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Behavioral and Biochemical Effects of Amperozide and Serotonin Agents on Nigrostriatal and Mesolimbic Dopamine Systems

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Abstract

Central dopaminergic system serves two major physiological functions, *i.e.*, motivation activation and motor coordination. The evidence that serotonergic system could modulate these two pathways suggests that serotonin (5-HT) and related agents may possess potential therapeutic values against certain mental or motor disorders caused by dopamine malfunction. This study presents novel modulatory role for serotonergic agents in rat behaviors which have been speculated to be associated with forebrain dopamine system. Three serotonergic agents, including DOI (5-HT_2 agonist), ritanserin (5-HT_2 antagonist) and amperozide (5-HT_2/ D_2 antagonist) were evaluated, focused particularly on the atypical antipsychotic amperozide. It was found that both amperozide and ritanserin could inhibit amphetamine-induced hyperlocomotion, and only amperozide inhibited nomifensine-induced hyperlocomotion. Amperozide could also reduce significantly the rearing but not sniffing behaviors. Furthermore, DOI and amperozide, but not ritanserin, reduced the haloperidol-induced catalepsy When animals were unilaterally radiofrequency lesioned in either caudate putamen (CP) or nucleus accumbens (NA), amperozide reduced both the ipsi- and contralateral turns in CP-lesioned, but reduced only ipsilateral turns in NA-lesioned rats. *Via in vivo* microdialysis, we demonstrated that amperozide could increase the extracellular dopamine release in both CP and NA in either intact or para-chlorophenylalanine (p-CPA) serotonin-depleted rats. Overall, we conclude that the modulatory role of amperozide on forebrain dopamine system requires not only 5-HT_2/D_2 antagonistic but also the blockade of dopamine transporter.

Key Words: amperozide, 5-HT_2 antagonism, dopamine transporter blocker, caudate putamen, nucleus accumbens

Introduction

The development of new antipsychotic drugs has focused on drugs having a selective or preferential inhibitory effect on limbic versus motor dopamine function. Clozapine, a prototype limbic-selective

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atypical antipsychotic, has no propensity to induce extrapyramidal syndrome (EPS), which resembles the symptoms of Parkinson's Disease and frequently triggered by typical antipsychotics, *e.g.* haloperidol. Those drug-induced catalepsy (the impairment of movement initiation) in rodents actually serves as a valid animal model for EPS, due to the severe blockade of dopamine D_2 receptors (10, 35, 61). Hence, the cataleptic immobility in rodents induced by typical neuroleptics provides a behavioral basis to study the striatal dopamine function which known to be modulated by various neuronal systems, includes cholinergic, serotonergic and nitrergic neurons (50, 51). A standard guideline to evaluate novel antipsychotics which carry D_2 receptor antagonist property depends on how drug would reduce the potential EPS syndromes (46).

The EPS syndrome accounts for a motor disorder associated with nigrostriatal dopamine system, of which its cell bodies locate in the substantia nigra (SN) with fibers project to the dorsal striatum (caudate putamen; CP) (26). On the other hand, the limbic dysfunction observed in various psychotic diseases results from abnormal dopamine function mainly in the medial prefrontal cortex (mPFC) and ventral striatum (nucleus accumbens; NA), of which their cell bodies derive from the ventral tegmental area (VTA) (60). Other than dopamine autoregulation in both nigrostriatal and mesolimbic/mesocortical dopamine systems (9, 34), notably the serotonin, have axonal projections to both dopamine terminals and somatodendritic area (43, 45), thus profoundly influence dopamine excitability (6, 59, 63) and release (6, 22). Despite the wealth of information on serotonin anatomy in the brain, the role of serotonin transmission in these brain areas remains largely unknown (6, 63).

The evaluation of limbic functions is routinely assessed by the measurement of locomotor activity in rodents (19, 52). Lesion or microinjection studies have provided evidence that hyperlocomotion induced by d-amphetamine is mediated preferentially by release of dopamine in the NA (7, 32). As a potent psychostimulant, amphetamine inhibits dopamine re-uptake (28, 62) and promote non-vesicular dopamine efflux by reverse transport through monoamine transporters (27, 54). The other dopamine transporter blocker, nomifensine, demonstrated also to induce hyperlocomotor activity could similarly enhance dopamine release in the NA (17).

