Effect of Pretreatment of (-)-3-PPP on the Haloperidol-Induced Extracellular Dopamine Concentrations in the Nucleus Accumbens of Rats

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白鼠 중격측좌핵에서 Haloperidol로 유발된 세포외 도파민 농도 변화에 대한 (-)-3-PPP 전처치 효과

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ABSTRACT

Objectives : To investigate the effects of (-)-3-PPP(0.5, 2, and 10mg/kg, s.c.) and haloperidol(0.1, 0.5, and 2mg/kg, s.c.) on the extracellular dopamine concentrations, and the effect of pretreatment with (-)-3-PPP(2mg/kg) on the haloperidol(2mg/kg)-induced extracellular dopamine concentrations in the nucleus accumbens(NAS) of free moving rats.

Methods : Dopamine levels in dialysate were determined with high pressure liquid chromatography(HPLC) with electrochemical detection(ECD).

Results : (1)(-)-3-PPP had dual actions depending on the doses: at 2mg/kg, it decreased and at 10mg/kg, increased extracellular dopamine concentrations ; (2) haloperidol at all doses increased dopamine levels with higher dose having a greater increase; and (3) pretreatment of (-)-3-PPP reduced the increase in dopamine levels elicited by acute treatment with haloperidol.

Conclusions : These findings suggest that pretreatment of (-)-3-PPP in low dose could accelerate the onset of therapeutic effect of haloperidol by diminishing the haloperidol-induced dopamine release in the limbic system.

KEY WORDS : (-)-3-PPP · Haloperidol · Dopamine · Nucleus accumbens.

Introduction

Since the discovery of the antipsychotic property of chlorpromazine by Delay and Deniker in 1952, many new antipsychotics with different mode of actions and good tolerability have been developed. These new antipsychotic drugs have contributed greatly to the improvement in the quality of treatment for patients with psychotic disorders. However, improvement in the onset of response is needed for these drugs as well as the older, typical antipsychotics.

Assuming that antipsychotics exert their therapeutic effects through postsynaptic dopamine D_2 receptor blockade, the delay in onset of therapeutic effects can be attributed, in part, to the concomitant blockade of presynaptic dopamine autoreceptors

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by antipsychotics. As dopamine autoreceptors have pharmacological characteristics identical to those of postsynaptic D_2 receptor(Roth, 1979 ; Helmreich et al., 1982), antipsychotics block not only postsynaptic D_2 receptors, but also dopamine autoreceptors located on the dopaminergic nerve terminals and cell bodies, thus increasing firing rates of dopamine neurons and dopamine release(Imperato and Di Chiara, 1985 ; Zetterstrom et al., 1985). This initial increase has been hypothesized to counteract the blockade of postsynaptic D_2 receptors and delay the onset of therapeutic effects(Chido and Bunney, 1983, 1985 ; Blaha and Lane, 1987 ; Grace, 1991). In chronic treatment, however, gradual reduction of dopamine release occurs with the development of supersensitivity of dopamine autoreceptors (Scatton et al., 1976) and depolarization block of dopaminergic neurons(Chido and Bunney, 1983). Development of supersensitivity of dopamine autoreceptors and depolarization block of dopaminergic neurons are reported to begin within one(Asper et al., 1973) and two-three weeks(Jiang et al., 1988) respectively. When the effects of blockade of postsynaptic D_2 receptors become prominent by this gradual reduction of dopamine release over time, psychotic symptoms improve(Chiodo and Bunney, 1985 ; Blaha and Lane, 1987). Considering all the results of the studies described above, it can be assumed that a cause for the delayed therapeutic effects of antipsychotics is the initial increase of dopamine release triggered by the blockade of presynaptic dopamine autoreceptors. In this regard, we hypothesized that the pretreatment of dopamine D_2 autoreceptor agonists prior to the administration of antipsychotics could block the initial increase of dopamine release induced by them and this, in turn, could possibly accelerate their onset of therapeutic action. This strategy is worthy of trial, considering that faster onset of therapeutic effects has been noted by combination treatment of selective serotonin reuptake inhibitors(SSRI) and pindolol, 5-HT1A autoreceptor agonist(Blier and Bergeron, 1995). Furthermore, to our knowledge, there have been no studies examining our hypothesis in both preclinical and clinical form.

