Br. J. Pharmacol. (1993), **110**, 713–718

Enhancement of D_2 receptor agonist-induced inhibition by D_1 receptor agonist in the ventral tegmental area

Toshihiko Momiyama, *[1]Masashi Sasa & Shuji Takaori

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606-01, and *Department of Pharmacology, Hiroshima University School of Medicine, Hiroshima 734, Japan

1 A microiontophoretic study was performed on chloral hydrate-anaesthetized rats to examine the role of D_1 receptors in the ventral tegmental area (VTA) neurones, which are inhibited by autoreceptor and D_2 receptor agonists.

2 Inhibition by microiontophoretic application of quinpirole (a D_2 agonist) of antidromic spikes elicited by stimulation of the nucleus accumbens in dopaminergic neurones of the VTA, was significantly enhanced by simultaneous application of SKF 38393 (D_1 agonist), although SKF 38393 alone had little effect on the neurones.

3 In addition, quinpirole-induced inhibition was antagonized by iontophoretic application of domperidone (D_2 antagonist), but was not affected by SCH 23390 (D_1 antagonist).

4 Furthermore, SKF 38393-induced enhancement of inhibition by quinpirole was antagonized by simultaneous application of SCH 23390.

5 These results suggest that activation of D_1 receptors located on the VTA dopaminergic neurones or on non-dopaminergic nerve terminals is not essential for inducing inhibition of the dopaminergic neurones, but enhances D_2 receptor-mediated inhibition directly or indirectly via inhibitory neurones.

Keywords: Ventral tegmental area; D_1 receptor; D_2 receptor; SKF 38393; quinpirole; domperidone; SCH 23390

Introduction

The ventral tegmental area (VTA) is composed of dopamine-containing neurones and non-dopaminergic neurones. Both neurone types project to limbic areas such as the nucleus accumbens (Acc) and frontal cortex (Anden *et al.*, 1966; Beckstead *et al.*, 1979; Simon *et al.*, 1979). The spontaneous and/or glutamate-induced firing of VTA dopaminergic neurones are reported to be inhibited by dopamine as well as dopamine agonists such as apomorphine, LY 141865, (+)-amphetamine and quinpirole (LY 171555), through dopamine autoreceptors (Aghajanian & Bunney, 1977; Wang, 1981b; White & Wang, 1984a,b). Recently, we have also found that dopamine, a D_2 agonist, talipexole (B-HT 920) and a dopamine autoreceptor agonist, OPC-4392, produced D_2 receptor antagonist-reversible inhibition of the antidromic spike induced by stimulation of the Acc in dopaminergic neurones of the VTA (Momiyama *et al.*, 1990; 1991). Similar results in the VTA neurones were also obtained by Seutin *et al.* (1990) with systemic injection of talipexole. However, an autoradiographic study shows that in addition to D_2 receptors, D_1 receptors also exist in the VTA of man and rats (Boyson *et al.*, 1986; Dawson *et al.*, 1986; 1988; Cortes *et al.*, 1989). Furthermore, a recent biochemical study has demonstrated that neurones, probably in the ventral tegmental area, superio-medial to the lateral mammillary nuclei, express mRNAs of a subtype of the D_1 receptor (D_{1B}) (Tiberi *et al.*, 1991). Therefore, a microiontophoretic study was performed to elucidate the role of D_1 receptors in the VTA.

Methods

Twenty-eight male Wistar rats weighing 270–350 g were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.). After tracheal cannulation, the animals were fixed in a stereotaxic apparatus and immobilized with gallamine triethiodide (80 mg/animal, i.p.) under artificial respiration. ECG (II lead) was continuously recorded to monitor the anaesthetic condition of the animal. When the experiments exceeded 5 h, a supplemental dose of 200 mg kg^{-1} chloral hydrate was administered. Gallamine triethiodide (40 mg/animal) was also given when required. Lignocaine was sprayed on all pressure points and incision sites repeatedly throughout the experiments. Body temperature was maintained between 36.5–37.5°C with a heating pad.

A bipolar stimulating electrode with a tip 0.1 mm in diameter was inserted into the Acc (1.7–2.2 mm anterior to bregma, 1.5–2.0 mm lateral to midline, 7.5–8.0 mm from the cortical surface) (Paxinos & Watson, 1986) ipsilateral to the recording site to activate the VTA neurones antidromically. A stimulus composed of a rectangular pulse (0.1 ms duration and 0.1–0.3 mA) was applied to the Acc every 1.6 s. The stimulus intensity was 1.5 times higher than the threshold which was the minimum current to produce an antidromic action potential in the VTA neurone. Single neuronal activities were extracellularly recorded in the VTA (4.7–5.0 mm posterior to bregma, 0.5–1.0 mm lateral to midline, 7.5–8.8 mm from the cortical surface) (Paxinos & Watson, 1986) using a glass-insulated silver wire microelectrode (electrical resistance; 1–2 MΩ) attached along a seven-barreled micropipette with an outer diameter of 3–5 μm. The distance between the tips of the recording electrode and micropipette was 20–30 μm.

