Biphasic firing response of nucleus accumbens neurons elicited by THPB-18 and its correlation with DA receptor subtypes[1]

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KEY WORDS berberine; dopamine D1 receptors; dopamine D2 receptors; nucleus accumbens

ABSTRACT

AIM: To investigate the possibility whether THPB-18 (*l*-12-shloroscoulerine) possesses the D_1 agonist-D_2 antagonist action on meso-accumbens-mPFC DA system. **METHODS:** Single unit spontaneous firing activity was recorded in the nucleus accumbens (NAc) neurons of naïve and unilateral-6-hydroxydopamine (6-OHDA)-lesioned Sprague-Dawley rats. The effects of drugs applied intravenously or iontophoretically were determined by the change of firing rates. **RESULTS:** Under normal conditions, the systemic administration of THPB-18 produced a decrease-increase biphasic firing pattern in the NAc neurons during cumulative doses. High dose of THPB-18 was capable of reversing the inhibition induced by both D_2 agonist LY171555 and D_1/D_2 agonist APO on NAc firing activity. Spiperone pretreatment could not block the high dose of THPB-18-induced firing rate increase, which was reversed by the D_1 selective antagonist SCH23390. The tested NAc neurons were effectively inhibited by iontophoretically applied THPB-18 in 90 % of 6-OHDA-lesioned rats, while THPB-18 caused variable effects on the firing of NAc neurons in the neurons of unlesioned rats. The inhibitory effect of THPB-18 was blocked by iontophoretic application of SCH23390, but not D_2 antagonist spiperone. **CONCLUSION:** Similar to *l*-stepholidine, THPB-18 also possesses the "D_1 agonistic-D_2 antagonistic" dual action on the VTA-NAc DA system.

INTRODUCTION

Our previous studies have demonstrated that (-)-stepholidine (SPD), a leading compound of the tetrahydroprotoberberines (THPB)[1,2], acts as a D_1 agonist-D_2 antagonist in the nigro-striatal dopamine (DA) system[3,4]. Moreover, it has been shown that SPD selectively produced a depolarization inactivation (DI) of the DA neurons in the ventral tegmental area (VTA, A_{10}), but not in the substantia nigra pars compacta (SNC, A_9) DA neurons, and behaves with a low incidence of extrapyramidal side-effects[5,6], which revealed that SPD possesses some atypical neuroleptic properties. Recently, we also observed that SPD could produce a biphasic effect (decrease followed by increase) on the firing activity of nucleus accumbens (NAc) neurons. The biphasic effect was closely related to its antagonistic action to D_2 receptors and agonistic effect to D_1 receptors, respectively in the meso-accumbens DA system[7].

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Structurally, THPB-18 and SPD both belong to dihydroxy-THPB. THPB-18, chemically synthesized based on the pharmacological studies of SPD, has more potent action on DA receptors[8,9] as well as protective action on DA neurons[10] as previous studies showed. Since THPB-18 also acts as a D_1 agonist-D_2 antagonist in the nigro-striatal DA system, it is necessary to reveal its action in the meso-accumbens-mPFC DA system which is involved in the pathogenesis of schizophrenia. Thus, the present study was carried out to investigate the possibility whether THPB-18 possessed the D_1 agonist-D_2 antagonist action on meso-accumbens-mPFC DA system.

MATERIALS AND METHODS

Rats and unilateral MFB lesion Sprague-Dawley rats (male, 170±10 g, Grade II, Shanghai Experimental Animal Center, Certificate No 005 conferred by Animal Management Committee, Chinese Academy of Sciences) were used. Rats for lesion of forebrain bundle (MFB, coordination: AP -4.3 mm, ML 1.5 mm, DV -7.9 mm according to the rat Atlas)[11] were anesthetized with pentobarbital (40 mg/kg, ip), and then 4 μL of 6-OHDA-HBr (Sigma, 8 μg 6-OHDA free base, in 0.1 % ascorbic acid and 0.9 % NaCl solution) was injected into the right side SNC as described previously[12]. One month later, the lesioned rats were screened according to their response to SPD (Shanghai Insititute of Materia Medica, CAS) and D_1/D_2 mixed agonist apomorphine (Shenyang Pharmaceutical Co, APO). Only those rats showing steady contralateral rotation (more than 6 turns per min) to both APO (0.2 mg/kg, ip) and SPD (4 mg/kg, ip) were used in the subsequent experiments.

Single-unit recording The animal pre-treatment and the electrophysiological recording techniques have been detailed elsewhere[13]. Briefly, single-barrel glass electrode (filled with 2 mol/L NaCl containing 2 % Pontamine Sky Blue dye, impedance measured 4-9 MΩ at 60 Hz *in vitro*) was passed through a small burr hole drilled in the skull at the level of the NAc (AP+1.4-2.2 mm, ML 0.8-1.5 mm, V -5.5– -7.5 mm) with a pulse-motor microdriver (Narishige, Japan). Electrical signals were amplified and displayed on a storage oscilloscope (Nicolet 2090-1, USA), then led into a window discriminator and an audio monitor. A computer was used to collect the firing activity and constructed the firing rate histogram on-line. The NAc neurons were identified by the following electrophysiological characteristics[14]: 1) spontaneous firing rate, typically between 0.5-8 Hz; 2) apparent biphasic waveforms, most (approximately 90 % of the NAc neurons) exhibiting negative/positive responses with a short duration of 0.5-1.4 ms (Type I cell) and the others exhibiting positive/negative with a duration of 1.0-2.5 ms (Type II cell), only Type I neurons were tested in this study; 3) periodic bursts that are separated by long silent period.