Therefore, as a preclinical study, we tried to examine the effects of pretreatment of dopamine D_2 autoreceptor agonists on the extracellular dopamine levels in the nucleus accumbens (NAS) of rats, induced by antipsychotics. Among many dopamine D_2 autoreceptor agonists, (-)-3-PPP was selected because it is one of the more intensively studied dopamine autoreceptor modulatory agents. However, its action as a dopamine D_2 autoreceptor agonist has not been confirmed by the studies using an in vivo microdialysis although there are many other supportive results obtained with different methods such as behavioral, electrophysiological, and in vitro tissue measurements. Hence, the present study was designed to investigate the following three purposes, using microdialysis and high pressure liquid chromatography(HPLC) with electrochemical detection(ECD).

First, by measuring the effects of (-)-3-PPP in different doses on the extracellular levels of dopamine in the NAS of free moving rats, we tried to determine whether (-)-3-PPP has an action of dopamine D_2 autoreceptor agonist, assuming reduction of dopamine release is indicative of a stimulation of dopamine autoreceptors. Second, we measured the effects of haloperidol in different doses on the extracellular levels of dopamine in the NAS of rats. Third, we tried to determine what effect the pretreatment of (-)-3-PPP in the dose showing an action of dopamine dopamine D_2 autoreceptor agonist has on the changes of extracellular levels of dopamine in the NAS induced by haloperidol.

Materials and Methods

1. Animals

Male Sprague-Dawley rats(DHAC, Seoul, Korea) weighing 250-300g were used throughout the study. Rats were housed two per cage and were maintained on a 12-h light/dark cycle and under constant temperature at 22℃ with free access to food and water.

2. Surgery

Rats were anesthetized with sodium pentobarbital(50mg/kg i.p.) and mounted in a stereotaxic frame(Stoelting, Wood Dale, Illinois, USA). Intracerebral guide cannulae and stylets(Bioanalytical Systems, Inc., Indiana, USA) were placed and fixed by cranioplastic cement onto the cortex 2mm dorsal to the left NAC. Stereotaxic coordinate of NAC was A +2.0, L +1.7, and V -7.5mm relative to bregma according to the atlas of Paxinos and Watson(1986).

3. Microdialysis

Three days after cannulation, the stylet was removed and the microdialysis probe with 2mm membrane(Bioanalytical Systems, Inc., Indiana, USA) was implanted into the NAC. The rat was placed in a plexiglas bowl and connected to BAS Raturn system(Bioanalytical Systems, Inc., Indiana, USA) for freely moving animals. The input tube of the dialysis probe was connected to a syringe pump(MD-1001, Bioanalytical Systems, Inc., Indiana, USA) which delivered an unbuffered artificial cerebrospinal fluid containing 150mM NaCl, 3mM KCl, 1.7mM $CaCl_2$ and 0.9mM $MgCl_2$(pH 7.4) to the probe at a rate of 1 μl/min. After overnight perfusion, the perfusion flow rate was increased to 2.0 μl/min and output tube of the dialysis probe was

attached to an electrically actuated switching valve(Pollen-8 On-Line Injector, Bioanalytical Systems, Inc., Indiana, USA). One hour later, collected dialysate samples(5μl) were automatically injected to HPLC system every 30 min. After obtaining stable baseline values in the dialysate such that a percentage of S.E.M. of the three consecutive dopamine values in the dialysate differed less than 10% of the dopamine values, each drug or vehicle was administered s.c. to rats. The effect of the drug was followed for another 180min. The procedures applied in this study were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Chonbuk National University, School of Medicine.

4. Biochemical assay

Dopamine concentrations in dialysate samples were determined by HPLC with LC-4C amperometric detector(Bioanalytical Systems, Inc., Indiana, USA).

