

Effects of Perospirone, a Novel Antipsychotic Agent, on the Dopaminergic Neurons in the Rat Ventral Tegmental Area

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ABSTRACT—An electrophysiological study was performed to investigate the effects of *cis-N*-[4-[4-(1,2-benz-isoxazole-3-yl)-1-piperazinyl]butyl]cyclohexane-1,2-dicarboximide hydrochloride (perospirone), a novel antipsychotic agent with high affinities for D₂/5-HT₂-receptors, on the dopaminergic (DA) neurons in the ventral tegmental area (VTA) using chloral hydrate-anesthetized rats. DA neurons and non-DA neurons in VTA were identified according to the configurations of their action potentials and firing rates. Spontaneous firing of DA neurons was dose-dependently decreased by i.v. injection of methamphetamine (MAP). Most non-DA neurons were unaffected by MAP up to 2 mg/kg, but the firing was increased with MAP in 2 of 7 neurons. Perospirone injected intravenously reversed the MAP-induced decrease in spontaneous firing of DA neurons in a dose-dependent manner. In addition, i.v. injection of perospirone also inhibited the MAP-induced increase in firing of the 2 non-DA neurons. Similarly, inhibition of spontaneous firing in DA neurons by microiontophoretically applied DA was antagonized during iontophoretic application of perospirone. However, the firing of non-DA neurons, which were insensitive to DA, was not affected by iontophoretically applied perospirone. Since the DA neurons are inhibited by DA via D₂-receptors, these findings suggest that perospirone acts on the D₂-receptors to antagonize the dopaminergic inhibition of DA neurons in VTA.

Keywords: Electrophysiology, Ventral tegmental area, Methamphetamine, Perospirone, Dopaminergic D₂-receptor

The blockade of central dopamine (DA) D₂-receptors is considered to mitigate positive schizophrenic symptoms (1, 2). Also, the blockade of central 5-hydroxytryptamine 2 (5-HT₂) receptors might ameliorate negative schizophrenic symptoms and attenuate the incidence of extrapyramidal side effects associated with neuroleptic maintenance therapy (3–5). Perospirone (*cis-N*-[4-[4-(1,2-benz-isoxazole-3-yl)-1-piperazinyl]butyl]cyclohexane-1,2-dicarboximide hydrochloride), which differs in chemical structure from analogs of known antipsychotic agents such as butyrophenone, phenothiazine and benzamide derivatives (Fig. 1), is a novel antipsychotic drug. Behavioral and biochemical studies have demonstrated that perospirone exhibits high antagonistic activities for both 5-HT₂- and D₂-receptors (K_i value=0.61 and 1.4 nM, respectively) with only weak cataleptogenic actions in animals (6, 7). Therefore, perospirone may mitigate both positive and negative symptoms without marked extra-

pyramidal side effects in schizophrenic patients. In addition to the 5-HT₂-blocking activity, perospirone, like other atypical neuroleptics (e.g., clozapine), increases DA turnover to a similar extent both in the cerebral cortex and striatum through a D₂-blocking action, whereas typical neuroleptics (e.g., haloperidol) preferentially enhance the striatal DA turnover (8). These properties may also in part account for the lower incidence of extrapyramidal side effects with perospirone than typical neuroleptics.

Ventral tegmental area (VTA) DA neurons are the principal cells forming the mesolimbic and mesocortical

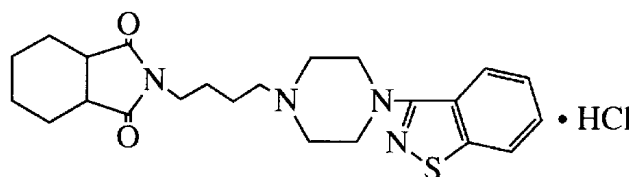


Fig. 1. Chemical structure of perospirone.

