. Post-hoc test revealed that LY341495 dose-dependently produced significantly enhanced increase of locomotion in both saline and AMPH pre-exposed rats ($p < 0.001$). However, these effects were produced significantly greater in AMPH than saline pre-exposed rats at two doses of LY341495 (1 and 3 ㎎/㎏; $p < 0.001$ and $p = 0.001$, respectively).

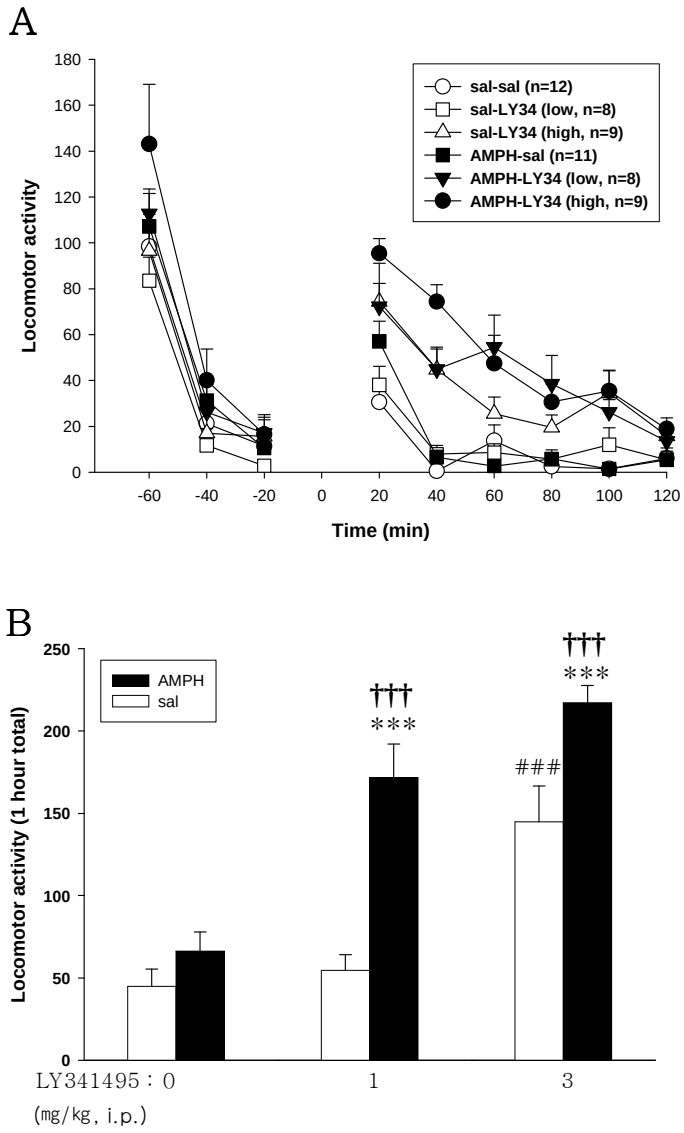


**Figure 1.** The selective group II mGluR antagonist LY341495 produces enhanced increase of locomotion when systemically administered to AMPH compared to saline pre-exposed rats. (A) Pre- and post-injection time course for locomotor activity in different groups is shown as means (+SEM). Rats were injected at time 0. (B) Data are shown as group means (+SEM) of the first hour total

activity counts. ###, $p<0.001$, sal-sal vs. sal-LY341495(3㎎/㎏, i.p.). ***, $p<0.001$, AMPH-sal vs. AMPH- LY341495(1㎎/㎏, i.p.), AMPH-sal vs. AMPH-LY341495(3㎎/㎏, i.p.). ††† , $p<0.001$, AMPH-LY341495(1㎎/㎏, i.p.) vs. sal-LY341495(1㎎/㎏, i.p.), AMPH-LY341495(3㎎/㎏, i.p.) vs. sal-LY341495((3㎎/㎏, i.p.).