Each seven-barreled micropipette was filled with 0.2 M dopamine hydrochloride (Sigma, pH 5.5), 30 mM SKF 38393 (2, 3, 4, 5-tetrahydro-1-phenyl-1 H-3-benzazepine-7,8-diol hydrochloride; Smith Kline and French Lab., pH 6.0), 10 mM quinpirole (LY 171555; Eli Lilly Co., pH 5.5), 5 mM SCH 23390 ((**R**)-(+)-8-chloro-2, 3, 4, 5-tetrahydro-3-methyl-5-[phenyl-1H-3-benzazepine-7-ol hemimaleate; Schering Corp., dissolved in 0.3% tartrate, pH 4.0), 30 mM domperidone (Kyowa Hakko, dissolved in 1% lactate, pH 4.5), 1 M monosodium L-glutamate (Sigma, pH 7.4) and 3 M NaCl. These chemicals were applied iontophoretically to the immediate vicinity of the target neurone being recorded, with a microiontophoresis programmer (WP-I, model 160). NaCl solution (3 M) was used for automatic current-balancing and for checking the current effects. Retaining currents of ap-

[1] Author for correspondence.

proximately − 20 nA were used between application periods for all these drugs except L-glutamate, which was retained with a + 20 nA current and applied as an anion.

The successive responses to Acc stimulation displayed on an oscilloscope (Nihon-Kohden VC-11) were photographed. The mean number and latency of spikes produced by Acc stimulation in each neurone were obtained by 10 successive responses before and during iontophoretic application of each drug; then, the mean spike number and latency of each group of neurones were calculated from the mean value of each neurone. The statistical significance of the data was determined by the paired t test. The positions of the stimulating and recording electrode tips were marked by passing a direct current of 0.3 mA for 10 s and 20 μA for 2 min, respectively, and histologically checked by staining with cresyl violet. Typical examples of the locations of recording and stimulating electrodes are shown in Figure 1a and b, respectively. Other details of the experiment have been previously reported (Akaike *et al.*, 1984; Momiyama *et al.*, 1990; 1991).

Results

Forty-five neurones were antidromically activated by Acc stimulation. Of these 45 neurones, 39 were histologically confirmed to be located in the VTA. As previously reported (Momiyama *et al.*, 1990; 1991), VTA neurones activated by antidromic Acc stimulation were classified into type I and II neurones according to their responses to Acc stimulation; presumably corresponding to dopaminergic and non-dopaminergic neurones, respectively, since they had long and short latencies of the antidromic spike, respectively (Figure 2a and b).

Effects of agonists

The effects of microiontophoretic application of dopamine, quinpirole and SKF 38393 were examined in 16 type I neurones and 13 type II neurones. In 15 of 16 type I neurones, antidromic spike generation was inhibited by dopamine at a current of 40 nA, although that of the remaining one neurone was not affected (Figure 3b). When dopamine (40 nA) was applied for 60 s, the mean spike number of 16 type I neurones significantly ($P<0.01$) decreased to 0.41 ± 0.05 from the control (1.06 ± 0.02), while the spike latency was not affected (Table 1a). Quinpirole also inhibited antidromic spike generation current-dependently in type I neurones without affecting the spike latency; the inhibition of the antidromic spike generation during application of 20, 40 and 70 nA of quinpirole was seen in 7, 11 and 14 of 16 type I neurones, respectively (Figure 3c,d and e). The mean spike number of 16 type I neurones upon Acc stimulation current-dependently and significantly ($P<0.01$) decreased from 1.06 ± 0.02 to 0.83 ± 0.04, 0.68 ± 0.05 and 0.43 ± 0.06 during application of quinpirole at currents of 20, 40 and 70 nA, respectively (Table 1a). However, SKF 38393 at a current of 40 nA did not significantly affect antidromic spikes in 15 of 16 type I neurones, although an inhibition of the spike generation was seen in the remaining neurone and at a current of 70 nA, the drug did not affect any of the 16 type I neurones tested (Figure 2f and g). The mean spike number of 16 type I neurones was unaffected by SKF at a current of 40 or 70 nA, (Table 1a). In contrast, simultaneous application of SKF 38393 with quinpirole enhanced the quinpirole-induced inhibition of antidromic spikes of type I neurones upon Acc stimulation without affecting the spike latency. When SKF 38393 at a current of 70 nA was simultaneously applied, significant ($P<0.05$) enhancement of quinpirole (20 nA)-induced inhibition was observed in 6 of 16 type I neurones, compared with the spike number during application of quinpirole alone at a current of 20 nA (Figure