Microiontophoresis A five-barrel micropipette was pulled (Narishige PE-2, Japan) and broken mechanically to an overall tip diameter of 10-18 mm, adjacently glued with a single-barrel recording electrode, which extended 10-20 mm beyond the drug pipette tip. The impedance of the iontophoretic barrels was between 20 and 70 MΩ. One of the iontophoretic barrels was filled with a 2 mol/L NaCl solution for automatic current balancing, and one barrel was always filled with *l*-glutamic acid monosodium (Sigma, Glu, 50 mmol/L in 10 mmol/L NaCl, pH 8) for activating the quiescent NAc neurons. The remaining three iontophoretic barrels contained different drug solutions (all at pH 4): DA (Sigma, 20 mmol/L), SKF38393 (RBI, 10 mmol/L), LY171555 (RBI, 10 mmol/L), SCH23390 (RBI, 30 mmol/L), spiperone (RBI, 30 mmol/L, dissolved in *NN*'-Dimethyl formamide solution) or THPB-18 (30 mmol/L). The drugs were iontophoretically applied using a five-channel Neuro Phore System (Medical Systems Corp, USA; Model BH-2).

A positive current of 10-80 nA was passed through the selected barrel(s) for drug ejection except the glutamic acid. In some rats anesthetized with chloral hydrate, quiescent NAc neurons were activated to fire (2-8 spikes/s) by iontophoretic administration of Glu (negative ejection currents up to 10-30 nA). Retaining currents (positive for Glu and negative for others) of 8-10 nA were applied to drug-containing barrels between ejection periods.

Determination of drug effects on NAc neurons The responses of NAc neurons to iv or microiontophoresis of drugs were expressed as the percentage of firing rate (raw data, ie, spikes/s) at the last 60 sec during the drug application of each dose. The basal firing (less than about 20 % of average rate) was recorded at least 3-5 min before the onset of drug administration. The DA agonists and the tested drug were applied continuously on a dose regimen in which each dose (or iontophoretic current) was doubled every 2 min. Antagonists were usually given in a single dose, which was chosen to insure maximum efficacy

as determined in previous experiments. For glutamate-activated neurons, glutamate was automatically ejected with a pulse paradigm in 30 s 'on' / 30-40 s 'off' period, and their responses to iontophoretic tested drugs were compared with the amplitude of glutamate-induced firing. A total of 1-5 NAc neurons in each rat were tested using microiontophoresis, only one cell was tested for iv administration.

Histology At the end of each experiment, the final recording site was marked by passing a cathodal current (30 mA, 20-30 min) through the recording electrode to deposit a discrete spot of Pontamine Sky Blue dye. The rats were then perfused transcardially with 0.9 % NaCl followed by 10 % buffered formalin. The brains were removed and stored in formalin for at least 48 h until the serial frozen sections of 40 mm thickness could be made and stained with cresyl violet and neutral red. The dye spot was verified using a light microscope.

Statistics The data were expressed as mean±SD. One-way analysis of variance (ANOVA) and an unpaired *t*-test were used for statistical analysis. $P<0.05$ was accepted as statistical significant.

RESULTS

All the tested NAc neurons in the control group (n=37) showed a firing rate of 2.4±0.7 spikes/s with a short-duration of action potential 1.3±0.4 ms and displayed an irregular pattern of activity with bursts of 2 to 6 action potentials.

Effects of THPB-18 and DA agonists on the spontaneous firing of NAc neurons THPB-18 (iv) produced a consistent biphasic action, ie, with the increasing cumulative doses, it produced a decrease followed by an increase on the firing activity (called decrease-increase response) of NAc neurons (Fig 1, n=8). At the low cumulative doses of THPB-18 (0.125–0.5 mg/kg, iv), the maximum decrease was -55.3 %±11.3 % against baseline (range -15.4 %– -100 %, $P<0.01$); and at the high cumulative doses (1-2 mg/kg), THPB-18 showed a maximum increase of 199.9 %±57.9 % (range 45.3 %–458.2 %, $P<0.01$) (Fig 2).