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DA systems which are involved in pathogenesis of schizophrenia. The neuronal activity of DA neurons in VTA, at least a part, is regulated by DA acting on auto-receptors, which have the pharmacological characteristics of D₂-receptors (9–18). To elucidate whether or not perospirone has D₂-antagonistic action, in vivo electrophysiological studies were performed using the DA neurons in the VTA of rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–350 g were used; they were housed under standard laboratory conditions with continuous access to food and water. The rats were anesthetized with chloral hydrate (300 mg/kg, i.p.) and injected methyl atropine (0.1 mg/kg, i.v.) for prevention of tracheal secretion. Jugular cannulation was performed only for rats to whom drugs were intravenously injected. Then the animals were fixed in a stereotaxic instrument after tracheal cannulation. A part of the cranium and the dura were removed for insertion of a recording electrode. Thereafter, the animals were immobilized with gallamine triethiodide (200 mg/kg, i.p.) under artificial respiration. All pressure points and surgical wounds were locally anesthetized using 8% lidocaine spray repeatedly throughout the experiment. The electrocardiogram (II lead) and heart rate were continuously monitored to confirm that the animals were free from pain. A supplemental dose (50 mg/kg, i.p.) of chloral hydrate was given to the animal when the experiment extended over 6 hr. Body temperature was maintained at 36.5–37.5°C with a heating pad placed beneath the animal.

Recording

Single neuron activities in the VTA (4.8–5.3-mm posterior to Bregma, 0.5–1.0-mm lateral to the midline, 7.5–8.4 mm from the cortical surface) (19) were extracellularly recorded with a glass-insulated silver wire micro-electrode (electrical resistance: approx. 1–2 MΩ) attached along a seven-barreled micropipette, the outer diameter of which was 6–8 μm. The distance between tips of the recording electrode and micropipette was 20–40 μm. The spontaneous firing obtained in the VTA neurons was displayed on an oscilloscope (VC-10; Nihon Kohden, Tokyo). Then the spontaneous firings of DA neurons and non-DA neurons were continuously recorded with the recticorder (RJG-4124, Nihon Kohden) through the spike counter (DSE-325P; Dia Medical System, Tokyo). After the termination of each experiment, the recording sites were marked by passing a cathodal current of 20 μA for 2 min and then histologically checked by staining with cresyl violet. Further details of the procedures have been

reported elsewhere (16, 17).

Drugs

Intravenous injection of drugs: Methamphetamine hydrochloride (Dainippon Pharm., Co., Osaka) at doses of 0.5, 1.0 and 2.0 mg/kg followed by perospirone hydrochloride (Sumitomo, Osaka; dissolved in isotonic sodium chloride solution) at doses of 0.1, 0.2 and 0.4 mg/kg was intravenously and cumulatively injected every 5 min through the cannule inserted into the jugular vein.

Microiontophoretic application of drugs: Each pipette of the seven-barreled micropipette was filled with 0.2 M DA hydrochloride (Wako Pure Chemical Ind., Osaka; pH 5.5), 0.01 M perospirone hydrochloride (dissolved in distilled water, pH 4.5), 1 M monosodium L-glutamate (Wako Pure Chemical Ind., pH 7.4) and 2 M NaCl. These chemicals were microiontophoretically applied to the immediate vicinity of the target neuron being recorded using a four-channel Micro Constant Current Supply (S-5125B, Nihon Kohden).

Statistics

In the intravenous injection experiment, the mean control number of spontaneous firings in each neuron was calculated from the number of firings recorded for 2 min before drug injection. The mean number of spontaneous firings after drug application in each neuron was calculated from the number of firings recorded for 2 min starting 3 min after injection of each dose of drug. In the microiontophoretic experiment, the mean control number of spontaneous firings in each neuron was calculated from the number of firings recorded for 40 sec before drug application. The mean number of spontaneous firings after drug application in each neuron was calculated from the number of firings recorded for 40 sec starting 20 sec after the iontophoretic application of each dose of drug. The statistical significance of the data was determined by the paired Student's *t*-test.