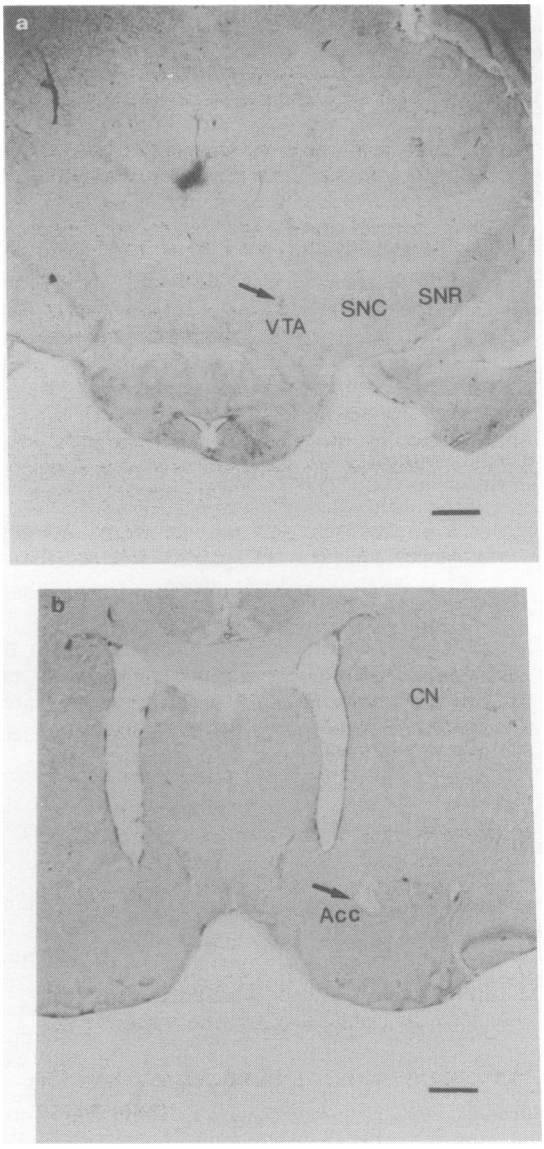

Figure 1 (a) The location of the recording electrode (arrow) within the ventral tegmental area (VTA). (b) The location of stimulating electrode (arrow) within the nucleus accumbens (Acc). Abbreviations: CN, caudate nucleus; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata. Calibration bar: 1.0 mm.

Figure 2 Spikes of type I (a) and type II (b) neurones in the ventral tegmental area elicited by stimulation of the nucleus accumbens antidromically: (▲) indicate stimulus artifacts. Calibration: 5 ms, 1 mV.

Table 1 Effects of dopamine, quinpirole (LY 171555) and SKF 38393 on antidromic spikes elicited by stimulation of the nucleus accumbens in type I (a) and type II (b) neurones of the ventral tegmental area

	Number of spikes		*Latency* (ms)
a *Type I neurone* ($n = 16$)			
Control	1.06 ± 0.02		9.36 ± 0.40
DA (40 nA)[a]	0.41 ± 0.05*	(15)[b]	9.34 ± 0.40
LY (20 nA)	0.83 ± 0.04*	(7)	9.36 ± 0.40
LY (40 nA)	0.68 ± 0.05*	(11)	9.35 ± 0.43
LY (70 nA)	0.43 ± 0.06*	(14)	9.35 ± 0.43
SKF (40 nA)	1.03 ± 0.06	(1)	9.37 ± 0.40
SKF (70 nA)	1.05 ± 0.05	(0)	9.36 ± 0.40
SKF (70 nA) + LY (20 nA)	0.46 ± 0.06†	(13)[b] (6)[c]	9.36 ± 0.40
SKF (40 nA) + LY (40 nA)	0.44 ± 0.06†	(14)[b] (3)[c]	9.35 ± 0.43
b *Type II neurone* ($n = 13$)			
Control	1.08 ± 0.08		2.85 ± 0.43
DA (40 nA)[a]	1.05 ± 0.09	(1)[b]	2.85 ± 0.43
LY (20 nA)	1.06 ± 0.10	(0)	2.85 ± 0.42
LY (40 nA)	0.98 ± 0.10	(2)	2.79 ± 0.50
LY (70 nA)	0.98 ± 0.10	(2)	2.79 ± 0.50
SKF (40 nA)	1.03 ± 0.09	(1)	2.85 ± 0.43
SKF (70 nA)	1.04 ± 0.09	(1)	2.85 ± 0.43
SKF (70 nA) + LY (20 nA)	0.98 ± 0.10	(2)[b] (1)[c]	2.88 ± 0.43
SKF (40 nA) + LY (40 nA)	0.95 ± 0.12	(2)[b] (1)[c]	2.83 ± 0.55