## 2. Repeated AMPH alters the expression levels of mGluRs in the NAcc and the striatum but not in the VTA.

We examined the contribution of group Ⅱ mGluRs to AMPH-induced locomotor sensitization by measuring their expression levels in the nucleus accumbens (NAcc), striatum and ventral tegmental area (VTA). Rats in different groups were administered five injections of saline or AMPH (1.5 ㎎/㎏, i.p), one injection every two to three days. Two weeks after, rats were decapitated and NAcc, striatum and VTA were punched out and frozen. Proteins were isolated and Western blots were performed with antibodies for different subtypes of mGluRs (group I, II, III). In the NAcc, expression levels of group II mGluRs are significantly increased in AMPH compared to saline pre-exposed rats (Fig. 2). In the striatum, furthermore, chronic AMPH administration produces a change in the expression levels of group I and Ⅱ mGluRs (Fig. 3) but, in the VTA, another important region for addiction, expression level for all subtypes of mGluRs have not been changed (Fig. 4).

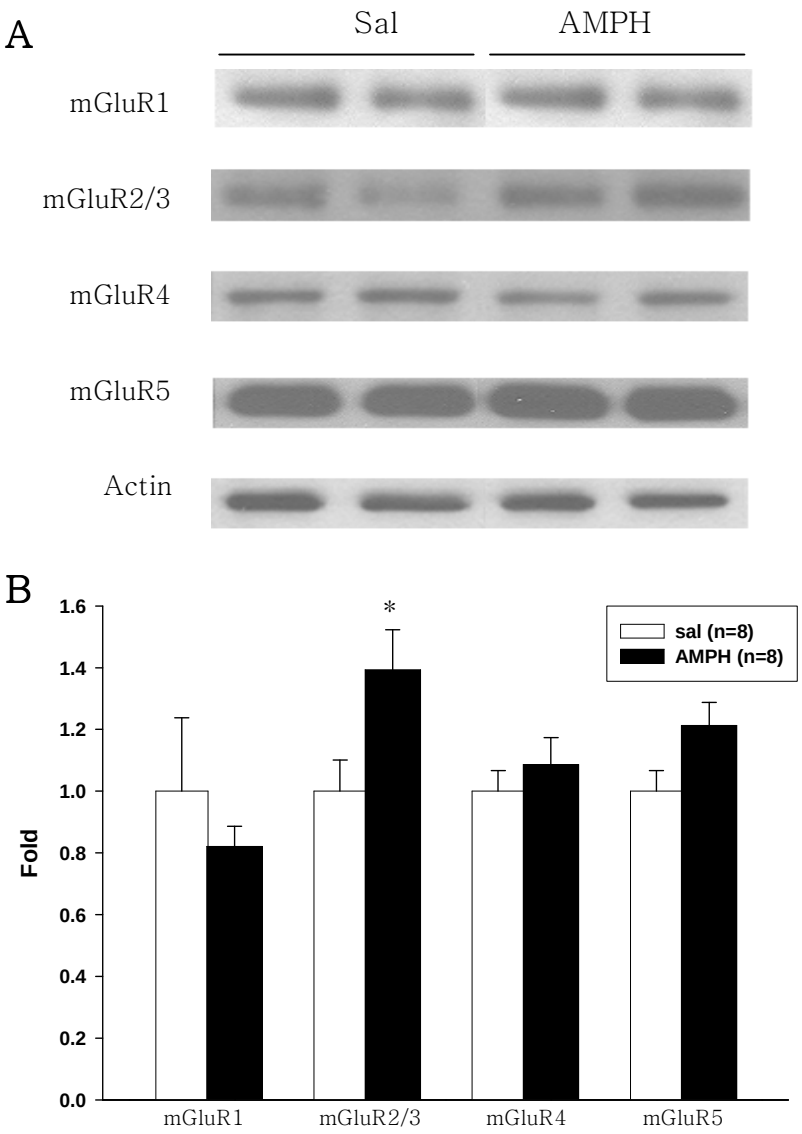


**Figure 2.** Repeated AMPH produces a change in the expression levels of mGluR2/3 in the nucleus accumbens. Immunoblot(A) and quantitative densitometric measurements of mGluR levels(B) in the NAcc of rats decapitated 2 weeks after AMPH or sal pre-exposure. The numbers in each bar graph is the number of determination in each group. *, $p<0.05$, compared the saline to AMPH groups using Student's *t*-test