Interestingly, in contrast to THPB-18, selective D_2 agonist LY171555 (iv) caused an 'increase to decrease' biphasic response in the NAc neurons tested during the cumulative doses (Fig 3A, n=5). At the low cumulative doses of 0.025–0.2 mg/kg, LY171555 exhibited an increase of firing by 258.9 %±42.0 % *vs* baseline (range

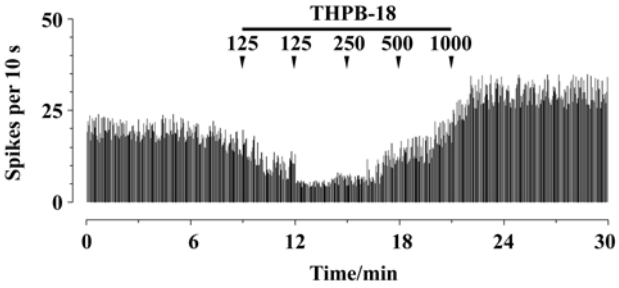

Fig 1. Examples of the effects produced by cumulative doses of THPB-18 tested on NAc neurons recorded from chloral hydrate-anesthetized rats. Arrows indicate time of injection. Numbers above arrows indicate dosages expressed in μg/kg, iv. Note the biphasic (decrease-increase) firing pattern elicited by THPB-18.

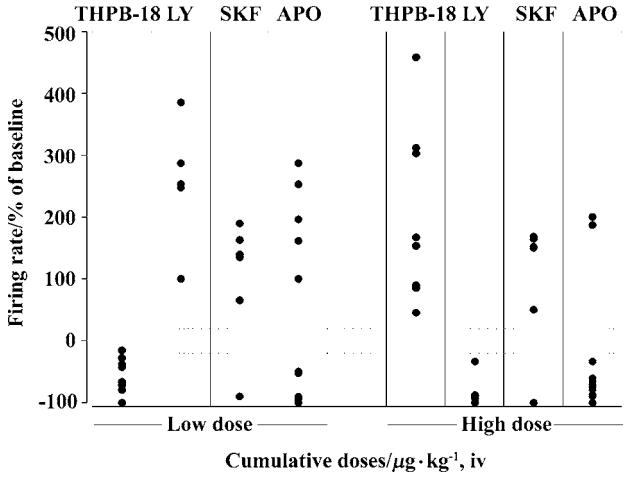

Fig 2. Effects of THPB-18, SKF-38393 (SKF), LY171555 (LY) and apomorpine (APO) on the firing rates of NAc neurons. The range of responses (<20 % of baseline) indicated by the dotted line, was considered to be of no significant change. Black points on each column represented, respectively, the maximum response of single neuron during drug administration (iv).

100 %-385.3 %, $P<0.01$), and followed by a decrease (-80.8 %±11.0 % of baseline, range -33.4 %– -100 %, $P<0.05$) at the high cumulative doses (Fig 2). However, SKF38393 (D_1 selective agonist) produced a relatively consistent increasing firing by low (0.025–0.2 mg/kg) and high (0.4–1.6 mg/kg) doses. Most neurons (n=5) had a maximum increase by 146.9 %±15.3 % (range 65.3 %–189.7 %, $P<0.01$) of baseline. Only one neuron showed a decrease charge of -90.1 % of baseline at the low cumulative doses (Fig 2).

Unlike SKF38393 or LY171555, APO (D_1/D_2 agonist) produced various actions, namely excitation or inhibition on the firing of NAc neurons (Fig 2, n=12).

Seven out of 12 neurons showed inhibition significantly (-77.3 %±8.6 % *vs* baseline Fig 3B) at either low or high cumulative doses, but an increase in firing was seen in two tested neurons (186.2 %±7.1 %). The remaining neurons resulted in a biphasic firing pattern just similar to D_2 agonistic effect that an increase was followed by a decrease in firing with increasing cumulative doses.

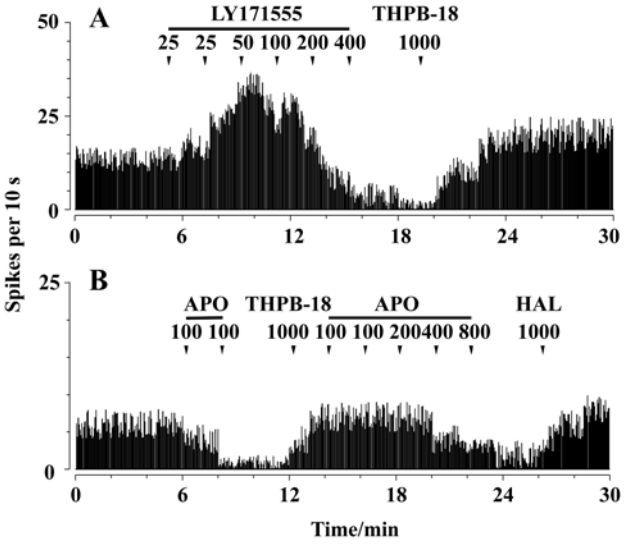

Fig 3. Examples of the reversal effects of THPB-18 on the firing activities of NAc neurons induced by cumulative doses of DA agonist recorded from chloral hydrate-anesthetized rats. A. Biphasic firing of increase-decrease elicited by D_2 agonist LY171555 (0.025–0.8 mg/kg), opposing to THPB-18, and the inhibition was completely reversed by THPB-18 (1.0 mg/kg). B. Neuron firing completely inhibited by APO (0.1–0.2 mg/kg) was fully reversed by THPB-18 (1.0 mg/kg). And the subsequent inhibition at higher doses of APO (0.1–1.6 mg/kg) was still completely reversed by D_2 antagonist haloperidol (HAL, 1.0 mg/kg, iv). Arrows indicate time of injection. Numbers above arrows indicate dosages were expressed in μg/kg, iv.