The discovery of clozapine as novel antipsychotics that carry less EPS syndrome provides a new direction for atypical drug design. Subsequently, atypical antipsychotics were proved to have higher serotonin 5-HT_2 receptor binding than dopamine D_2 receptor and appears to be limbic selective (56). Several atypical antipsychotics which have been developed include the amperozide (41). Amperozide (5-HT_2/D_2 antagonist) is originally developed as a compound to decrease the aggressiveness (3, 18). Later, animal studies indicate that amperozide has antidepressant effects since the drug decreased the duration of immobility in a despair test (20). The drug not only carries high binding affinity of 5-HT_{2A} (K_i = 16.5-23 nM) than D_2 receptors (K_i = 403-540 nM) in the cerebral cortex, striatum and limbic forebrain (57), but also display ability to block serotonin transporter (14). Application of amperozide is shown to down-regulate the cortical serotonin 5-HT_{2A} receptors (58), but not dopamine D_2 receptors (55). Recently, the drug was found that it can attenuate the behavioral effect of NMDA antagonist, MK-801 (16). Animal studies depict that amperozide could suppress food-reinforcement (2, 12), inhibit the conditioned avoidance (12), block the amphetamine-induced hyperlocomotion but not stereotypy (13, 21). Amperozide by itself did not produce acute catalepsy (21) and sedation (20), and it, has minimal effects on jaw movements after chronic administration (53). In practice, amperozide reduces volitional alcohol consumption (40, 44, 47) and volitional cocaine intake (39). The above experimental evidence implicates that amperozide might act multifunctionally at different brain regions to combat both negative and positive syndromes of various psychiatric disorders. However, other than its known function on mPFC and NA dopamine neuron, very few studies focus on the evaluation of the drug effect between nigrostriatal and mesolimibic dopamine system.

In the present study, we intent to investigate the effect of amperozide on several behavioral and neurochemical tests, *i.e.* amphetamine- and nomifensine-induced hyperactivity, amphetamine-induced rotation in lesioned animals as well as haloperidol-induced catalepsy and compare those behavioral outcomes with ritanserin (5-HT_2 antagonist) and DOI (5-HT_2 agonist). The purpose is to understand if amperozide would function differentially in between nigrostriatal and mesolimbic dopamine system.

Materials and Methods

Animals and Drugs

A total of 80 male Sprague-Dawley rats were used in this study, weighing from 300 to 350 g. For each experiment, a group of 8 rats was treated either with drugs or with vehicle as control. Rats were housed 3 per cage in a colony room with an ambient temperature of 20-25°C and a 12-h light/dark cycle (lights on at 0700 h). Behavioral observations were conducted during the light phase. Standard rat chow and water were available *ad libitum*. The experimental protocol was approved by the National Defense Medical Center

Animal Care and Use Committe, and the experiments were conducted following the guidelines provided by the National Institutes of Health. The following drugs were dissolved in saline: amperozide (Kabi Pharmacia Therapeutics AB, Sweden), DOI (1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; RBI, Natick MA, USA), d-amphetamine sulfate (National Bureau of Controlled Drugs, Taiwan, ROC), nomifensine maleate (RBI), and chloral hydrate (Sigma, ST. Louis, MO, USA). Ritanserin (RBI) was dissolved in methanol. Haloperidol (RBI) was dissolved by titration with 0.1N hydrochloric acid solution, and p-CPA (para-chlorophenylalanine, Sigma) was suspended in a 1% aqueous solution of Tween 80.

Drug-Induced Locomotor Activity and Stereotyped Behaviors

Animals were tested for locomotor activity after injection of amphetamine (2.0 mg/kg, i.p.) or nomifensine (5.0 mg/kg, i.p.) with or without pretreatment of amperozide (10.0 mg/kg, i.p.), ritanserin (2.5 mg/kg, i.p.) or DOI (1.0 mg/kg, i.p.) for 30 min. Thirty min after amphetamine or nomifensine injection, the locomotor activity was recorded for 150 min in a black plastic activity chamber (45 × 45 × 30 cm) analyzed by a computer-assisted Video Tracking System (San Diego Instrument, San Diego, CA, USA). A procedure was modified from Costall and Naylor to quantify the stereotyped behaviors (8). The following behavioral parameters were recorded: sniffing (not moving but sniffing the walls or floor of the apparatus) and rearing (body inclined vertically with hind-paws on the floor and forepaws on the wall of the cage). Both behaviors were observed and recorded at 1 min period for a total of 150 min. Behavior was scored from 0 to 5 as persistent time spending: 0 (0 min), 1 (1-30 min), 2 (31-60 min), 3 (61-90 min), 4 (91-120 min) and 5 (121-150 min).

Radiofrequency Lesion of the Caudate-Putamen and Nucleus Accumbens

To produce brain lesion, we used radiofrequency (RF) apparatus (Radionics Inc., Burlington, MA, USA) through a RFG-4A lesion maker and TCZ electrodes, which destroyed both neurons and fibers of passage. After anesthetized with chloral hydrate (400 mg/kg, i.p.), each rat was placed in a stereotaxic apparatus that had the incisor bar set at 3.3 mm below the interaural line. The rat atlas (48) was used to analyze and locate the lesions. The stereotaxic coordinates for the nucleus accumbens lesions relative to bregma were: AP +2.2 mm, ML ± 1.6 mm, DV –7.0 mm from surface of the brain; for the caudate putamen: AP 1.2 ± 1.0 mm, ML 2.5 mm, DV –5.0 mm. After a midline incision (15-20 mm), two burr holes were made on the right side of the skull. An RF electrode (tip diameter of 0.25 mm, tip exposure of 0.25 mm) was lowered into designated brain regions through a burr hole. Tip temperature was set at 55°C for 90 s to create the lesions. For sham lesion, the electrode was lowered to 1.0 mm above the target regions and no heat was generated. The incision was closed with silk suture. Six to 8 days after the surgery, animals that showed no behavioral abnormality were selected for amphetamine-induced rotation test.