RESULTS

As previously described by others and us (9–13, 16), DA neurons in the VTA showed an action potential with a notch on the rising spike phase and a long duration of over 2.5 msec and regular low spontaneous firing (0.5–5/sec). Non-DA neurons exhibit an action potential with a short duration and high firing rates (>10/sec). According to these characteristics of the neurons in the VTA, the neurons which showed spontaneous firing rates of less than 10/sec, duration of action potential more than 2.5 msec and a notch on the rising phase of the spike were determined as DA neurons, and other neurons, which have high frequency spontaneous firing of more

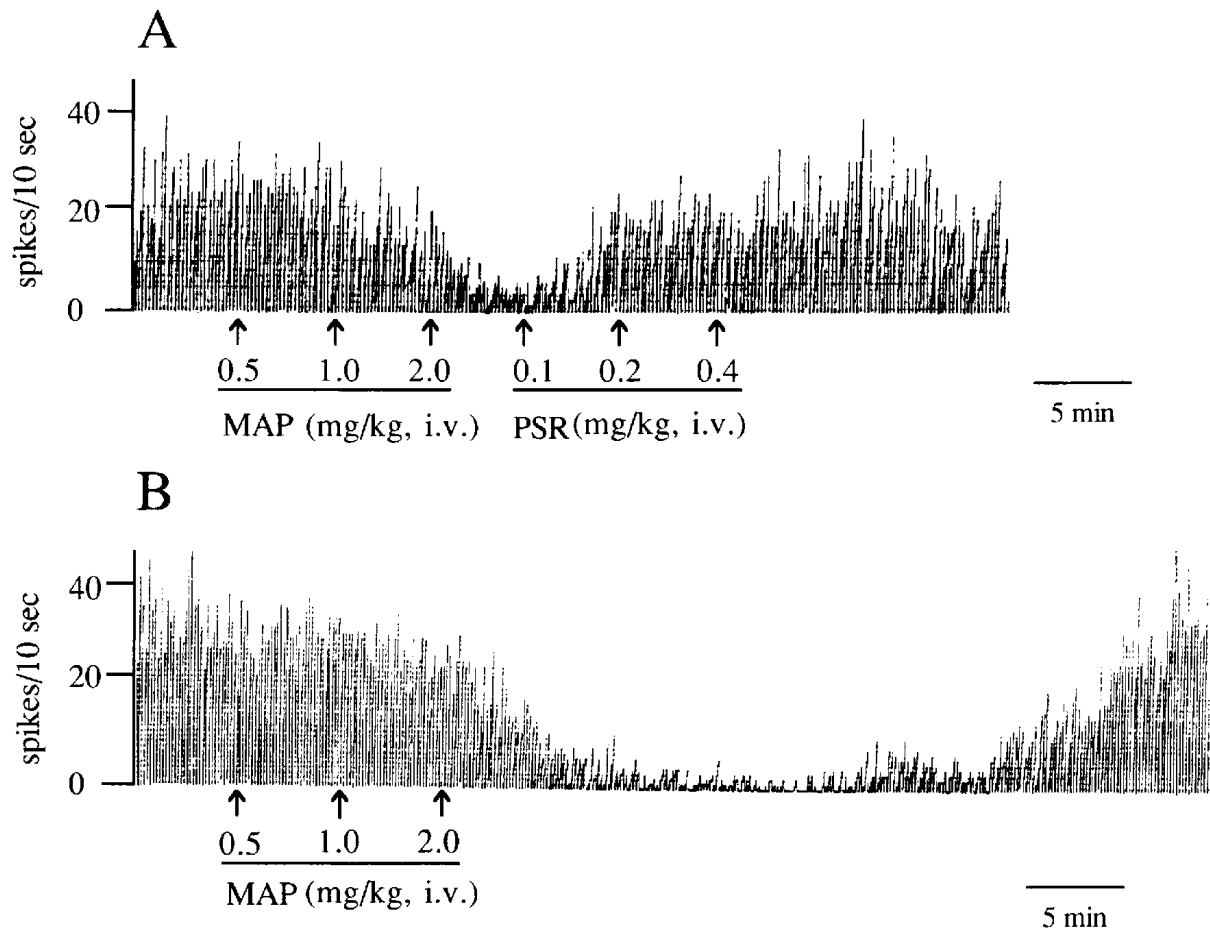


Fig. 2. Effects of intravenous injection of methamphetamine (MAP) and perospirone (PSR) on spontaneous firing of dopamine (DA) neurons in the ventral tegmental area. A: Effects of PSR on MAP-induced inhibition of firing. B: Time course of MAP-induced inhibition. Arrows indicate the intravenous injection of drugs.

Table 1. Effects of intravenous injection of perospirone (PSR) on dopamine (DA) and non-DA neurons in the ventral tegmental area following injection of methamphetamine (MAP)

No. of neurons	Control	MAP (mg/kg, i.v.)			After MAP injection (min)		
		0.5	1.0	2.0	20	25	30
Injection of MAP alone							
DA neurons: 4	28.5 ± 2.2	20.3 ± 2.9** (4/4) ↓	14.8 ± 4.3** (4/4) ↓	9.9 ± 2.4** (4/4) ↓	5.7 ± 0.3** (4/4) ↓	3.8 ± 0.4** (4/4) ↓	2.9 ± 1.1** (4/4) ↓
No. of neurons	Control	MAP (mg/kg, i.v.)			PSR (mg/kg, i.v.)		
		0.5	1.0	2.0	0.1	0.2	0.4
Injection of PSR following MAP							