D_1/D_2 inhibition of NAc neurons firing reversed by THPB-18 When the firing cessation of significant decrease was elicited by injection of D_2 agonist LY171555 (n=5) or D_1/D_2 agonist APO (n=6), the inhibition was completely reversed by a high dose of THPB-18 (1-2 mg/kg, iv) 2-3 min later (Fig 3A, 3B). The reversing rates of THPB-18 were 118.4 %±23.3% to LY171555 and 124.6 %±12.3 % to APO, respectively. However, the subsequent high cumulative doses of APO (0.1-1.6 mg/kg, iv) was still capable of reducing firing rate of NAc neurons (Fig 3B). These results suggest that THPB-18 could antagonize the function of D_2 receptors on the NAc neurons.

However, high dose of THPB-18 (1-2 mg, n=6) also had a reversing effect on D_1 agonist SKF-38393 induced NAc neuron firing activity (either exciting or inhibition, Fig 4A, 4B). The reversing rate of THPB-18 was 123.6 %±16.9 % *vs* baseline (P<0.05). The effect is similar to D_1 antagonist SCH-23390, suggesting THPB-18 also possesses an antagonism to D_1 agonist.

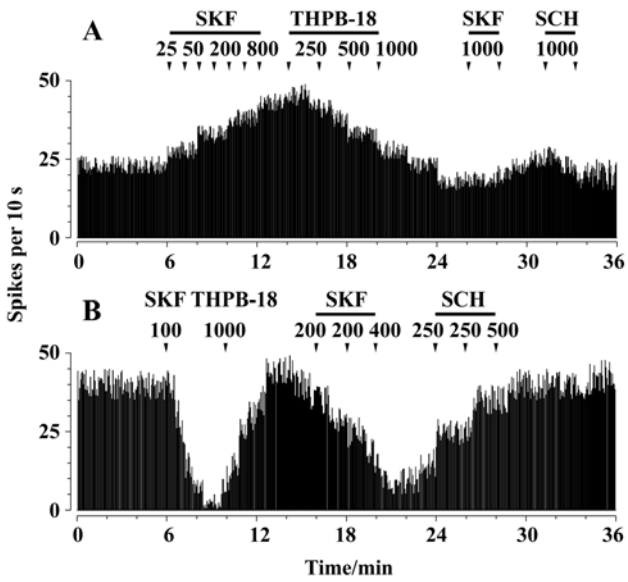

Fig 4. Examples of the reversal effects of THPB-18 on the firing activities of NAc neurons induced by D_1 agonist SKF-38393 recorded from chloral hydrate-anesthetized rats. A. A cumulative doses (0.025–1.6 mg/kg) of SKF-38393 produced an increment of NAc neuron firing which was inhibited by THPB-18 (0.25–2 mg/kg). B. The inhibition of NAc neuron firing elicited by SKF-38393 (0.1 mg/kg) was also reversed by THPB-18 (1.0 mg/kg) and the selective D_1 antagonist SCH-23390 (0.25–1 mg/kg). Arrows indicate time of injection. Numbers above arrows indicate dosages expressed in μg/kg, iv.

Effect of THPB-18 on NAc neurons pretreated with a D_1 or D_2 antagonist Unlike THPB-18-induced biphasic firing response, the selective D_2 antagonist spiperone (0.5-2 mg/kg, iv) *per se*, produced a mild inhibition of NAc firing (-44.9 %±9.2 % of baseline, P<0.01, n=5), lasting for 10-20 min, then followed by a slight recovery (Fig 5A, inner box). However, when the rats (n=5) were pretreated with spiperone (0.5 mg/kg, iv), THPB-18 still increased the NAc firing activity significantly by -28.6 %±8.0 % *vs* pre-THPB-18 (P<0.01) at cumulative doses (0.5-2 mg/kg) (Fig 5). Furthermore, the increase of SPD could be reversed by the D_1 selective antagonist SCH23390 (1-2 mg/kg, iv) to the baseline