Each value represents the mean ± s.e.
Each drug was applied iontophoretically for 60 s.
[a]Current applied; [b]number of neurones, in which spike generation by stimulation of the nucleus accumbens was significantly ($P < 0.05$) inhibited, when tested in 16 type I and 13 type II neurones; [c]number of neurones in which quinpirole-induced inhibition was enhanced by SKF, when tested in 16 type I and 13 type II neurones.
*$P < 0.01$, significantly different from control;
†$P < 0.01$, significantly different from LY (20 nA) or LY (40 nA) alone.
DA: dopamine, LY: quinpirole, SKF: SKF 38393.
SKF + LY: simultaneous application of SKF 38393 and quinpirole.

3h, Table 2). The inhibition of the antidromic spike generation was seen in 13 of 16 type I neurones when SKF 38393 at a current of 70 nA and quinpirole at a current of 20 nA were simultaneously applied (Tables 1a and 2). Similar effects were observed during simultaneous application of SKF 38393 at a current of 40 nA with quinpirole at a current of 40 nA (Figure 3i, Table 2). The mean spike number of 16 type I neurones significantly ($P < 0.01$) decreased from 0.83 ± 0.04 to 0.46 ± 0.06 during simultaneous application of SKF 38393 (70 nA) and quinpirole (20 nA), and from 0.68 ± 0.05 to 0.44 ± 0.06 with SKF 38393 (40 nA) plus quinpirole (40 nA), compared with the controls by quinpirole alone (Tables 1a and 2). However, when dopamine (40 nA), quinpirole (20, 40 and 70 nA) or SKF 39393 (40 and 70 nA) alone was given to 13 type II neurones, there were no effects on the antidromic

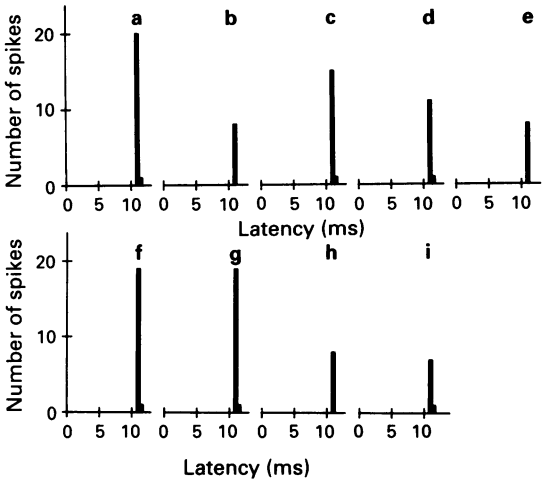

Figure 3 Effects of microiontophoretic application of dopamine, quinpirole and SKF 38393 on antidromic spikes elicited by stimulation of the nucleus accumbens in a type I neurone in the ventral tegmental area. The poststimulus latency histogram was obtained from 20 successive responses of the neurones. (a) Control; (b) during iontophoretic application of dopamine at a dose of 40 nA; (c), (d) and (e): during application of quinpirole at doses of 20, 40 and 70 nA, respectively; (f) and (g) during application of SKF 38393 at doses of 40 and 70 nA; (h) and (i) simultaneous application of SKF 38393 (70 nA) with quinpirole (20 nA) and SKF 38393 (40 nA) with quinpirole (40 nA), respectively.

Table 2 Effects of quinpirole (LY) in the presence of SKF 38393 (SKF) on number of antidromic spikes elicited by stimulation of the nucleus accumbens in type I neurones in the ventral tegmental area

Neurone no.	*Control*	*LY* (20 nA)	*LY* (40 nA)	*SKF* (70 nA) + *LY* (20 nA)	*SKF* (40 nA) + *LY* (40 nA)
1	1.0[n]	1.0	0.8	0.5**	0.5**
2	1.0	0.7*	0.7*	0.4**	0.4**
3	1.2	0.7*	0.6**	0.4**	0.2**
4	1.0	0.9	0.7*	0.2**	0.6*
5	1.0	0.4**	0.5**	0.5**	0.2**
6	1.0	0.8	0.6*	0.4**	0.4**
7	1.1	0.6*	0.5**	0.3**	0.3**
8	1.2	1.1	0.9	0.4**	0.4**
9	1.0	0.9	0.5**	0.2**	0.3**
10	1.0	0.9	0.6*	0.3**	0.3**
11	1.0	1.0	1.0	0.9	0.7*
12	1.2	0.8*	0.7**	0.4**	0.4**
13	1.0	0.8	0.9	0.8	0.8
14	1.0	0.7*	0.3**	0.3**	0.1**
15	1.0	1.0	1.0	0.9	0.9
16	1.2	0.9*	0.6*	0.5**	0.6**
Mean	1.06	0.83**	0.68**	0.46††	0.44††
s.e.	0.02	0.04	0.05	0.06	0.06