Amphetamine-Induced Rotation Test

The behavioral effects of amperozide on dopamine imbalance were assessed using amphetamine-induced rotation followed by one-side RF lesion. Sham or RF-lesioned rats were injected with amperozide (10.0 mg/kg, i.p.) and allowed to habituate in the test plastic cylinder for 30 min, where they were tethered to an automated rotometer (Coulbourn Instruments). Turns performed in the direction ipsilateral and contralateral to the lesion were recorded separately for a total of 60 min following immediate amphetamine administration (2.0 mg/kg i.p.).

Haloperidol-Induced Catalepsy

The catalepsy experiments were performed using the horizontal bar method (23). Haloperidol (0.75 mg/kg, s.c.) was administered at time zero and the test drugs were administered at 90 min. This dose of haloperidol was chosen to produce only a moderate degree of catalepsy so that reversal or potentiality of catalepsy would be measured. Catalepsy was assessed by placing the front limbs of the rats over a horizontal bar with the hind limbs extended and abducted. The time that the rat maintained this posture was recorded. Catalepsy was scored from 0 to 5 as follows: score 0 (<15 sec), score 1 (16-115 sec), score 2 (116-215 sec), score 3 (216-315 sec), score 4 (316-415 sec) and score 5 (>415 sec). Following haloperidol with or without testing drug administration, rats were given three trials on the horizontal bar every 30 min for 3 h for the presence of catalepsy. The maximum catalepsy scores for each time interval were used for statistical comparisons.

In Vivo Brain Microdialysis and HPLC-ECD Detection

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame (Stoetling, Wood Dale, IL). Two 21-gauge guide stainless cannula with dummy probes were placed onto the caudate putamen (CP) and nucleus accumbens (NA) and fixed by cranioplastic cement (Plastic One, Roanoke, VA) on the skull. Concentric-shaped dialysis

probes were constructed according to the method of Kuroki *et al*. (33). The probe diameter is 310 μm, 4 mm exposure distance in striatum and 2 mm in nucleus accumbens. The rat atlas (48) was used to analyze and locate the probe. The stereotaxic coordinates for probes were AP: +0.2, ML: –2.8, DV: –7.4 for CP and AP: +2.2, ML: +1.6, DV: –7.8 mm for NA, relative to bregma (48). The locations of the dialysis probes were verified at the end of each experiment. Because of the probe size used in this study, it is likely that the dialyzed area included both the shell and core regions of the NA. Two hours following the dialysis probes were implanted, rats were under slight anesthesia with chloral hydrate (100-150 mg/kg/h, femoral vein injection) and began the low flow rate perfusion (0.2 μl/min). The perfusion medium was artificial cerebrospinal fluid solution (ACSF: 140 mM NaCl, 3.0 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.04 mM ascorbic acid pH = 7.4). After 2 h perfusion, the flow rate was increased to 1.0 μl/min and dialysates were collected every 30 min. After obtaining stable dopamine baseline values by high-performance liquid chromatography (HPLC) analysis, amperozide (0.5, 0.5, 1.0, 2.0, 4.0, 8.0 mg/kg/15 min consecutive doses, femoral vein injection) or vehicle was administered to the rats. The dialyzed samples were analyzed by HPLC coupled to electrochemical detector (HPLC-ECD). Dialysate samples (15 μl) were directly applied to an HPLC bearing with a 20 μl sample loop and analyzed for dopamine with an integrator (BAS-480, West Lafayette, IN, USA). Dopamine was separated on a stainless steel, reversed phase column (BDS Hypersil 3-μm C18, 2.0 × 100 mm, Keystone Scientific, Bellefonte, PA, USA) at 35°C maintained by column heater and temperature controller (LC-22C, BAS). The mobile phase consisted of sodium phosphate 20.5 g/l containing EDTA-Na_2 (185 mg/l) and sodium octyl sulfate (150 mg/l, Kodak, Rochester, NY, USA) adjusted to pH 6.7 with concentrated phosphoric acid, and 12% (v/v) methanol and was pumped at the flow rate of 0.2 ml/min (PM-80, BAS, West Lafayette, IN, USA). Dopamine was detected by a dual glassy carbon-working electrode (MF-1000, BAS), set at +0.65 V (LC-4C, BAS) *vs*. Ag/AgCl reference electrode. Dopamine standards were prepared fresh and injected every 5 sample runs.

Histology

After completion of amphetamine-induced rotation and microdialysis experiments, animals were deeply anesthetized and perfused intracardially with 0.9% saline followed by 10% formalin. Brains were removed immediately and stored in 10% formalin. Vibratome sections were made at 50 μm in the coronal plane through the targeted regions, and stained with cresyl violet. The rat atlas (48) was used to analyze and depict the lesions.

Statistical Analysis

Repeated-measures two-way analysis of variance (ANOVA) was used to determine if there was an overall significance in catalepsy experiment. Post-hoc Duncan's multiple range test was used to determine if there was a significant change in particular group as compared to control. Student's *t*-test was used in the other experiments. A level of 0.05 was considered to be significant.