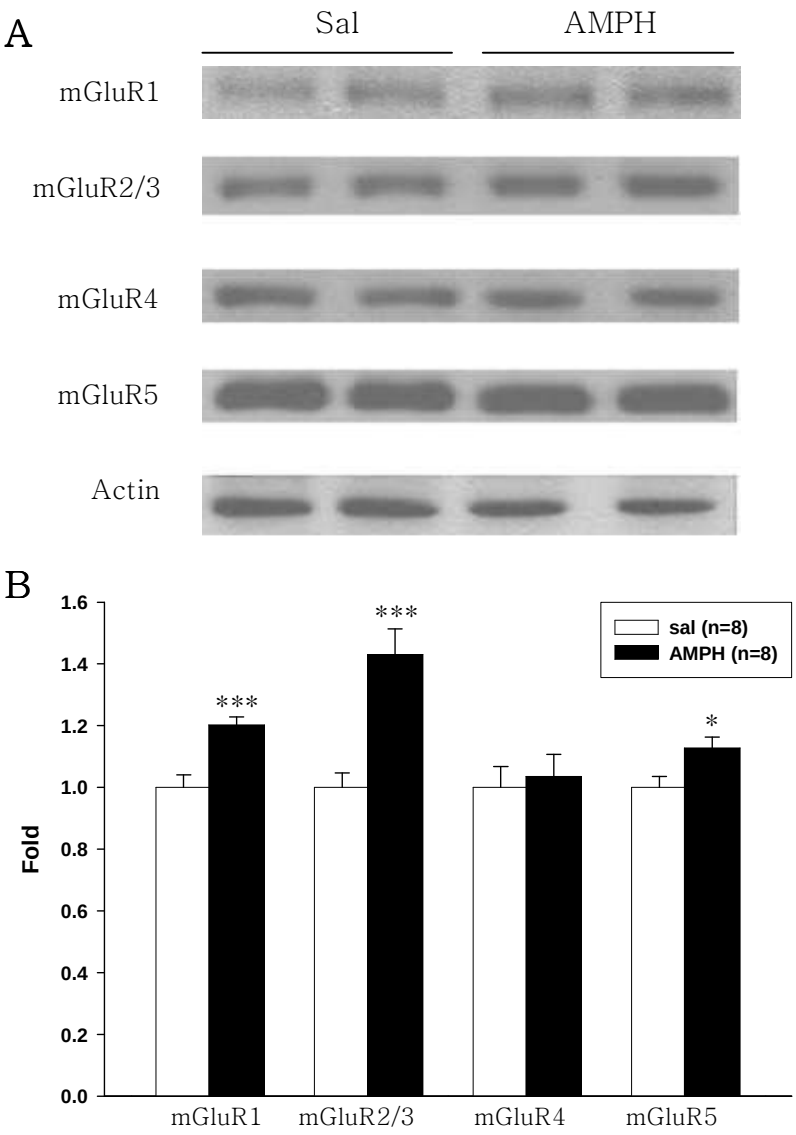


**Figure 3.** Repeated AMPH produces a change in the expression level of group Ⅰand Ⅱ mGluRs in the striatum. Immunoblot(A) and quantitative densitometric measurements of mGluR levels(B) in the striatum of rats decapitated 2 weeks after AMPH or sal pre-exposure. *, $p<0.05$, ***, $p < 0.001$, compared the saline to AMPH groups using Student's *t*-test