n: mean spike number of 10 successive responses.
*$P < 0.05$, significantly different from the control. **$P < 0.01$, significantly different from the control.
††$P < 0.01$, significantly different from LY (20 nA) or LY (40 nA) alone.

spike elicited by Acc stimulation in most of the neurones examined, although the inhibition was seen in 1–2 neurones (Figures 4b–g, Table 1b). In addition, SKF 38393 given simultaneously with quinpirole had no effect on the antidromic spikes in 12 of 13 type II neurones tested, although enhancement of the inhibition was seen in the remaining one neurone (Figure 4h and i, Table 1b).

Effects of antagonists

When domperidone (40 nA) was applied 30 s prior to the application of quinpirole (40 nA), the quinpirole-induced inhibition was antagonized during application of domperidone in 5 of 10 type I neurones (Figure 5a–d). The mean spike number of 10 type I neurones significantly ($P<0.01$) decreased to 0.69 ± 0.03 from 1.09 ± 0.05 during microiontophoretic application of quinpirole, and then increased to 1.07 ± 0.05 in the presence of domperidone (Table 3).

To examine whether or not activation of D_1 receptors by intrinsic dopamine enhances the inhibition resulting from D_2 receptor stimulation in the VTA type I neurones, the effects of SCH 23390 (D_1 antagonist) on the quinpirole-induced inhibition were investigated. When SCH 23390 at currents up to 70 nA was applied 30 s prior to, and then simultaneously with quinpirole (40 and 70 nA), quinpirole-induced inhibition was not antagonized in any of 10 type I neurones (Figure 5e and f). There was no significant difference in the mean spike number between application of SCH 23390 plus quinpirole and quinpirole alone (Table 3).

Effect of SCH 23390 on SKF 38393-induced enhancement

SCH 23390 at a current of 40 nA was applied iontophoretically for 30 s and then SKF 38393 and quinpirole at a current of 40 nA were given simultaneously in the presence of SCH 23390. Under these conditions, an enhancement by SKF 38393 of quinpirole-induced inhibition of antidromic spike generation in VTA type I neurones was antagonized by SCH 23390. As shown in Table 4, the mean number of antidromic spikes of 10 type I neurones tested was significantly ($P<0.01$) decreased by iontophoretic application of quinpirole (40 nA) from 1.13 ± 0.08 to 0.68 ± 0.06 (Table 4). The inhibitory effect induced by quinpirole was significantly ($P<0.01$) enhanced from 0.68 ± 0.06 to

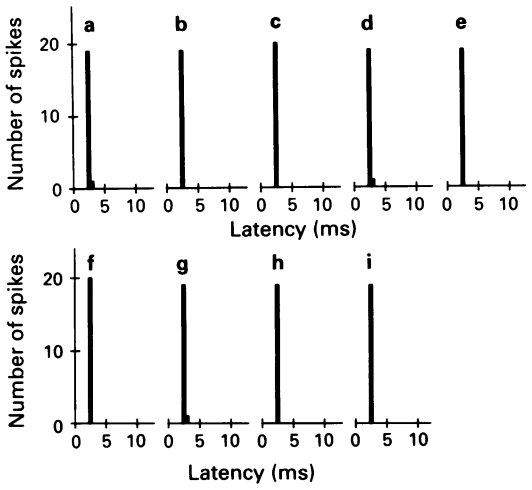

Figure 4 Effect of microiontophoretic application of dopamine, quinpirole and SKF 38393 on antidromic spikes elicited by nucleus accumberns stimulation in a type II neurone in the ventral tegmental area. The poststimulus latency histogram was obtained from 20 successive responses of the neurone. (a) Control; (b) during iontophoretic application of dopamine at a dose of 40 nA; (c), (d) and (e) during application of quinpirole at doses of 20, 40 and 70 nA, respectively; (f) and (g) during application of SKF 38393 at doses of 40 and 70 nA; (h) and (i) simultaneous application of SKF 38393 (70 nA) with quinpirole (20 nA) and SKF 38393 (40 nA) with quinpirole (40 nA), respectively.