Results

Amperozide Inhibited Amphetamine- and Nomifensine-Induced Hyperlocomotor Activity and Rearing Behavior

Low doses of acute amphetamine (2.0 mg/kg, i.p.) or nomifensine (5.0 mg/kg, i.p.) induced a time-dependent hyperlocomotor activity in testing animals with peak activation apparently faster in nomifensine (20 min) than in amphetamine (40 min) after 30 min drug administration (Fig. 1A). The total distance (m) evoked by either amphetamine (365 ± 16) or nomifensine (147 ± 8) during 150 min testing session was significantly higher than that of the saline controls (62 ± 5) (Fig. 1B). In addition, higher amphetamine dosage (5.0 mg) induced more stereotyped behaviors than locomotor activity (data not shown) which is consistent with previous reports (1, 32). Hence, the dose of 2.0 mg/kg amphetamine and 5.0 mg/kg nomifensine were chosen for the subsequent studies to characterize drug effect of amperozide.

Pretreatment with 5-HT_2/D_2-antagonist-amperozide and 5-HT_2-antagonist- ritanserin both inhibited significantly the amphetamine-induced hyperlocomotor activity (total distance: 88 ± 5 *vs*. 380 ± 20 of controls, $P < 0.01$; 237 ± 17 *vs*. 352 ± 32 of controls, $P < 0.05$, respectively). On the other hand, the inhibition on nomifensin-induced hyperlocomotor activity was only inhibited by amperozide (92 ± 5 *vs*. 196 ± 10 of controls, $P < 0.01$), but not ritanserin. Pretreatment with the 5-HT_2-agonist-DOI had no effect on either amphetamine- or nomifensin-induced hyperlocomotion (320 ± 47 *vs*. 375 ± 21 of control and 202 ± 20 *vs*. 192 ± 8 of control; respectively) (Fig. 2). Pretreatment of amperozide also significantly attenuated the rearing behavior induced by amphetamine (0.4 ± 0.1 *vs*. 3.5 ± 0.5 of control, $P < 0.01$) or nomifensine (0.3 ± 0.3 *vs*. 1.2 ± 0.3 of control, $P < 0.05$), but no effect on amphetamine- (4.9 ± 0.1 *vs*. 4.0 ± 0.5 of control) or nomifensine-induced sniffing (4.7 ± 0.2 *vs*. 4.2 ± 0.2 of control) (Fig. 3).

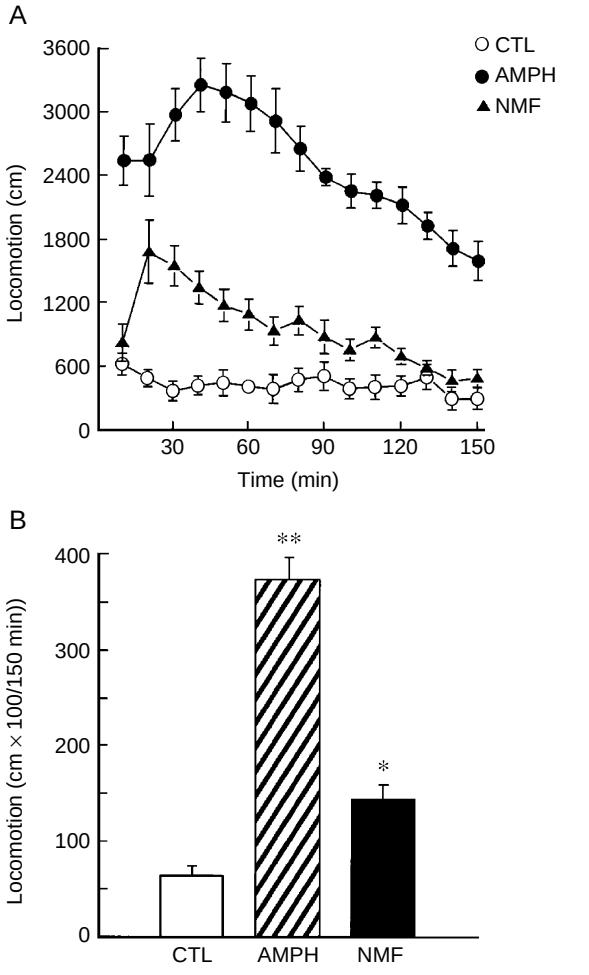

Fig. 1. Amphetamine- and nomifensine-induced hyperlocomotor activity in rats. (A) amphetamine (AMPH, 2.0 mg/kg), nomifensine (NMF, 5.0 mg/kg) or vehicle (saline, CTL) was injected (i.p.) 30 min before behavioral measurement. The locomotion distance (mean ± S.E.M.) was measured every 10 min for a total session of 150 min. (B) The cumulative distance (mean ± S.E.M.) of 150 min recording was counted from (A). Eight rats were tested in each group. $^{**}P < 0.01$, $^{*}P < 0.05$, significant difference compared to vehicle control.