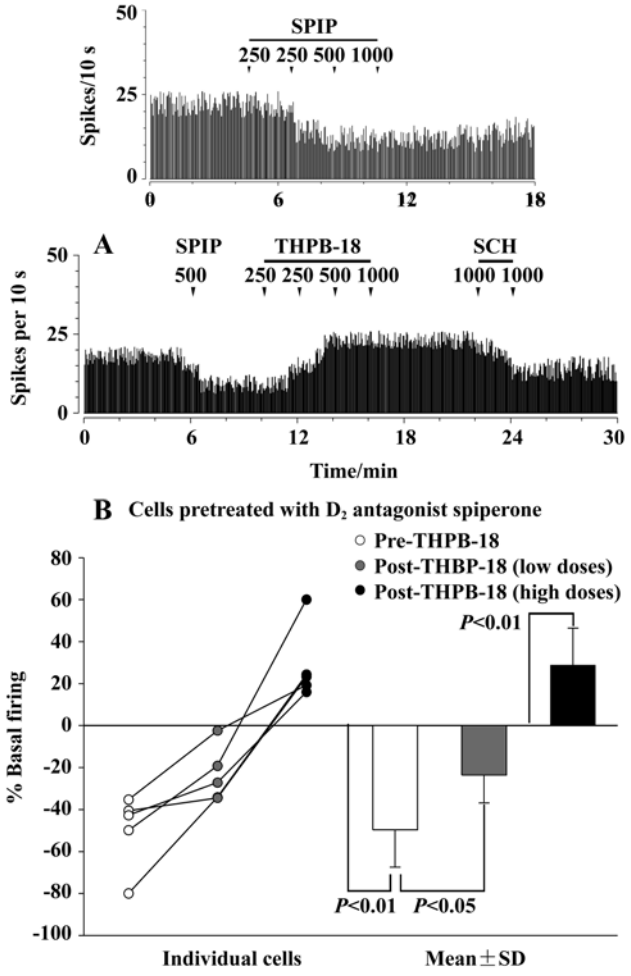

Fig 5. Effect of THPB-18 (iv) on the firing activity of NAc neuron in rats with pre-blockade of D_2 receptors. A. Histogram showing the enhancing effect of THPB-18 during cumulative doses on a NAc neuron pretreated with D_2 antagonist spiperone (0.5 mg/kg, iv), and the subsequent enhancement was back to baseline by D_1 antagonist SCH 23390 (2 mg/kg). The upper box shows the effect of spiperone *per se*, on NAc firing with cumulative doses. B. Graph showing the firing augmentation induced by THPB-18 (low doses: 0.0625-0.25 mg/kg, high doses: 0.5–2 mg/kg) of five NAc neurons in rats pretreated with spiperone (0.5 mg/kg, iv).

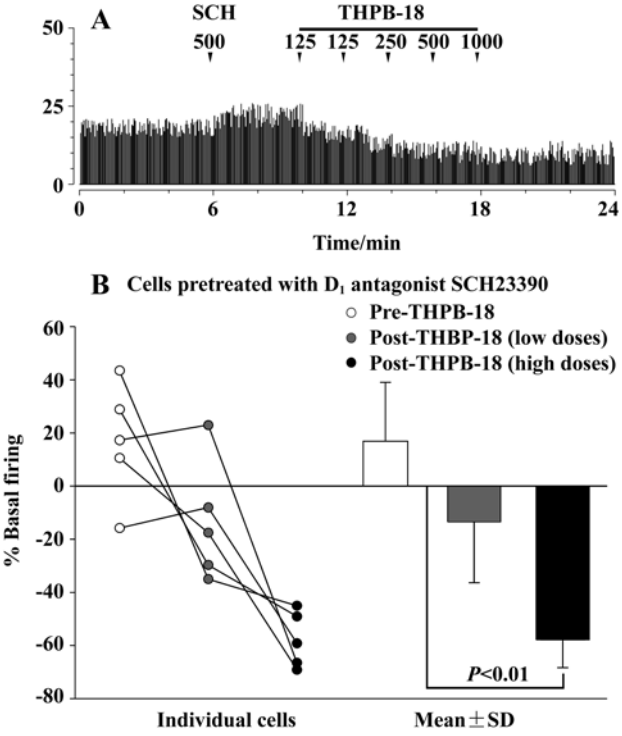

Fig 6. Effect of THPB-18 (iv) on the firing activity of NAc neuron in rats with pre-blockade of D_1 receptors. A) Histogram showing the inhibitory effect of THPB-18 with cumulative doses on a NAc neuron pretreated with D_1 antagonist SCH-23390 (0.5 mg/kg, iv). Arrows indicate time of injection. Numbers above arrows indicate dosages expressed in mg/kg, iv. B) Graph showing the firing decrease induced by THPB-18 (low doses: 0.0625–0.25 mg/kg, high doses: 0.5-2 mg/kg) of five NAc neurons in rats pretreated with SCH-23390 (0.5 mg/kg, iv).

(Fig 5A). In contrast, in the rats (n=5) pretreated with SCH23390 (0.5 mg/kg, iv), the firing rate of NAc neurons did not show any significant change, but showed an inhibition of 57.8 %±4.7 %, P<0.01 *vs* baseline) after injection of THPB-18 at high cumulative doses (0.4-2.0 mg/kg, Fig 6). These results imply that the excitatory effect of THPB-18 on NAc firing resulted from its D_1 agonistic action after deprivation of the D_2 antagonistic effect of SPD by spiperone.