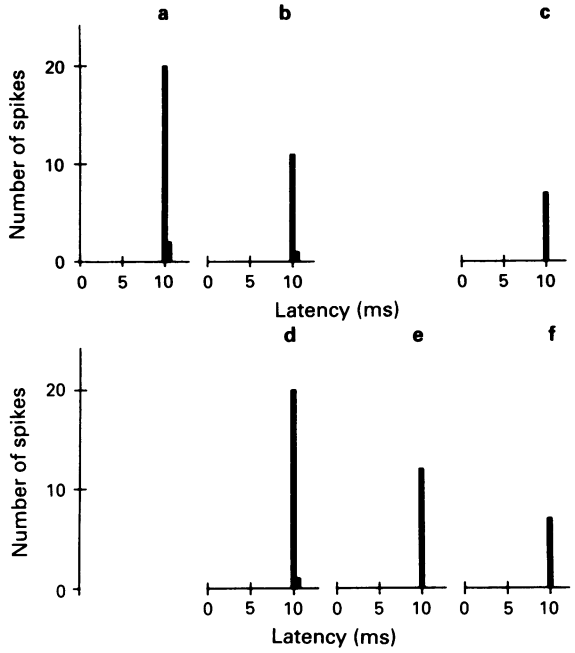

Figure 5 Antagonistic effects of domperidone and SCH 23390 on quinpirole-induced inhibition of antidromic spikes elicited by stimulation of nucleus accumbens in a type I neurone in the ventral tegmental area. The poststimulus latency histogram was obtained from 20 successive responses of the neurone. (a) Control; (b) and (c) during iontophoretic application of quinpirole at doses of 40 and 70 nA, respectively; (d) simultaneous application of domperidone at a dose of 40 nA with quinpirole (40 nA); (e) during simultaneous application of SCH 23390 at a dose of 40 nA with quinpirole (40 nA); (f) during simultaneous application of SCH 23390 (70 nA) and quinpirole (70 nA).

Table 3 Effect of quinpirole (LY) on antidromic spikes of type I neurones in the VTA upon Acc stimulation, and antagonistic effects of domperidone (Dom) and SCH 23390 (SCH) on quinpirole-induced inhibition of the spike

	Number of spikes	*Latency* (ms)
Control	1.09 ± 0.05	10.01 ± 0.43
LY (40 nA)[a]	0.69 ± 0.03* (9)[b]	10.05 ± 0.40
LY (70 nA)	0.41 ± 0.04* (10)	10.07 ± 0.40
Dom (40 nA) + LY (40 nA)	1.07 ± 0.05† (0)	10.12 ± 0.39
SCH (40 nA) + LY (40 nA)	0.68 ± 0.05* (9)	10.06 ± 0.40
SCH (70 nA) + LY (70 nA)	0.36 ± 0.04* (10)	10.06 ± 0.41

$n = 10$
Each value represents the mean ± s.e.
Each drug was applied iontophoretically for 60 s.
*$P<0.01$, significantly different from control.
†$P<0.01$, significantly different from LY alone.
[a]Current applied; [b]number of neurones, in which spike generation by stimulation of the nucleus accumbens was significantly ($P<0.05$) inhibited.
Dom + LY and SCH + LY: simultaneous application of domperidone with quinpirole, and SCH 23390 with quinpirole, respectively.

Table 4 Effect of SCH 23390 (SCH) on SKF 38393 (SKF) -induced enhancement of inhibition by quinpirole (LY) in type I neurones

	Number of spikes	*Latency* (ms)
Control	1.13 ± 0.08	11.39 ± 0.59
DA (40 nA)[a]	0.46 ± 0.05*	11.44 ± 0.59
LY (40 nA)	0.68 ± 0.06*	11.51 ± 0.56
SKF (40 nA)	1.08 ± 0.06	11.46 ± 0.54
SKF (40 nA) + LY (40 nA)	0.46 ± 0.04††	11.56 ± 0.55
SCH (40 nA) + SKF (40 nA) + LY (40 nA)	0.73 ± 0.08†	11.43 ± 0.56

$n = 10$.
Each value represents the mean ± s.e.
Each drug was applied iontophoretically for 60 s.
*$P < 0.01$, significantly different from control.
††$P < 0.01$, significantly different from LY alone.
†$P < 0.01$, significantly different from the value without SCH.
[a]Current applied.
SKF + LY: simultaneous application of LY with SKF.
SCH + SKF + LY: simultaneous application of SCH with SKF and LY.