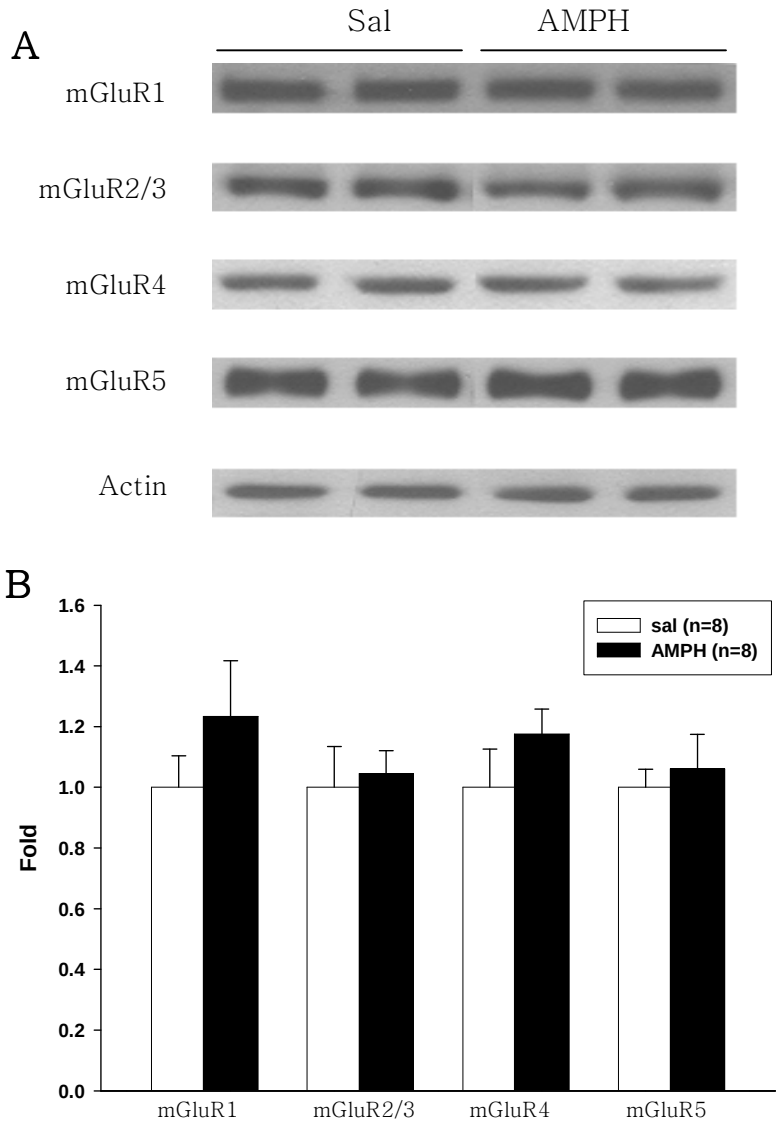


**Figure 4.** Repeated AMPH did not alter in the expression level of all subtypes of mGluRs in the VTA. Immunoblot(A) and quantitative densitometric measurements of mGluR levels(B) in the VTA of rats decapitated 2 weeks after AMPH or sal pre-exposure.

### 3. LY341495 increases locomotor activity equally in AMPH and saline pre-exposed rats.

When challenged with LY341495 (0.1, 0.5, 2.5㎍/0.5㎕/side) in the NAcc 2 weeks following the last drug pre-exposure injection, it was found that this selective group Ⅱ mGluR antagonist produced increased locomotion (Fig. 5). However, no significant difference between saline and AMPH pre-exposed rats was observed in a range of dose we used. None of the ANOVA conducted on the present locomotor data revealed at any time a significant difference between groups, indicating that prior exposure to AMPH did not affect the subsequent locomotor responses to NAcc LY341495 in this dose ranges.