Dopamine was separated on the microbore reversed-phase column(BAS Sep-Stick, 3μm octadecylsilane, 100×1.0mm I.D., C18, Bioanalytical Systems, Inc., Indiana, USA). The composition of the mobile phase was 0.1 M monochloracetic acid, 0.5mM EDTA, 0.15g/l sodium octyl sulfate, 5% acetonitrile, and 0.7% tetrahydrofuran(pH 3.1). The flow rate was 70μl/min and the potentials of a dual glassy carbon working electrode positioned in serial were +0.7V and +0.05V with respect to Ag/AgCl reference electrode. The in vitro recovery of probes for dopamine were 10 - 15% and the detection limit was 0.05 fmol/μl for dopamine at a 3∶1 signal-to-noise ratio. All reagents used for HPLC were purchased from Sigma Chemical Co.(St. Louis, MO, USA) and Fisher Scientific(Pittsburgh, PA, USA). The chromatograms were integrated with EZChromTM Chromatography Data System(Scientific Software, Inc, San Ramon, CA, USA)

5. Histology

The histological identification of probe placement was performed as follows∶after the termination of each experiment, the animal was decapitated, and the brain was removed. Serial fresh frozen sections were cut at 30μm intervals, stained with hematoxylin-eosin, and examined microscopically.

6. Drug

Haloperidol and (-)-3-PPP were obtained from Sigma Chemical Co.(St. Louis, MO, USA) and Research Biochemicals International(Natick, MA, USA) respectively. Haloperidol was dissolved in 0.1 M tartaric acid(pH 4.5) and (-)-3-PPP in distilled water. The injected doses were 0.1, 0.5, and 2mg/kg for haloperidol and 0.5, 2, and 10mg/kg for (-)-3-PPP which were decided by referring to other studies. Drugs or vehicle(0.1 M tartaric acid or distilled water) in a volume of 1.0ml/kg were administered s.c. to randomly assigned rats.

7. Data analysis

The average concentration of three stable samples(<10% variation) was considered the control and was defined as 100%. All values given are expressed as percent of controls. Data were analyzed using StatView 4.50(Abacus Concepts Inc., Berkeley, CA, USA). The time-dependent effect of (-)-3-PPP or haloperidol was analyzed by one-way repeated ANOVA followed by post-hoc Turkey test for multiple comparisons when appropriate. The effects of time and combination treatment groups[vehicle+vehicle, (-)-3-PPP+haloperidol, vehicle+haloperidol, and (-)-3-PPP+haloperidol] on the extracellular dopamine concentrations were analyzed by two-way repeated ANOVA followed by post-hoc Turkey test for multiple comparisons when appropriate. Comparisons of different groups of combination treatment at each time point were carried out by one-way ANOVA followed by post-hoc Turkey test for multiple comparisons. Probability within .05 was considered to indicate a significant difference.

Results

1. Effects of (-)-3-PPP on extracellular dopamine concentrations in the NAC(Fig. 1)

Basal concentrations of dopamine in the NAC were 1.9±

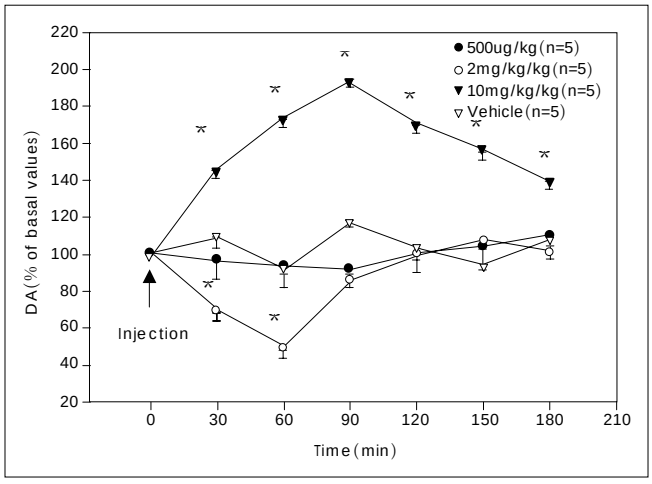

Fig. 1. Effects of (-)-3-PPP on extracellular dopamine(DA) concentrations in the nucleus accumbens. For each time points, means(S.E.) are expressed as percent of the respective basal dopamine value.
*∶ p<.05 in respect to basal value in each group.

0.9 fmol/μl(mean±S.E.). Significant time-dependent effects on extracellular dopamine concentrations were noted($p<.05$) with 2 and 10mg/kg(-)-3-PPP but not with 0.5mg/kg(-)-3-PPP and vehicle. The results of post-hoc tests were as follows : 2mg/kg(-)-3-PPP decreased extracellular dopamine concentrations significantly($p<.05$) at 30 and 60 min compared to baseline while 10mg/kg(-)-3-PPP increased extracellular dopamine concentrations significantly($p<.05$) at all time points compared to baseline.