0.46 ± 0.04 by simultaneous application of SKF 38393 (40 nA) with quinpirole (Table 4). Furthermore, when quinpirole (40 nA), SKF 38393 (40 nA) and SCH 23390 (40 nA) were simultaneously applied to these 10 type I neurones, the mean number of antidromic spikes was significantly ($P < 0.01$) increased from 0.46 ± 0.04 to 0.73 ± 0.08, compared with the value without SCH 23390 (Table 4).

Discussion

The present study confirmed the previous findings that the VTA neurones projecting to the Acc are composed of two types (Wang, 1981a; Momiyama *et al.*, 1990; 1991): type I and type II neurones, probably corresponding to dopaminergic and non-dopaminergic neurones, respectively, since in the former neurones, an antidromic spike elicited by Acc stimulation had a long duration and latency while the latter neurones, in contrast, had a short duration and latency (Grace & Bunney, 1980; Wang, 1981a; Momiyama *et al.*, 1990; 1991).

Microiontophoretically applied dopamine and quinpirole inhibited antidromic spikes elicited by Acc stimulation in type I neurones. The quinpirole-induced inhibition was antagonized by simultaneous application of domperidone, suggesting that this inhibition is mediated by D_2 receptors located in dopaminergic neurones of the VTA, as shown in our previous studies (Momiyama *et al.*, 1990; 1991). These results are also in accordance with those obtained by White & Wang with D_2 agonists, LY 141865 and bromocriptine, as well as a D_2 antagonist, sulpiride, on spontaneous firing of the VTA neurones (White & Wang, 1984a,b).

On the other hand, microiontophoretic application of SKF 38393 alone had little effect on antidromic spikes of type I neurones. The quinpirole-induced inhibition of the antidromic spikes was not affected by blocking D_1 receptors with SCH 23390. This suggests that D_1 receptors alone in the VTA are not essential for dopamine-mediated inhibition of dopaminergic neurones. However, the inhibitory effects of quinpirole were significantly enhanced in the presence of SKF 38393, suggesting that activation of D_1 receptors induces synergistic effects on D_2 receptor-mediated inhibition in the VTA. Similar synergistic effects by activation of both D_1 and D_2 receptors have also been found in globus pallidus neurones (Walters *et al.*, 1987), the caudate nucleus and the Acc neurones (Wachtel *et al.*, 1989), although the effects of D_1 and D_2 receptor activation were opposite in some caudate nucleus neurones in our studies (Akaike *et al.*, 1987; Ohno *et al.*, 1987). However, Wachtel *et al.* (1989) have reported that the ability of dopamine agonists to stimulate D_2 receptors in the VTA neurones was not altered by manipulation of D_1 receptor occupation. These differences are probably due to the low currents of the D_1 agonist applied in the presence of the D_2 agonist. Furthermore, in the present study, enhancement of quinpirole-induced inhibition by SKF 38393 was antagonized by simultaneous application of SCH 23390, a selective D_1 antagonist, indicating that this enhancement by SKF 38393 is mediated by D_1 type receptors. Our electrophysiological study suggests that D_1 type receptors are distributed in the VTA which have some modulatory function, although it is not as essential as that of D_2 receptors. One possibility suggested by our results is that under normal conditions, dopamine acts on the D_2 receptors located on the dendrites of dopaminergic neurones in the VTA, and that excess dopamine released from dendrites may also activate D_1 receptors, thereby enhancing the D_2 receptor-mediated inhibitory effects, although activation of the D_1 receptors is not always necessary to inhibit these neurones.

However, contradictory results have been reported regarding the existence of D_1 receptors in the VTA. One autoradiographic study found no D_1 receptors in the pars compacta of the substantia nigra or the VTA (Alter & Hauser, 1987); however, other autoradiographic studies indicated the existence of both D_1 and D_2 receptors in the VTA (Boyson *et al.*, 1988; Dawson *et al.*, 1986; 1988; Cortes *et al.*, 1989). *In situ* hybridization histochemistry studies have demonstrated that the D_1 receptor mRNA was not expressed in the VTA dopaminergic neurones (Dearry *et al.*, 1990; Sunahara *et al.*, 1990; Weiner *et al.*, 1991; Meador-Woodruff *et al.*, 1992). Recently, however, Mansour *et al.* (1992) have reported that there are marked discrepancies between D_1 receptor binding and mRNA in some brain regions; D_1 receptor binding can be observed in the VTA, but no D_1 receptor mRNA can be detected there. They have discussed whether these discrepancies may be due to technical problems such as ligand and probe sensitivity, and to specific transport of dopamine receptors synthesized. Therefore, conflicting results – that D_1 receptors in the VTA dopaminergic neurones are observed by autoradiographic studies, but their mRNA cannot be detected by *in situ* hybridization – appear not always to mean the lack of D_1 rceptors.