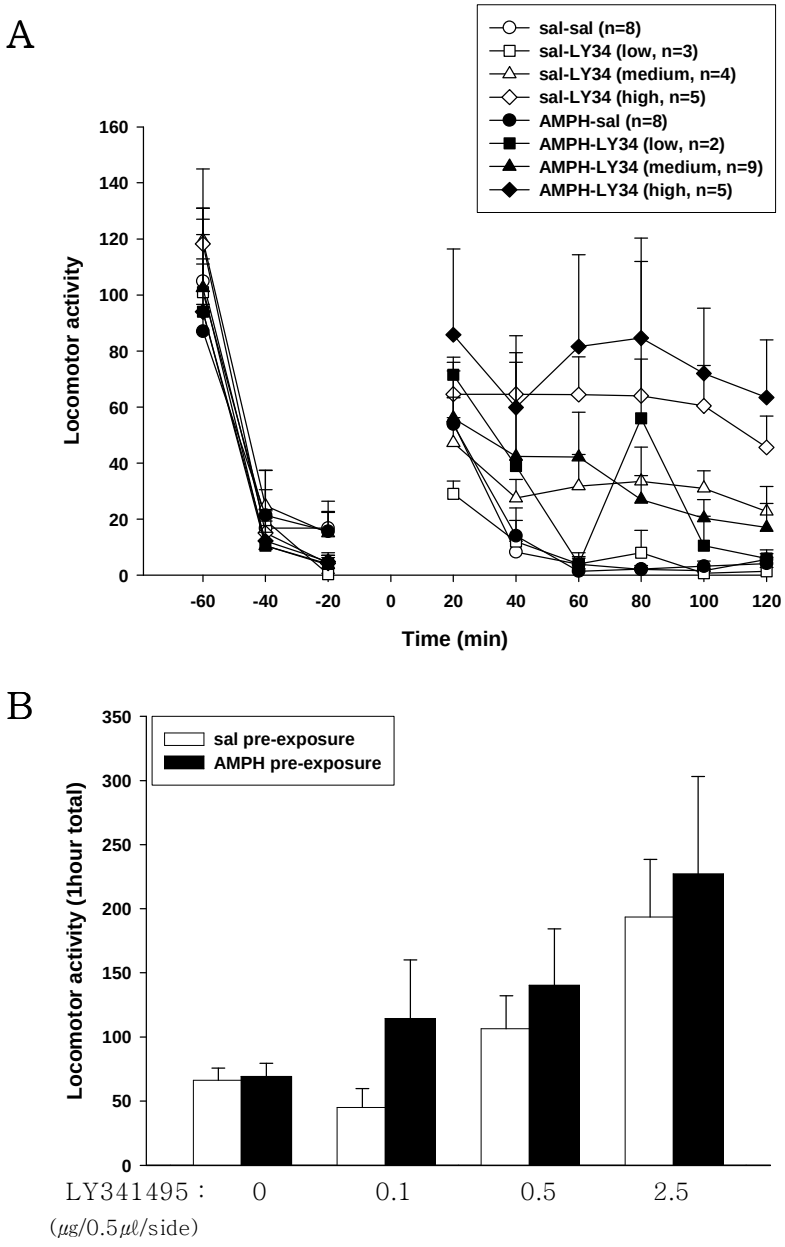


**Figure 5.** The selective group II mGluR antagonist LY341495 produces increased locomotion when injected into the NAcc of AMPH pre-exposed rats. (A) Pre- and postinjection time course for locomotor activity in different groups is shown as means (+ SEM). Rats were injected at time 0. (B) Data are shown

as group means (+ SEM) of the first hour total activity counts. None of the ANOVA conducted on these data at any time detected significant differences between groups.

## 4. Histology

Of the total of 44 rats tested, only rat with injection cannula tips located bilaterally in the NAcc were included in the data analyses. No neuronal damage was observed other than the mechanical damage produced by the insertion of the cannulas.

## IV. Discussion

The present results demonstrate that repeated AMPH produces cross-sensitization to a group II mGluR antagonist LY341495 in a dose-dependent manner. Furthermore, repeated exposure to AMPH produced marked alterations in the expression level of mGluRs. Together, these results further support the notion that repeated AMPH may alter glutamatergic neurotransmission mediated by group II mGluRs in the NAcc.

### *1. Change of locomotor activity by blockade of group II mGluR, LY341495, in the AMPH-pre-exposed rats.*

It has already been shown that (RS)-MCPG has no effect on locomotor activity when it is injected alone into the NAcc of drug-naive rats[20,25]. Interestingly, however, injection of (RS)-MCPG into the NAcc produced increased locomotor activity in AMPH-pre-exposed rats[20]. It was suggested that this finding may reflect a role for group II mGluR autoreceptors[21]. Supporting this, it was recently reported that group II mGluR-selective antagonist LY341495 dose-dependently increased the locomotor activity without any pre-exposure[26] and it also elicits a greater locomotor response in AMPH compared to saline pre-exposed rats[22]. The present study confirmed and extended these findings by using two doses of LY341495 (1 or 3 ㎎/㎏, i.p.). When systemically injected, LY341495 produced a significant increase of locomotion in a dose-dependent manner. More importantly, systemic LY341495 produced a greater hyper-locomotion in AMPH compared to saline pre-exposed rats. These findings suggest that group II mGluR play an important role

in the expression of AMPH-induced sensitization.

### *2. Repeated AMPH alters the expression levels of mGluRs in the NAcc and the striatum but not in the VTA.*

Figure 1. shows that repeated exposure to AMPH develops cross-sensitization to the systemic injection of the group II mGluR antagonist LY341495, suggesting that group II mGluR mediated glutamate neurotransmission is altered by AMPH pre-exposure. The present study further examined the contribution of group II mGluRs to AMPH-induced locomotor sensitization by measuring their expression levels in the NAcc, striatum and VTA. It was reported that all subtypes of mGluRs are expressed in these brain areas in rat[13-16]. Previous studies have shown that repeated AMPH reduced mGluR5 mRNA expression level in striatum and NAcc[27]. And chronic cocaine produced an enduring reduction in mGluR2/3 function in the NAcc[28]. Unlike with previous studies, the present immunoblotting study found that repeated AMPH produced increase in the expression levels of group II mGluR (mGluR2/3) in the NAcc, group I (mGluR1 and 5) and group II mGluR in the striatum, while any mGluR subtypes did not significantly change in the VTA in AMPH pre-exposed rat. The discrepancies among these studies may result from such experimental differences as doses, route of administration, treatment schedule, length of withdrawal, brain area examined. However, the present data are consistent with some of the previously observed changes in extracellular glutamate levels associated with repeated cocaine administration. For example, the repeated administration of cocaine has been shown that basal pre-injection levels of extracellular glutamate in the NAcc have reduced[29-30]. Also, while