2. Effects of haloperidol on extracellular dopamine concentrations in the NAC(Fig. 2)

Significant time-dependent effects on extracellular dopamine concentrations were significant($p<.05$) with all doses of haloperidol(0.1, 0.5, and 2mg/kg) but not with vehicle. The post-hoc tests showed each dose of haloperidol increased($p<.05$) extracellular dopamine concentrations significantly at all time points compared to its basal value. The highest levels of dopamine increase were 156, 163, and 201% for 0.1, 0.5, and 2mg/kg haloperidol respectively.

3. Effects of pretreatment of (-)-3-PPP on the halo-peridol-induced extracellular dopamine concentrations in the NAC(Fig. 3)

There were significant differences($p<.05$) between treatment groups or time points(for group×time interaction : $F=35.47$, $df=18$, $p<.001$). Post-hoc tests showed that in (-)-3-PPP+haloperidol group, there were significantly lower dopamine concentrations at all time points compared to the corresponding ones of vehicle+haloperidol group($p<.05$) and significantly higher dopamine concentrations at 30 and 60min compared to the corresponding ones of vehicle+vehicle group($p<.05$). Above results indicate that pretreatment of (-)-3-PPP reduced the increased dopamine concentrations elicited by acute treatment with haloperidol(2mg/kg).

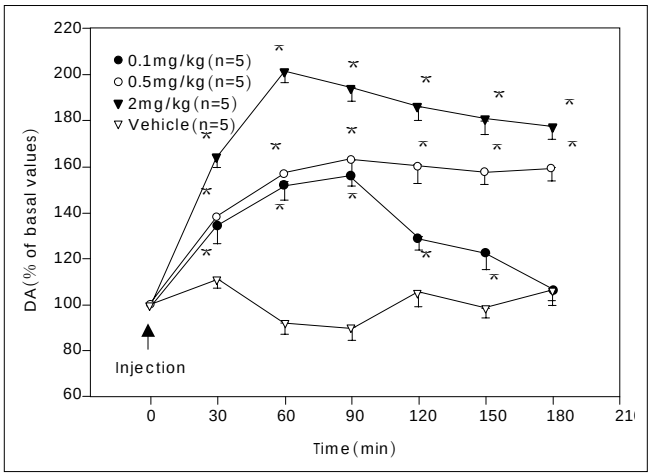

Fig. 2. Effects of haloperidol on extracellular dopamine(DA) concentrations in the nucleus accumbens. For each time points, means(S.E.) are expressed as percentage of the respective basal dopamine value.
*: $p<.05$ in respect to basal value in each group.

Discussion

The reason for selecting (-)-3-PPP as dopamine autoreceptor agonist for this study was that it has a highly preferential limbic action(Hjorth, 1983) and was demonstrated to have a therapeutic effect for patients with schizophrenia (Lahti et al., 1998). (-)-3-PPP has been reported to act as presyna-ptic dopamine autoreceptor agonist in low dose (<8 μmol/kg) and postsynaptic dopamine receptor antagonist in moderate to high dose(>16 μmol/kg)(Lundström et al., 1992). The action of (-)-3-PPP in low dose as presynaptic dopamine autore-ceptor agonist was supported by several studies showing that it decreased spontaneous locomotion (Schaefer et al., 1986), the firing rate of dopaminergic neurons in the zona compacta of substantia nigra(Clark et al., 1985_a) and blocked the increase of dopamine synthesis in the neostriatum induced by γ-butyrolactone(Clark et al., 1985_b). However, this action was not confirmed by the more directive method measuring ex-tracellular dopamine concentrations, i.e., microdialysis. Imperato et al.(1988), for example, failed to