DA neurons: 15	27.3 ± 2.8	16.9 ± 1.6** (15/15) ↓	11.1 ± 1.1** (15/15) ↓	7.0 ± 1.2** (14/15) ↓	12.9 ± 2.4 [#] (9/15) ↑	13.7 ± 3.6 [#] (9/15) ↑	13.8 ± 3.7 [#] (10/15) ↑
Non-DA neurons: 7	25.7 ± 2.6	30.2 ± 4.5 (0/7)	31.5 ± 4.3 (2/7) ↑	35.0 ± 6.5 (2/7) ^	28.3 ± 4.8 [#] (2/7) ↓	21.8 ± 2.6 [#] (2/7) ↓	18.6 ± 2.8 [#] (4/7) ↓

Each value represents the mean ± S.E. of spikes/10 sec of DA neurons and spikes/sec of non-DA neurons. **P < 0.01, significantly different from the respective control value. [#]P < 0.05, ^{##}P < 0.01, significantly different from the value in the MAP (2.0 mg/kg)-treated group. (): number of neurons, out of the total examined, in which firing was increased (↑) or decreased (↓) by drugs compared to the respective untreated control in the groups treated with MAP only or compared to the MAP (2.0 mg/kg, i.v.)-treated group in the groups treated with PSR after MAP treatment.

than 10/sec, duration of action potential of less than 2.5 msec and no notch on rising spike phase, were considered to be non-DA neurons in the present study.

Effects of perospirone injected intravenously on spontaneous firing of VTA neurons

The effects of perospirone injected intravenously on spontaneous firing were examined in 15 DA neurons. When methamphetamine (MAP) was intravenously administered at doses of 0.5, 1.0 and 2.0 mg/kg every 5 min, a dose-dependent decrease in firing was observed in 14 neurons (Fig. 2A). In the remaining one neuron, the firing was reduced by 0.5 and 1.0 mg/kg of MAP. The mean firing rate (spikes/10 sec) of 15 DA neurons was sig-