AMPH acutely increased glutamate overflow in the NAcc[31-32], this effect was sensitized in rats pre-exposed to AMPH two weeks earlier[33]. Therefore, as in the present study, the up-regulation of mGluR 2/3 receptors in the NAcc and striatum may reflect the changes resulted from the compensatory responses after chronic AMPH pre-exposure.

### *3. LY341495 increases locomotor activity equally in AMPH and saline pre-exposed rats.*

Figure 1. shows that AMPH compared to saline pre-exposed rat showed an enhance hyper-locomotor activity to LY341495 (1 or 3 ㎎/㎏, i.p.) in a dose-dependent manner. The present study further examined the contribution of group II mGluRs to AMPH-induced locomotor sensitization by blocking these receptors directly in the NAcc. Consistent with previous data from systemic challenge, LY341495 (0.1, 0.5, 2.5 ㎍ /0.5㎕/side) increased locomotor activity when injected into the NAcc in a dose-dependent manner, however, no significant difference between saline and AMPH pre-exposed rats was observed in a range of doses we used. Interestingly, it was previously shown that NAcc LY341495 produced a significantly different locomotor activity in AMPH compared to saline pre-exposed rats when given at a much higher dose (10 ㎍/0.5 ㎕/side)[34]. Together, these findings suggest that repeated AMPH alters glutamatergic neurotransmission mediated by group II mGluRs in the NAcc, but they require relatively high amount of antagonist in the NAcc to produce hyper-locomotion effect.

## V. Conclusion

We investigated the role of the NAcc group II mGluRs in the expression of AMPH-induced behavioral sensitization by measuring the locomotor activity after systemic or microinjection into the NAcc of group II mGluR antagonist LY341495 and their expression levels in the AMPH sensitized rat brain. The results obtained are summarized as follows.

1. Repeated AMPH produces cross-sensitization to a group II mGluR antagonist LY341495 in a dose-dependent manner.

2. In the NAcc, expression levels of group II (mGluR2/3) mGluR are increased in AMPH compared to saline pre-exposed rats.

3. In the striatum, expression levels of group I (mGluR1 and 5) and II (mGluR2/3) subtypes of th mGluR are increased in AMPH compared to saline pre-exposed rats.

4. In the VTA, expression level of all subtypes of mGluRs have not been changed.

5. LY341495 increases locomotor activity equally in AMPH and saline pre-exposed rats.

These findings indicate that repeated AMPH produces hyper-locomotion to a group II mGluR antagonist LY341495, and this behavioral change may result from the altered glutamatergic neurotransmission mediated by group II mGluRs in the NAcc.

## References

1. Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. 1986 Jun;396(2):157-198

2. Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev. 1991 Sep-Dec;16(3):223-244

3. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 1993 Sep-Dec;18(3):247-291

4. Piazza PV, Deminiere JM, Le Moal, M and Simon, H. Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res, 1990; 514:22 - 26.

5. Pierre PJ and Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology 1997; 129:277 - 284.

6. Lorrain DS, Arnold GM and Vezina P. Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. Behav Brain Res 2000 ; 107:, 9 - 19.

7. Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology. 1989; 98: 357 - 362.

8. Shippenberg TS and Heidbreder CH. Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther 1995; 273:808 - 815.

9. Perugini M, Vezina P. Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. J Pharmacol Exp Ther. 1994 Aug;270(2):690-696.

10. Cador M, Bjijou Y, Stinus L. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience. 1995 Mar;65(2):385-395.

11. Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM.. Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. Neuroscience. 1987 Aug;22(2):425-439.

12. Meredith GE, Pennartz CM, Groenewegen HJ. The cellular framework for chemical signalling in the nucleus accumbens. Prog Brain Res. 1993;99:3-24.

13. Albin RL, Makowiec RL, Hollingsworth ZR, Dure LS 4th, Penney JB,

Young AB. Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. Neuroscience. 1992;46(1):35-48.

14. Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. Neuroscience. 1993 Apr;53(4):1009-1018.

15. Testa CM, Standaert DG, Young AB, Penney JB Jr. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. J Neurosci. 1994 May;14(5 Pt 2):3005-3018.

16. Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J Comp Neurol. 1995 May 8;355(3):455-469.

17. Palucha A., Pilc A.: Metabotropic glutamatergic receptors (mGluR). In: Excitatory Amino Acids 2000 - Physiological and Pathological Aspects, and the Therapy of Neuropsychiatric Diseases (Polish). XVII Winter School of Institute of Pharmacology, Polish Academy of Sciences, 2000. Eds. Pilc A., Popik P., Institute of Pharmacology, Polish Academy of Sciences, Kraków, 2000, 53 - 63.

18. Senkowska A, Ossowska K. Role of metabotropic glutamate receptors in animal models of Parkinson's disease. Pol J Pharmacol. 2003 Nov-Dec;55(6):935-950.

19. Kim JH, Vezina P. Metabotropic glutamate receptors are necessary for sensitization by amphetamine. Neuroreport.1998 Feb 16;9(3):403-6.

20. Kim, J-H and Vezina, P, The metabotropic glutamate receptor antagonist (RS)-MCPG produces hyperlocomotion in amphetamine pre-exposed rats. Neuropharmacology. 1998;37(2):189-197

21. Vezina P, Kim JH. Metabotropic glutamate receptors and the generation of locomotor activity: interactions with midbrain dopamine. Neurosci Biobehav Rev. 1999 Mar;23(4):577-589.

22. Kim, J-H and Vezina, P, Group II metabotropic glutamate receptor blockade produces hyper-locomotion in amphetamine pre-exposed rats. Soc Neurosci Abst 2001 27, 878.10.

23. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 1951; 193:265 - 275.

24. Perrine SA, Schroeder JA, Unterwald EM. Behavioral sensitization to binge-pattern cocaine administration is not associated with changes in protein levels of four major G-proteins. Brain Res Mol Brain Res. 2005 Feb 18;133(2):224-232.

25 Attarian S, Amalric M. Microinfusion of the metabotropic glutamate receptor agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid into the nucleus accumbens induces dopamine-dependent locomotor activation in the rat. Eur J Neurosci. 1997 Apr;9(4):809-816.

26. O'Neill MF, Heron-Maxwell C, Conway MW, Monn JA, Ornstein P. Group II metabotropic glutamate receptor antagonists LY341495 and LY366457 increase locomotor activity in mice. Neuropharmacology. 2003 Oct;45(5):565-574.

27. Mao L, Wang JQ. Activation of metabotropic glutamate receptor mediates upregulation of transcription factor mRNA expression in rat striatum induced by acute administration of amphetamine. Brain Res. 2002 Jan 11;924(2):167-175.

28. Xi ZX, Ramamoorthy S, Baker DA, Shen H, Samuvel DJ, Kalivas PW. Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. J Pharmacol Exp Ther. 2002 Nov;303(2):608-615.

29. Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci. 1996 Feb 15;16(4):1550-1560.

30. Bell K, Duffy P, Kalivas PW. Context-specific enhancement of glutamate transmission by cocaine. Neuropsychopharmacology. 2000 Sep;23(3):335-344.

31 Reid MS, Hsu K Jr, Berger SP. Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse. 1997 Oct;27(2):95-105.

32. Xue CJ, Ng JP, Li Y, Wolf ME. Acute and repeated systemic

amphetamine administration: effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. J Neurochem. 1996 Jul;67(1):352-363.

33. Kim JH, Austin JD, Tanabe L, Creekmore E, Vezina P. Activation of group II mGlu receptors blocks the enhanced drug taking induced by previous exposure to amphetamine. Eur J Neurosci. 2005 Jan;21(1):295-300.

34. H. Chi and Vezina, P, 2003. Group II metabotropic glutamate receptor blockade in the nucleus accumbens locomotion in rats previously exposed to amphetamine. Soc Neurosci Abst 113.5.