Microiontophoretic effects of THPB-18 on the firing of NAc neurons in 6-OHDA-lesioned and unlesioned rats In all the unlesioned rats, the iontophoresis of THPB-18 (20 mmol/L, 10-80 nA, n=10) caused variable effects (P>0.05) on the firing activity of NAc neurons (Tab 1). The majority of neurons (70 %) were not influenced by THPB-18 even with long ejection periods, and a part of neurons showed both inhibition (1/10, or 10 %) and excitation (2/10, or 20 %) when THPB-18 was ejected with high currents (40-80 nA). However, iontophoresis of either SKF38393 (n=6) or LY171555 (n=6) produced a very significant reduction of NAc firing (Fig 7) in the unlesioned rats. The inhibited rates of the tested neurons were 83.3 % (5/6) to SKF38393 (P<0.01 *vs* THPB-18) and 66.6 % (4/6) to LY171555 (P<0.01 *vs* THPB-18), respectively.

Interestingly, in the 6-OHDA-lesioned rats, the tested NAc neurons were effectively inhibited by iontophoretically applied THPB-18 at approximately

Tab 1. Effects of iontophoretically applied THPB-18 on the firing activity of NAc neurons in the 6-OHDA lesioned rats.

	Inhibition	No effect	Excitation	Total
Unlesioned	1*(10.0 %)	7 (70.0 %)	2 (20.0 %)	10
6-OHDA lesioned	9 (90.0 %)	1 (20 %)	-	10

* The number of NAc neurons responding to microiontophoretic administration of THPB-18 (inhibition, excitation, and no effect).

90 % (9/10) of the cases (Tab 1). In comparison with the unlesioned group (including the untreated control and the saline treated rats), the response of NAc neuron firing was more sensitive to the inhibition induced by iontophoresis of THPB-18 in the 6-OHDA-lesioned rats (P<0.01 *vs* control, Fig 8).

In order to examine whether the inhibitory effect of THPB-18 was related to its possible agonistic action on DA receptors in the 6-OHDA-lesioned rats, D_2 and D_1 antagonists were applied iontophoretically with high

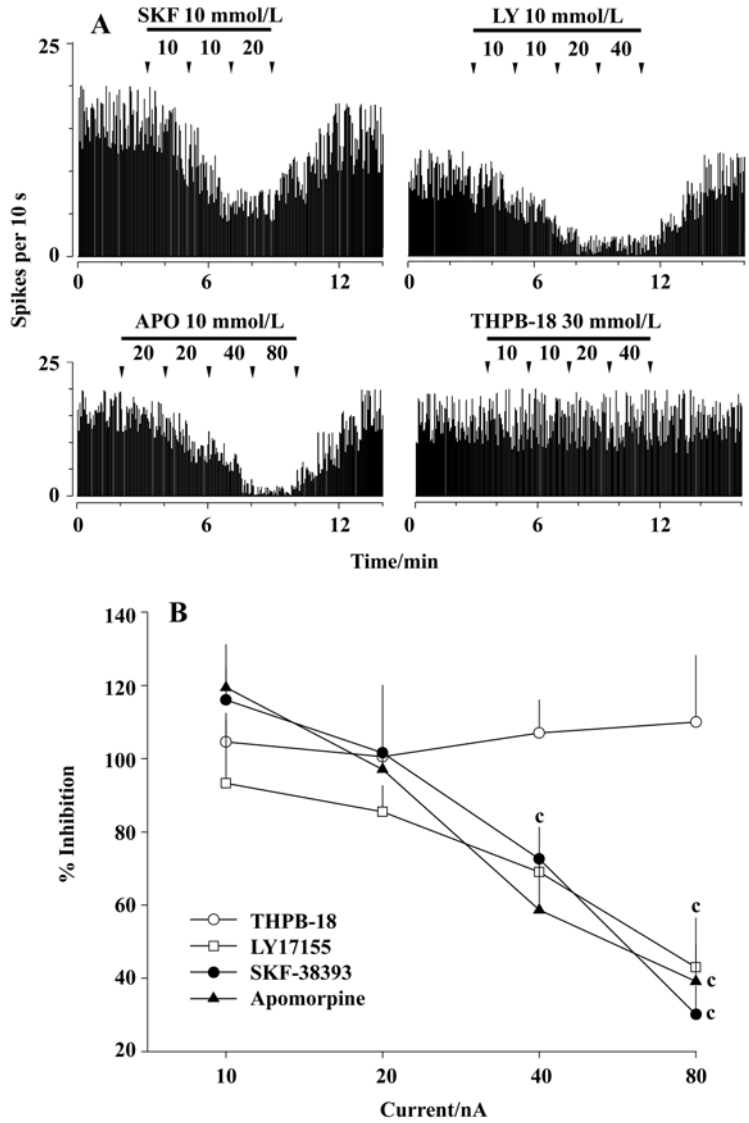

Fig 7. A) Firing histograms showing inotophoretic application of DA agonists and THPB-18 with cumulative doses (10-160 nA) on each NAc neuron in unlesioned rats. Arrows indicate time of injection and cessation. Numbers above arrows indicate current expressed in nA. B) Current-response curves illustrating the inhibition of iontophoretically ejected DA agonists (SKF-38393 for D_1, LY171555 for D_2, and D_1/D_2 agonist apomorphine, all were 10 mmol/L) on NAc neuron firing in the unlesioned groups, which were significantly more potent for the inhibition on NAc neuron activity than THPB-18 (30 mmol/L, ^cP<0.01).