nificantly ($P < 0.01$) and dose-dependently decreased from 27.3 ± 2.8 to 16.9 ± 1.6 , 11.1 ± 1.1 , and 7.0 ± 1.2 with 0.5, 1.0 and 2.0 mg/kg of MAP, respectively (Table 1). When perospirone was intravenously applied at doses of 0.1, 0.2 and 0.4 mg/kg every 5 min following injection of MAP, the firing was significantly increased in 9, 9 and 10 of 15 neurons tested, respectively (Fig. 2A). The mean firing number for 10 sec in 15 neurons was significantly ($P < 0.01$) increased to 12.9 ± 2.4 with 0.1 mg/kg of perospirone which was applied after MAP (2.0 mg/kg) injection (Table 1). When the recovery, from the inhibition induced by MAP at doses of 0.5, 1.0 and 2.0 mg/kg, was examined in 4 DA neurons, it was found that it took more than 30 min for firing to return to the control level

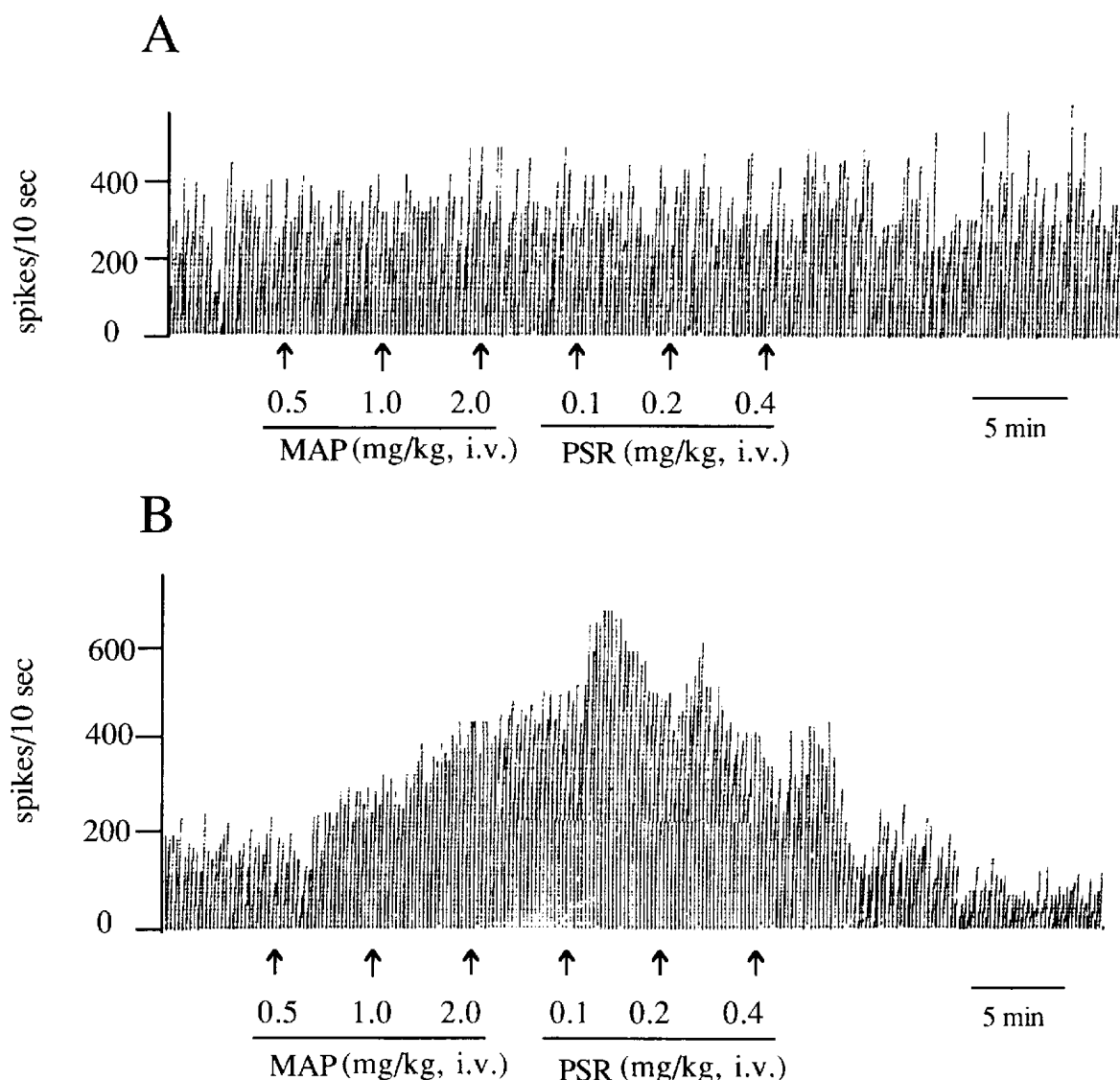


Fig. 3. Effects of intravenous injection of methamphetamine (MAP) and perospirone (PSR) on spontaneous firing of non-dopamine (DA) neurons in the ventral tegmental area. A: PSR had no effect on spontaneous firing of MAP-insensitive neuron. B: PSR inhibited the MAP-induced increase in firing. Arrows indicate the intravenous injection of drugs.