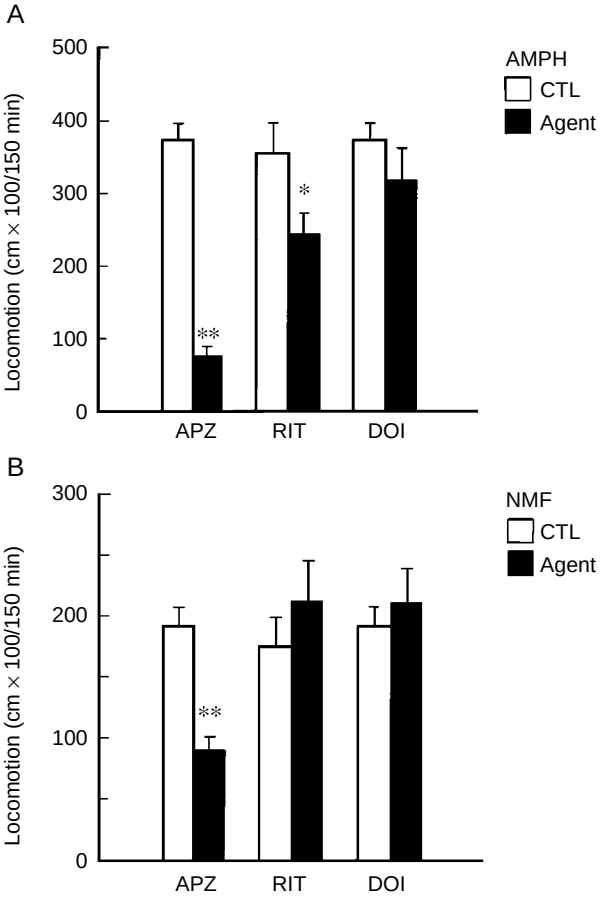

Fig. 2. Effects of amperozide (APZ), ritanserin (RIT) and DOI on (A) amphetamine (AMPH) or (B) nomifensine (NMF) induced hyperlocomotor activity. APZ (10.0 mg/kg), RIT (2.5 mg/kg), DOI (1.0 mg/kg) or vehicle was injected (i.p.) 30-min prior to AMPH (2.0 mg/kg) or NMF (5.0 mg/kg). The cumulative distance (mean ± S.E.M.) measured in a total of 150-min recording period. Control groups (CTL) were treated with vehicle and AMPH or NMF. Each group consists of 8 rats. $^{**}P < 0.01$, $^{*}P < 0.05$, significant difference compared to vehicle control.

Effects of Amperozide on Amphetamine-Induced Rotation in CP or NA Lesioned Rats

When the animals were lesioned at right caudate putamen (CP) or right nucleus accumbens (NA), systemic amphetamine challenge induced both contralateral (away from lesioned site) and ipsilateral (towards lesioned site) rotations with ipsilateral turns much intense than contralateral turns (Fig. 4, control). Pretreatment with amperozide significantly suppressed the amphetamine-induced ipsilateral (20 ± 3 *vs*. 88 ± 14 of controls, $P < 0.05$) and contralateral (3 ± 1 *vs*. 18 ± 3 of controls, $P < 0.05$) rotation in CP-lesioned rats. Alternatively, in the NA-lesioned rats, amperozide significantly decreased the amphetamine-induced ipsilateral rotation (35 ± 17 *vs*. 147 ± 35 of controls, $P < 0.05$), had no effect on contralateral rotation (19 ± 6 *vs*. 21 ± 4 of control) (Fig. 4).

Amperozide Attenuated Haloperidol-Induced Catalepsy

Systematic administration of haloperidol (0.75 mg/kg s.c.) induced muscle rigidity determined as an increased muscle resistance in rats' hind leg in response to passive extension and flexion at the ankle joint. That phenomenon was clearly visible during the period between 30 and 180 min after administration of

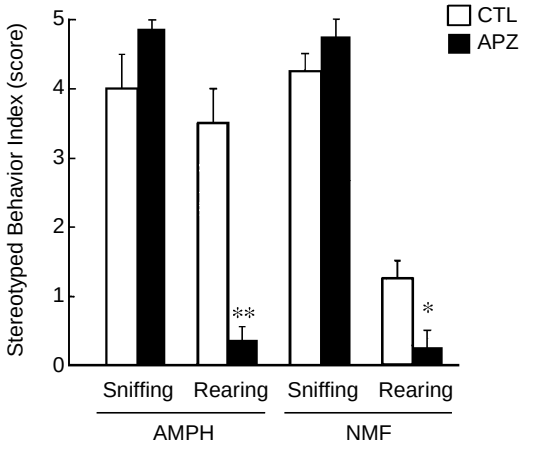

Fig. 3. Effects of amperozide (APZ) on amphetamine (AMPH) or nomifensine (NMF) induced rearing and sniffing behaviors. APZ (10.0 mg/kg) was injected (i.p.) 30 min prior to AMPH (2.0 mg/kg) or NMF (5.0 mg/kg). The intensity of individual behavior was recorded every 1 min for a total of 150-min period. The behavior index (mean ± S.E.M.) is scored for both controls (CTL) and APZ groups. Each group consists of 7 rats. $^{**}P < 0.01$, $^{*}P < 0.05$, significant difference compared to vehicle control.