## Abstract (in Korean)

### 암페타민에 의한 행동과민반응 형성 시 유도되는 대뇌 Group Ⅱ mGluR의 발현의 차이와 그에 따른 행동의 변화

<지도교수 김 정 훈>

연세대학교 대학원 의과학과

장 주 경

중독을 일으키는 여러 가지 약물 가운데에는 정신흥분제가 있는데, 이런 약물(e.g. AMPH, cocaine)에 반복적 그리고 간헐적으로 노출 되었을 때 그 약물에 대한 특이한 행동 반응이 증가되는 행동과민반응(behavioral sensitization)이 나타날 수 있고, 이는 약물 중독의 모델로 사용한다. 이런 약물 중독은 뇌의 보상 체계의 질병으로 여겨지는데 특히 측좌핵(NAcc)의 glutamatergic neurotransmission이 도파민과 함께 정신흥분제에 대한 행동과민반응의 발달(development)과 발현(expression)에 매우 중요한 역할을 한다고 알려져 있다. 그러므로 본 연구에서는 약물 중독에서 중요하게 여겨지는 mGluR의 기능에 대하여 알아보고자 하였다. 이전 연구에서 broad spectrum mGluR antagonist인 MCPG를 측좌핵 내에 acute하게 주입 하였을 때는 행동의 변화가 없지만, 복측피개(VTA)에 AMPH과 함께 반복적으로 주입하였을 때 AMPH에 의한 locomotor sensitization의 발달을 막았으며, AMPH를 미리 처리한 후 2주 뒤에 측좌핵 내로 MCPG를 직접 주입하였을 때 locomotor response가 크게 증가함을 관찰하였다 .이는 뇌에서의 mGluR에 의해 매개되는 신경전달이 AMPH에 의해 어떤 변화가 일어났을 가능성을 보여준다. 따라서 이번 연구에서는 1) 백서에게 AMPH을 반복적 간헐적으로 복강으로 전 처리한 뒤 약물 없는 기간(withdrawal period)을 거치고 나서, group Ⅱ mGluR-selective antagonist인 LY341495를(3mg/kg) 복강에 주입하였을 때 어떤 행동의 변화가 나타나는지, 2) 그리고 중독에

있어 중요한 뇌의 부분인 측좌핵에 직접 LY341495를(0.1, 0.5, 2.5㎍/0.5㎕/side) 주입하였을 때는 같은 반응이 일어나는지 알아보고, 마지막으로 3) 이런 약물 중독에 의해 뇌에서 일어나는 mGluR에 의한 신경전달의 변화를 알아보기 위해 saline에 대해서 AMPH 전 처리 그룹의 측좌핵(NAcc), 선조체(striatum), 복측피개(VTA)에서 달라진 mGluR의 양적 변화를 immunoblotting 방법을 통하여 체계적으로 조사하였다.

이상의 실험 과정을 통하여 AMPH을 전 처리한 집단에서 saline 전 처리한 집단에 비하여 LY341495에 대하여 농도에 따라 행동반응이 현저하게 증가하였다. 이러한 결과는 AMPH 전 처리에 의해서 mGluR에 의해 매개되는 glutamate의 신경전달에 변화가 생겼을 것을 시사한다. 다음으로 중독에 주요한 부위인 측좌핵, 선조체, 복측피개에서의 mGluR의 발현 정도는 측좌핵에서는 group Ⅱ mGluR이 선조체에서는 group Ⅰ과 Ⅱ mGluR의 발현이 각각 AMPH을 전 처리한 그룹에서 증가하였고, 복측피개에서는 mGluR의 발현의 변화는 관찰되지 않았다. 마지막으로 이러한 효과가 약물 보상 체계의 주요한 부위인 측좌핵에 의하여 직접 매개되는지 알아보기 위하여 AMPH을 전 처리한 뒤 LY341495로 측좌핵 내로 직접 microinjection 하였을 때 농도에 따라 행동 반응이 증가하였으나 AMPH 과 saline 집단간의 차이는 보이지 않았다. 그러나 흥미롭게도 상대적으로 매우 높은 농도로 주었을 때 행동반응이 증가된다는 보고가 있어 측좌핵 내의 group Ⅱ mGluR의 양적 변화가 이들 수용체의 antagonist에 의해 나타나는 행동 변화에 관련이 있음을 시사한다. 이상의 결과를 통하여 반복적인 AMPH은 측좌핵에서 group Ⅱ mGluR을 통한 glutamatergic neurontransmission을 바꾸는 것을 보여준다.

---

핵심 되는 말 : Metabotropic glutamate receptor(mGluR), 측좌핵, 복측피개, 암페타민, 행동과민반응