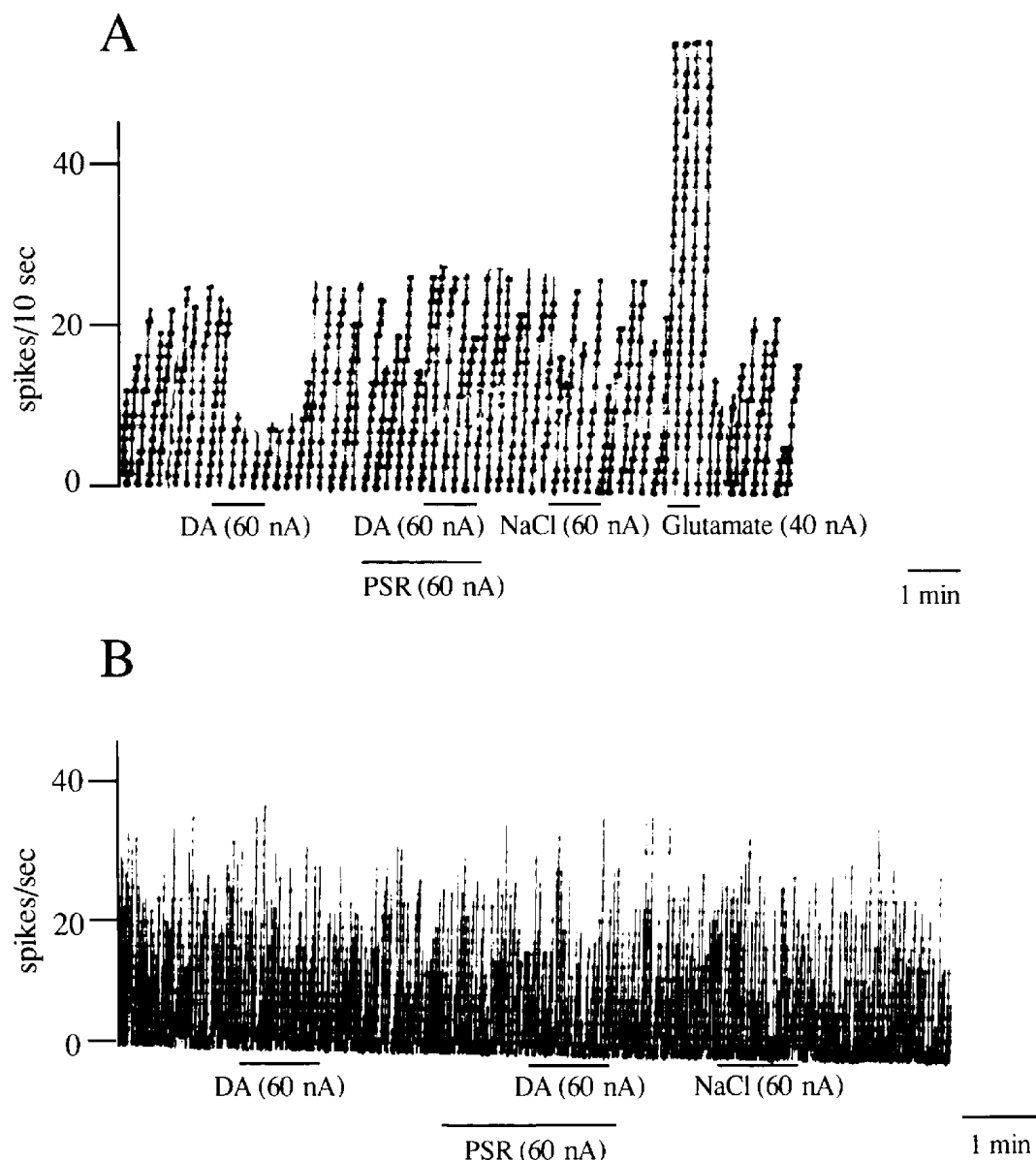


Fig. 4. Effects of iontophoretic application of dopamine (DA) and perospirone (PSR) on spontaneous firing of DA and non-DA neurons in the ventral tegmental area. **A:** Antagonized effects of perospirone (PSR) on DA-induced decrease in firing of DA neuron. **B:** No effects of PSR on spontaneous firing of non-DA neuron. Periods of drug application are indicated by horizontal bars, and the number under the bars show the current in nA.

(Fig. 2B, Table 1).

The effects of MAP and perospirone injected intravenously on non-DA neurons were examined in 7 non-DA neurons. Out of the 7 non-DA neurons, 5 neurons were unaffected by MAP at a dose of up to 2.0 mg/kg (Fig. 3A). However, in 2 non-DA neurons, the firing was increased by MAP at doses of 1.0 and 2.0 mg/kg, although the mean firing rate of 7 neurons remained unaltered (Fig. 3B). When perospirone was injected at doses of 0.1, 0.2 and 0.4 mg/kg 5 min after MAP application, the increased firing in 2 neurons was significantly antagonized

(Fig. 3B). A decrease in firing was observed in 2 out of the other 5 neurons with 0.4 mg/kg of perospirone, although the firing rate of the remaining 3 neurons remained unaltered with perospirone up to 0.4 mg/kg. Thus, the mean firing rate of 7 neurons was significantly ($P < 0.05$) reduced with 0.1, 0.2 and 0.4 mg/kg of perospirone (Table 1).