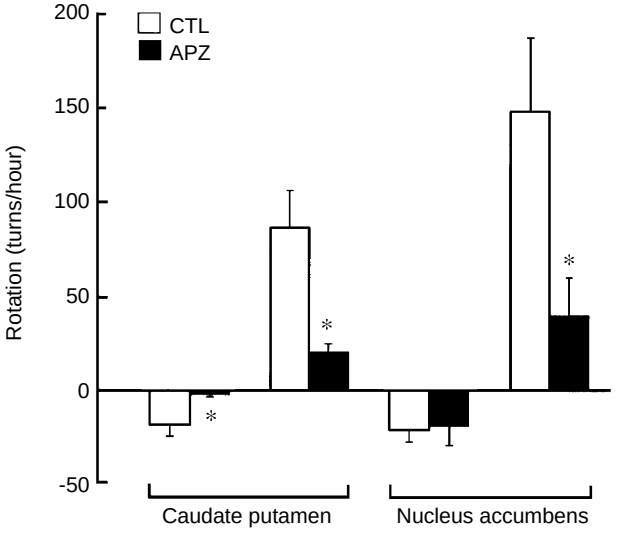

Fig. 4. Effects of amperozide (APZ) on rotation behavior induced by amphetamine (AMPH). Animals received the radiofrequency lesion on the right-hand side of either caudate putamen or nucleus accumbens (see text for details). APZ (10.0 mg/kg) was injected (i.p.) 30 min prior to AMPH (2.0 mg/kg). The induced-circling behaviors were expressed as mean ± S.E.M. for one hour recording session while positive scores are for ipsilateral and negative scores are for contralateral turns. Control groups (CTL) received vehicle. Each group consists of 8 rats. $^{*}P < 0.05$, significant difference compared to vehicle control.

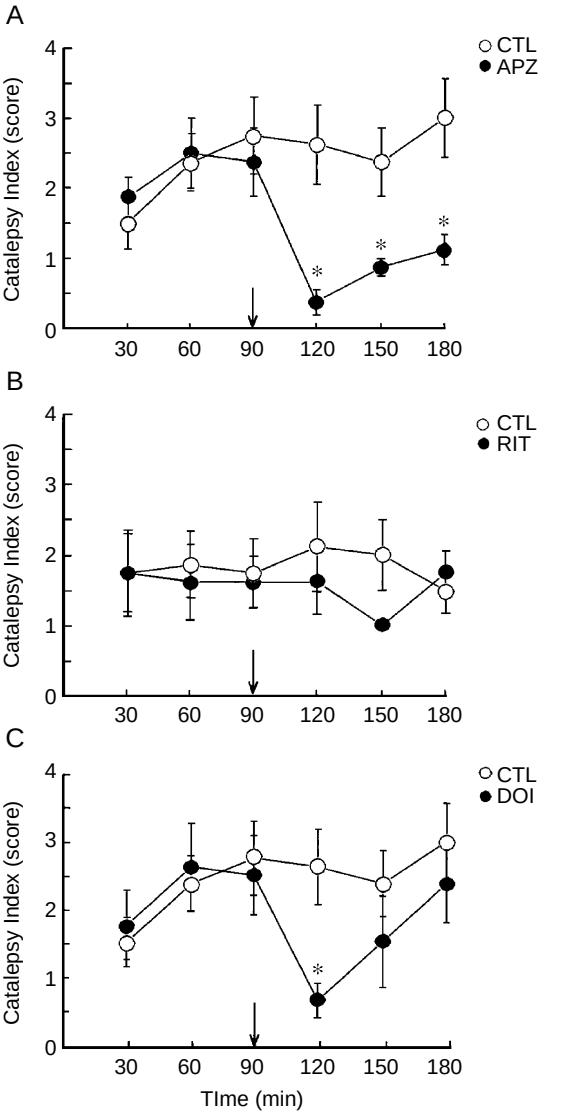

Fig. 5. Effects of amperozide (APZ), ritanserin (RIT) and DOI on catalepsy induced by haloperidol. APZ (10.0 mg/kg), RIT (2.5 mg/kg), DOI (1.0 mg/kg) or vehicle was injected (i.p.) at the 90th min after haloperidol (0.75 mg/kg, s.c.) induced catalepsy. The degree of animal rigidity was assessed by horizontal bar test and transferred the data to catalepsy score. The catalepsy score (mean ± S.E.M.) measured every 30-min time course in a total of 180-min period is indicated for control groups (CTL) receiving vehicle and for the groups receiving test drug APZ (A), RIT (B) and DOI (C). Each group consists of 8 rats. $^{*}P < 0.05$, significant difference compared to vehicle control.