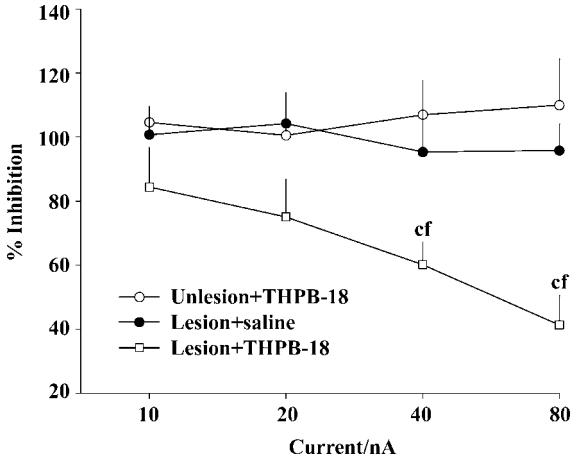

Fig 8. Current-response curves showing the different effects produced by iontophoretic administration of THPB-18 on NAc neurons in the 6-OHDA-lesioned and unlesioned rats. The activity of NAc neurons recorded in 6-OHDA rats were significantly more sensitive to the inhibitory effects induced by THPB-18 (30 mmol/L, 40-80 nA) as compared to either untreated or saline-treated rats. (^{c}*P*<0.01 *vs* untreated rats. ^{f}*P*<0.01 *vs* saline-treated rats)

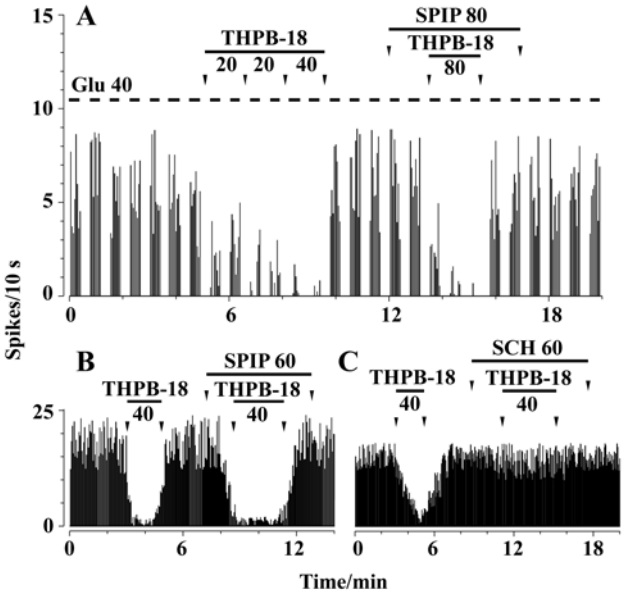

Fig 9. Representative cumulative rate histograms showing the inhibitory effects of iontophoretically ejected THPB-18 (30 mmol/L) on the glutamate (Glu, 40 mmol/L)-induced firing (A) and spontaneously active firing (B, C) of three NAc neurons in the 6-OHDA lesioned rats. The pre-iontophoretic application of spiperone (SPIP, 30 mmol/L) failed to block the inhibition of simultaneously ejected THPB-18 (A, B), while the inhibitory effect of THPB-18 was blocked by pre-iontophoretically applied SCH (30 mmol/L) (C). Arrows indicate time of injection and cessation. Numbers above arrows indicate current expressed in nA.

currents (40–80 nA) prior to the ejection of THPB-18. Iontophoresis of the D_2 antagonist spiperone (30 mmol/L, *n*=4) failed to prevent the THPB-18-induced firing suppressive effects on both glutamine-activated (Fig 9A) and spontaneously active NAc neurons (Fig 9B). On the contrary, this inhibitory effect of THPB-18 was blocked by iontophoretic application of D_1 antagonist SCH23390 (30 mmol/L, *n*=4, Fig 9C). These results suggest that THPB-18 possesses the D_1 agonistic action on NAc neurons firing in the 6-OHDA lesioned rats.

DISCUSSION

The nucleus accumbens (NAc), a forebrain structure that receives a dense innervation from meso-limbic DA neurons (A_{10}) in the ventral tegmental area (VTA), serves to integrate emotion-related inputs and plays an important role in motor-activation and the reinforcement effects of psychostimulant drugs[15]. The majority of psychotropic drugs such as neuroleptics exert their therapeutic action by depressing the DA activity in meso-limbic dopamine system especially in the NAc. Based on recent clinic and experimental studies, the pathogenesis of schizophrenia may involve the dysfunction of D_1 receptors (abnormally low DA activity) of prefrontal cortex (PFC), accompanied by secondary D_2 hyperactivity of subcortical regions such as the NAc, which may then produce some of the schizophrenia symptoms[16-19]. Hence, there are considerable findings to suggest that the strategy of exciting the D_1 receptors and blocking the D_2 receptors on the meso-mPFC-NAc DA system should contribute to the schizophrenia therapy.