Effects of perospirone applied microiontophoretically on spontaneous firing of VTA neurons

Microiontophoretic application of DA at a dose of 60

Table 2. Effects of microiontophoretic application of dopamine (DA) and perospirone (PSR) on spontaneous firing of DA and non-DA neurons in the ventral tegmental area

	No. of neurons	Control	DA (60 nA)	PSR	PSR + DA
DA neurons					
PSR (40 nA)	9	24.4 ± 3.3	10.4 ± 1.8** (9/9) ↓	21.4 ± 5.2	17.4 ± 3.2## (7/9) ↑
PSR (60 nA)	8	23.9 ± 3.2	11.6 ± 2.6** (8/8) ↓	17.9 ± 2.9* (2/8) ↓	17.8 ± 3.4# (7/8) ↑
Non-DA neurons					
PSR (40 nA)	4	28.9 ± 6.8	30.0 ± 7.3	30.7 ± 7.5	32.0 ± 8.3
PSR (60 nA)	5	21.5 ± 0.8	21.0 ± 0.4	21.5 ± 0.6	21.8 ± 0.7

Each value represents the mean ± S.E. of spikes/10 sec of DA neurons and spikes/sec of non-DA neurons. * $P < 0.05$, ** $P < 0.01$, significantly different from the respective control value. # $P < 0.05$, ## $P < 0.01$, significantly different from the value in the DA (60 nA)-treated group. (): number of neurons, out of the total examined, in which firing was increased (↑) or decreased (↓) by drugs compared to the untreated control in the groups treated with DA or PSR alone or compared to the DA-treated group in the group treated with both PSR and DA.