haloperidol. Post-treatment (90 min after haloperidol) of amperozide (5 mg/kg, i.p.) quickly induced an inhibition (30 min later) on haloperidol-induced muscle rigidity and lasted for 90 min (ANOVA, $F_{1,7} = 15.23$, $P < 0.01$, Fig. 5A). On the other hand, 5-HT_2-antagonist-ritanserin had no effect on haloperidol-induced

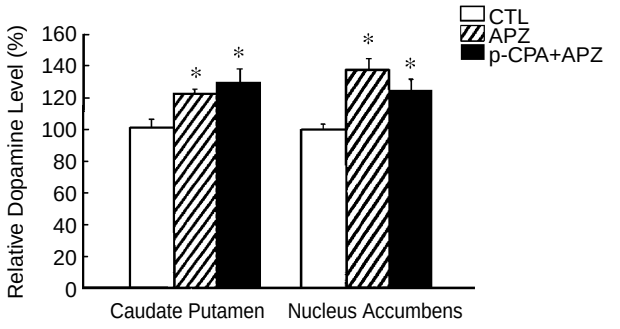

Fig. 6. Effects of amperozide (APZ) on extracellular dopamine level in the caudate putamen and nucleus accumbens. Rats were anesthetized and mounted in a stereotaxic frame, APZ was given by femoral intravenous injection with consecutive 6 doses (0.5, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg/15 min) for totally 75 min. Microdialysis solution (30 μl) was collected from either caudate putamen or nucleus accumbens before and after APZ treatment. Dialysates and the relative level of dopamine (set control as 100%) were analyzed by HPLC. The brain 5-HT was depleted with p-CPA (200.0 mg/kg/day × 3, i.p.). Sample number: CTL, 4; APZ, 8; p-CPA+APZ, 5. Data expressed as mean ± S.E.M. **P* < 0.05, significant difference compared to vehicle control (CTL).

catalepsy (Fig. 5B). Interestingly, post-treatment of 5-HT_2-agonist-DOI resulted in a transient attenuation on haloperidol-induced muscle rigidity (ANOVA, $F_{1,7} = 7.74$, $P < 0.05$, Fig. 5C).

Amperozide Increased Extracellular Dopamine Efflux in Intact or 5-HT Depleted Neuron

Using the *in vivo* microdialysis with HPLC-ECD, we were able to measure the extracellular dopamine release from both CP and NA in anesthetized rats. Femoral vein administration of amperozide (total cumulative dosage of 16.0 mg/kg in 90 min) increased significantly the dopamine efflux from both CP and NA as compared to saline controls (Fig. 6). Pretreatment with p-CPA for 3 days completely depleted the 5-HT content in both CP and NA as indicated by the loss of major acidic 5-HT metabolite 5-HIAA signals (data not shown). Interestingly, amperozide could still increase the extracellular dopamine both CP and NA in p-CPA-treated rats, with approximately similar levels between these two brain regions (Fig. 6).

Discussion

In the present study, we demonstrated that atypical amperozide had unique pattern on modulating the forebrain dopamine neurons. Although agree with previous reports that amperozide acts selectively on limbic system, we found that amperozide also affected striatum potently. That is, amperozide could suppress amphetamine-induced locomotor activity and stereotyped behaviors, inhibit amphetamine-evoked rotation in either caudate putamen (CP)- or nucleus accumbens (NA)-lesioned rats, as well as enhance dopamine efflux from both CP and NA. Nevertheless, amperozide apparently display anti-cataleptic ability to reverse the haloperidol-induced muscle rigidity, and exhibits selectivity on stereotyped behaviors, *i.e.* the suppression of amphetamine-induced rearing but not sniffing behavior. The finding that ritanserin could not modify the nomifensine-evoked hyperlocomotion or rescue haloperidol-induced catalepsy suggested that amperozide exerts its pharmacological effect depends more than 5-HT_2 receptor binding.

Amphetamine and nomifensine induced hyperlocomotor activity in rodents most likely through its action on dopamine transporter. This animal model is widely used to characterize the limbic dopamine function and as index to evaluate the antipsychotic efficacy (1, 42, 52). Amperozide and ritanserin, both play as 5-HT_2 antagonist, decreased the amphetamine-induced locomotor activity which is consistent with previous studies of this drug action (21, 24). On the other hand, we found that amperozide, but not ritanserin, could decrease nomifensine-induced locomotor activity indicated amperozide may target other than 5-HT_2 receptors. Amphetamine and nomifensine both could enhance synaptic dopamine strength but with different mechanisms. Amphetamine not only inhibits monoamine oxidase but promotes nonvesicular DA efflux by reverse transport through dopamine transporters (27, 54). However, nomifensine works dopamine transporter inhibitor only, which enhances the synaptic dopamine concentration once dopamine was released (17). Amperozide could not only act through 5-HT_2 receptor (like as ritanserin) to decrease amphetamine-induced hyperlocomotion, but also affect the extracellular dopamine level *via* blocking the dopamine re-uptake mechanisms (15, 63). Further study has evidenced that amperozide carries binding affinity towards dopamine transporter (49). In present studies, we found amperozide administration alone could not induce locomotor activity (data not shown). This finding could explain why amperozide can, but not ritanserin, block nomifensine-induced behavioral activation. That is, amperozide might compete with nomifensine at the dopamine transporter site to decrease nomifensine-induced locomotor activity.