As showed by our previous studies, SPD exerts D_1 agonitstic-D_2 antagonistic dual actions in meso-mPFC-NAc DA system[7,20]. The present studies first showed that the systemic administration of THPB-18, similar to SPD, also produced a decrease-increase biphasic firing pattern in the NAc neurons during cumulative doses, and its increase firing phase (excitatory effect) was predominant and lasted for a longer period, especially at high doses (Fig 1). Further experiments showed that THPB-18 was capable of reversing the inhibition induced by both D_2 agonist LY171555 and D_1/D_2 agonist APO on NAc firing activity, suggesting that THPB-18 might have an antagonistic action to D_2 receptors in the meso-limbic DA system. Moreover, according to the other reports[21], it has been suggested that the biphasic firing activity of the NAc neurons elic-

ited by DA or D_2 agonists should consists of two ingredients: the precedent one is associated with the somatodendritic D_2 autoreceptors in the VTA (A_{10}) DA neurons innervating the NAc, and the sequential one is directly from the D_2 receptors in the NAc. As the D_2 autoreceptors are more sensitive (3-10 fold) to DA agonists than the D_2 receptors within the NAc neurons, the low doses of DA or D_2 agonists (such as LY171555) preferentially inhibit the firing activity of VTA DA neurons mediated via the D_2 autoreceptors, and thereby decrease the release of DA from the nerve endings in the NAc, finally results in the increase of firing activity in the NAc neurons due to the disinhibitory effect from VTA DA neurons. In this view, the present results of LY171555 (iv) that it initially increased the firing activities of most NAc neurons at the low cumulative doses (Fig 2, 3A) could be explained. Similarly, it could be presumed that the low cumulative doses of THPB-18 depressed the NAc firing activity owing to its antagonistic action to the D_2 autoreceptors in the VTA DA neurons. In other words, to antagonize the D_2 autoreceptors in the VTA DA neurons could facilitate DA release from nerve terminals in the NAc[22], and then reduce or decline the firing activity of NAc neurons indirectly.

Nevertheless, at high cumulative doses, the excitatory effect of THPB-18 on NAc neuron firing should be considered as another factor involved in the action of THPB-18, except its antagonist to D_2 receptors. The results of D_1 selective agonist SKF-38393 supported this sight in the present work, which is consistent with the recent report that DA on D_1 receptor caused an excitatory effect on NAc neuron firing[23]. Furthermore, the increased firing rate of THPB-18 were nearly reversed by the D_1 selective antagonist SCH23390 (Fig 4A), and SCH23390 was capable of preventing THPB-18-induced rate increasing at high concentration, if it was pre-applied. These results indicate that the D_1 agonistic effect of THPB-18 enhances the NAc firing activity consisting of its dual action in the meso-limbic DA system. On the other hand, THPB-18 may be enough to block the D_2 receptors in NAc neurons besides VTA DA neurons, but the former blockade (direct disinhibition in the NAc) would counteract the inhibitory effect from the activation of VTA DA neurons via the blockade of D_2 autoreceptors. Thus, it would result in an increased firing activity of NAc neurons in this study. However, the action of D_2 selective antagonist spiperone could not support the above issue, spiperone *per se*, only produced mild inhibition of NAc neurons. It seldom displayed a biphasic firing pattern or an enhancement. When the pre-treatment with spiperone occluded the D_2 receptors in VTA DA neurons and NAc neurons, the intravenously applied THPB-18 still increased the firing rate of NAc neurons yet. So, the D_1 agonistic action of THPB-18 should be taken as an ultimate factor involved in the excitatory firing activity of the NAc neurons.

Similar to the action of THPB-18 in the nigro-striatum DA system, the iontophoresis of THPB-18 into the NAc in the 6-OHDA-lesioned VTA of rats caused almost all the tested neurons (90 %) to be inhibited, which can be completely blocked by the ejection of D_1 antagonist SCH-23390, but not D_2 antagonist spiperone, clearly indicating that THPB-18 possesses the D_1 agonistic property under the 6-OHDA-lesioning condition. However, it seemed that THPB-18 had no a definite direct D_1 agonistic effect in the NAc of unlesioned rats. We hypnotized that THPB-18 might preferentially act on the medial prefrontal cortex (mPFC) and exert its indirect D_1 agonistic action through the glutamatergic efferents projected into the NAc[24]. Thus, the mPFC would regulate the subcortical activities in the NAc.

Considering the current conception that the hyperactivity of the mesolimbic DA system (particularly the meso-NAc DA system) resulted from the hypoactivity of mPFC DA system might play an important role in the pathogenesis of schizophrenia[25,26]. Very recently, we have demonstrated that the D_1 agonistic action of THPB-18 on NAc neurons firing was from the mPFC (unpublished data). The D_1 agonistic action in the mPFC and D_2 antagonistic action in the subcortex are emphasized for the new idea in antipsychotic drug research.

So far as we know, THPB-18 acts much the same way as SPD does in the nigro-striatum, as well as the meso-NAc-mPFC DA system, only that THPB-18 possesses a more potent action on DA receptors[8,9], which would be a more efficient candidate for the potential novel antipsychotic drugs with dual D_1 receptor agonist and D_2 receptor antagonist actions[2]. Yet the underlying mechanisms need to be investigated and elucidated further.

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