nA for 60 sec inhibited the spontaneous firing of all 17 DA neurons tested (Fig. 4A, Table 2). DA (60 nA)-induced inhibition of firing was antagonized by the simultaneous application of perospirone at doses of 40 and 60 nA in 7 of 9 and in 7 of 8 DA neurons tested, respectively, although those of the remaining 2 and one neuron, respectively, were not significantly affected (Fig. 4A). Perospirone alone at a dose of 40 nA did not affect the spontaneous firing in any of the 9 DA neurons tested, but that at 60 nA significantly ($P < 0.05$) decreased the firing in 2 of the 8 DA neurons examined. In contrast, alteration of firing was not observed with microiontophoretic application of DA up to 60 nA or perospirone up to 60 nA in any of the 9 non-DA neurons tested (Fig. 4B, Table 2).

DISCUSSION

Spontaneous firing of the VTA DA neurons was dose-dependently inhibited by i.v. injection of MAP, and the inhibition was reversed by i.v. injection of perospirone. Since DA released by MAP from the dendrites of VTA DA neurons is considered to act on D_2 -receptors of the DA neurons to inhibit the activities (17, 18, 20), perospirone is suggested to block the DA-induced inhibition by acting on the D_2 -receptors (autoreceptors). Microiontophoretically applied perospirone also antagonized the inhibition by microiontophoretically applied DA of the VTA DA neurons. Furthermore, perospirone has high affinity for D_2 , although the affinity for D_1 -receptors is low (21). In behavioral studies, perospirone showed D_2 -receptor antagonistic effects, since it inhibited DA agonist-induced behavior such as MAP-induced hyperlocomotion in rats, apomorphine-induced stereotyped

behavior in rats and climbing behavior in mice (6). The present electrophysiological findings also indicate that perospirone blocks the DA D_2 -receptors involved in the behavioral changes mentioned above.

A decrease in firing was observed in 2 of 8 DA neurons with high doses of perospirone (60 nA) applied microiontophoretically in the present study. This effect does not appear to be due to the agonistic action, since perospirone did not inhibit acetylcholine release but antagonized the quinpirole-induced inhibition of the release evoked by the electrical stimulation (21). However, the possibility that blockade by perospirone of 5-HT₂-receptors in the VTA DA neurons is involved in the decrease of firing, can not be completely excluded, since 5-HT₂-receptors are known to exist in the VTA (22) to mediate an activation of the neurons (23), and perospirone has high affinity and antagonistic activity for 5-HT₂-receptors (6, 7).

In non-DA neurons, the decrease in firing was not obtained with either i.v. injection of MAP or microiontophoretic application of DA, as described previously (16, 17). In contrast, an increase in firing was observed in a few neurons with i.v. injection of MAP. Perospirone injected i.v. also inhibited the MAP-induced increase in firing of non-DA neurons. The effects of i.v. injection of MAP on non-DA neurons in the VTA are probably an indirect action on the neuron: a possible explanation is that the increase in firing of the VTA non-DA neurons is due to inhibition of GABA neurons in the nucleus accumbens by DA released with MAP from the terminal of DA neurons. The GABA neurons suppress the VTA non-DA neurons (24). Perospirone injected intravenously might have also acted on the postsynaptic DA D_2 -receptors of GABA neurons to block the DA-induced inhibition, thereby resulting in the activation of GABA neurons

to decrease firing of the VTA non-DA neurons. The findings that non-DA neurons were not affected by microiontophoretically applied MAP (25) or DA also support that the increase in firing by i.v. injection of MAP in the VTA non-DA neurons is an indirect action on the area outside the VTA via DA D₂-receptors.

In conclusion, perospirone is suggested to act on D₂-receptors of the VTA DA neurons to block the DA-induced inhibitory action.

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