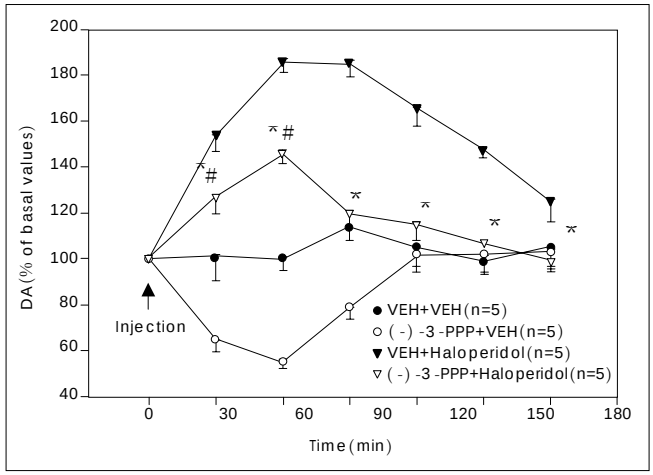

Fig. 3. Effects of the pretreatment of (-)-3-PPP on the haloperidol-induced extracellular dopamine(DA) concentrations in the nucleus accumbens. For each time points, means(S.E.) are expressed as percentage of the respective basal dopamine value. The pretreatment of VEH or (-)-3-PPP was done 10 min before the injection of VEH or haloperidol.
*: $p<.05$ in respect to the corresponding values in VEH+Haolperidol group.
#: $p<.05$ in respect to the corresponding values in VEH+VEH group.

demonstrate any significant changes of extracellular dopamine concentrations in the NAC of rats with (-)-3-PPP injected s.c. in low doses(62.5-250μg/kg, and 1mg/kg). In addition, See(1994) demon-strated only the action of (-)-3-PPP as a postsynaptic dopamine receptor antagonist observing increased dopamine levels by the infusion of (-)-3-PPP(1-100μM) through the dialysis membrane into the NAS and caudate putamen of the awake rat. Therefore, it is significant that we demonstrated the reduction of dopamine release by the administration of 2mg/kg(-)-3-PPP s.c., using microdialysis. Although the discrepancy with the results of Imperato et al. study (1988) can be partly explained by the differences in drug doses and experimental conditions, it warrants further replication. To further examine whether (-)-3-PPP has a greater effect on the terminal dopamine autoreceptor or the somatodendritic dopamine autoreceptor, direct local infusion of (-)-3-PPP into the ventral tegmentum and NAS is required.

Acute treatment of haloperidol at all doses increased the extracellular dopamine concentrations in the NAC with higher dose having a greater increase. This finding suggest that haloperidol in acute treatment block presynaptic dopamine autoreceptor and stimulate dopamine release. The highest levels of dopamine increased with 0.1 and 0.5mg/kg haloperidol were 156 and 163% which is similar to the results of other studies (Kuroki et al., 1999 ; Moghaddam and Bunney, 1990) but the effect of the 2mg/kg dose of haloperidol, 201%, is relatively higher than the result of Ichikawa and Meltzer's study(1991).

Combination treatment of (-)-3-PPP and haloperidol caused a lower dopamine concentrations at all time points compared to the corresponding ones in vehicle+haloperidol treatment group, indicating pretreatment of (-)-3-PPP reduced the increased dopamine concentrations elicited by acute treatment with haloperidol(2mg/kg). Thus, this finding suggests that (-)-3-PPP could have a possibility of accelerating the onset of therapeutic effect of haloperidol, assuming the initial increase of dopamine release by haloperidol is a major cause for the delay of therapeutic effect. To further examine the possibility of (-)-3-PPP as an accelerator for therapeutic effect, studies measuring intracellular effects of haloperidol such as *c-fos*, and cyclic-AMP, would be desirable since the intracellular effects of drug are more closely related to therapeutic effect. Also, a controlled clinical trial of combination treatment of (-)-3-PPP and haloperidol compared to either drug alone and placebo in patients with schizophrenia would be a good way to test this hypothesis clinically.

In conclusion, our finding that pretreatment with (-)-3-PPP reduced the haloperidol-stimulated dopamine release suggests it could possibly speed up the onset of therapeutic effect of haloperidol. Since atypical antipsychotic drugs, with the exception of clozapine, increase dopamine release in the NAS or other limbic nuclei after acute administration(Kuroki et al., 1999), it can be also assumed that treatment of patients with (-)-3-PPP prior to one of the other atypical antipsychotic drugs might be beneficial in accelerating their onset of action as well.

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