Previous studies indicate that amperozide displayed a greater ability to enhance dopamine efflux in the medial prefrontal cortex (mPFC) than in NA, possibly due to regional discrepancy between ratios of serotonin 5-HT_2 *vs*. dopamine D_2 receptors (33).

This anatomical difference provides platform to discriminate the efficacy of typical *vs*. atypical antipsychotics since the latter carry dual 5-HT_2/D_2 receptor binding while the former target only on D_2 receptor. Hence, atypical antipsychotics could eliminate the negative symptoms of schizophrenia due to its ability to elevate dopamine efflux in the mPFC (33). In addition, atypical antipsychotics were claimed to elicit less of extrapyramidal syndrome (EPS) as compared to typical antipsychotics (*i.e.* haloperidol or sulpiride), possibly due to its limbic selectivity (56). In addition to the effect of amperozide on amphetamine-induced locomotion, the present study demonstrated that amperozide attenuated both amphetamine and nomifensine induced rearing, but not sniffing behavior. Previous studies suggested that ambulatory behavior is mediated primarily by an increase in dopamine neurotransmission in the NA (30, 32). However, the dopamine neurotransmission in nigrostriatal pathways is involved in amphetamine-induced stereotyped behaviors that include sustained or repetitive rearing and sniffing (29, 32). Our results clearly indicate amperozide could suppress psychostimulant-induced hyperactivity in both nigrostriatal and mesolimbic dopamine pathway. In addition, the finding that amperozide could partially suppress the stereotyped behaviors (*i.e.* rearing *vs*. sniffing), though not clear, seems to be consistent with the notion that the drug acts more selective on limbic than motor system.

Further characterization of to the effect of amperozide on nigrostriatal *vs*. mesolimbic dopamine system by using amphetamine-induced rotation in lesioned animals, draw a similar conclusion. First, when animals received a unilateral radiofrequency lesion to either the CP or NA, they displayed enhanced rotations to challenged amphetamine. Previous reports with classical treatment of an indirect agonist (*e.g.*, amphetamine) in unilateral lesioned rats result in rotation towards the lesion side (ipsilateral turns) by reason of imbalance dopamine content (60). Our study found that amphetamine (2.0 mg/kg, i.p.) triggered both ipsilateral and contralateral rotation, with much stronger ipsilateral turns than contralateral turns. This result is compatible with previous reports. The administration of amperozide antagonized amphetamine-induced ipsilateral and contralateral rotations in CP but only ipsilateral rotations in NA lesioned rats. Those findings support our previous argument that amperozide might display different potency on nigrostriatal and mesolimbic dopamine modulation. Nevertheless, the present result agrees with an early study's findings (5) but further implicates a subtle difference in regional response that amperozide appears to regulate tightly in the NA than CP.

It has been reported previously that amperozide by itself would not produce catalepsy (21). The present study further demonstrated that amperozide at 10 mg/kg attenuated the haloperidol-induced catalepsy. A paradoxical result that 5-HT_2 agonist DOI also reversed the haloperidol-induced catalepsy agrees with an early report by Hicks (23), possibly due to the fact that both DOI and amperozide could increase extracellular dopamine in CP and NA (4, 25, 37). It has been suggested that 5-HT_2 antagonism may reduce EPS liability due to a tonic serotonin inhibition, deriving from the dorsal raphe, consequently results in a disinhibition on nigrostriatal dopamine neurons (31). In support of this hypothesis, the administration of amperozide enhanced dopamine efflux in both CP and NA in our studies; again, it is compatible with previous report (11). However, the administration of another 5-HT_2-antagonist-ritanserin 90 min after haloperidol (exhibits clear cataleptic behaviors) could not suppress haloperidol-induced catalepsy. This suggested a critical time window for ritanserin action since same drug given 30 min prior to haloperidol significantly reduced the neuroleptic-induced catalepsy (38). This anti-cataleptic effect of ritanserin was also linked to an alteration in striatal dopamine release (36). Interestingly, our results indicate that depletion of 5-HT with *p*-CPA would not affect the effect of amperozide on extracellular dopamine elevation in both CP and NA. We thus speculate that amperozide attenuated haloperidol-induced catalepsy possibly results from increasing extracellular dopamine level and directs 5-HT_2 antagonism on both nigrostriatal and mesolimbic presynaptic dopamine terminals.

We conclude that the modulatory effect of amperozide on forebrain dopamine system depends not only on its 5-HT_2 antagonistic actions but also on the blockade of both dopamine D_2 receptor and transporter. Amperozide acts selectively on limbic system to be a candidate of atypical antipsychotic drug. Alternatively, its potent effect on nigrostriatal system, *i.e.* reverse haloperidol-induced catalepsy implicates amperozide would combat the EPS syndromes. The global effect on both nigrostriatal and mesolimbic (or mesocortical, as reported from previous studies) dopamine systems suggested amperozide potential applications to multiple disease status, such as drug addiction, psychosis and Parkinson's Disease.

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