However, iontophoretically applied SKF 38393 does not influence only the neurone recorded, but also affects other neuronal elements in the vicinity of the iontophoretic pipette. Therefore, it is possible that the SKF 38393-induced response is mediated by non-dopaminergic elements within the VTA e.g. it may activate D_1 type receptors located on the terminals of GABAergic neurones originating in the Acc and enhance release of GABA, eventually potentiating D_2 receptor-mediated inhibition.

The authors are grateful to Smith Kline & Franch Laboratories (Philadelphia, PA, U.S.A.), Eli Lilly and Company (Indianapolis, U.S.A.), Schering-Plough Corporation (Bloomfield, NJ, U.S.A.) and Kyowa Hakko Company (Tokyo, Japan) for the gifts of SKF 38393, quinpirole, SCH 23390 and domperidone, respectively. This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

AGHAJANIAN, G.K. & BUNNEY, B.S. (1977). Dopamine 'autoreceptors': pharmacological characterization by microiontophoretic single cell recording studies. *Naunyn-Schmied. Arch. Pharmacol.*, **297**, 1–7.

AKAIKE, A., SASA, M. & TAKAORI, S. (1984). Microiontophoretic studies of the dopaminergic inhibition from the ventral tegmental area to the nucleus accumbens. *J. Pharmacol. Exp. Ther.*, **229**, 859–864.

AKAIKE, A., OHNO, Y., SASA, M. & TAKAORI, S. (1987). Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons *in vitro*. *Brain Res.*, **418**, 262–272.

ALTER, C.A. & HAUSER, K. (1987). Topography of substantia nigra innervation by D_1 receptor-containing striatal neurons. *Brain Res.*, **410**, 1–11.

ANDEN, N.E., DHALSTROM, A., FUXE, K., LARSON, K., OLSON, L. & UNGERSTEDT, U. (1966). Ascending monoamine neurons to telecephalon and diencephalon. *Acta Physiol. Scand.*, **67**, 313–326.

BECKSTEAD, R.M., DOMESICK, V.B. & NAUTA, W.J.H. (1979). Efferent connections of substantia nigra and ventral tegmental area in the rat. *Brain Res.*, **175**, 191–207.

BOYSON, S.J., MCGONIGLE, P. & MOLINOFF, P.B. (1986). Quantitative autoradiographic localization of D_1 and D_2 subtypes of dopamine receptors in rat brain. *J. Neurosci.*, **6**, 3177–3188.

CORTES, R., GUEYE, B., PAZOS, A., PROBST, A. & PALACIOS, J.M. (1989). Dopamine receptors in human brain: autoradiographic distribution of D_1 sites. *Neuroscience*, **28**, 263–273.

DAWSON, T.M., GEHLERT, D.R., MCCABE, R.T., BARNETT, A. & WAMSLEY, J.K. (1986). D-1 dopamine receptors in the rat brain: a quantitative autoradiographic analysis. *J. Neurosci.*, **6**, 2352–2365.

DAWSON, T.M., BARONE, P. & SIDHU, A. (1988). The D_1 dopamine receptor in the rat brain: quantitative autoradiographic localization using an iodinated ligand. *Neuroscience*, **26**, 83–100.

DEARRY, A., GINGRICH, J.A., FALARDEAU, P., FREMEAU R.T. Jr., BATES, M.D. & CARON, M.G. (1990). Molecular cloning and expression of the gene for a human D_1 dopamine receptor. *Nature*, **347**, 72–76.

GRACE, A.A. & BUNNEY, B.S. (1980). Nigral dopamine neurons: intracellular recording and identification with L-Dopa injection and histofluorescence. *Science*, **210**, 654–656.

MANSOUR, A., MEADOR-WOODRUFF, J.H., ZHOU, Q., CIVELLI, O., AKIL, H. & WATSON, S.J. (1992). A comparison of D_1 receptor binding and mRNA in rat brain using receptor autoradiographic and in situ hydridization techiques. *Neuroscience*, **48**, 959–971.

MEADOR-WOODRUFF, J.H., MANSOUR, A., GRANDY, D.K., DAMASK, S.P., CIVELLI, O. & WATSON, Jr. S.J. (1992). Distribution of D_5 dopamine receptor mRNA in rat brain. *Neurosci. Lett.*, **145**, 209–212.

MOMIYAMA, T., SASA, M. & TAKAORI, S. (1990). D-2 receptor-mediated inhibition by a substituted quinolinone derivative, 7-{3-[4-(2,3-dimethylphenyl) piperazinyl]propoxy}-2(1H)-quinolinone (OPC-4392), of dopaminergic neurons in the ventral tegmental area. *Life Sci.*, **47**, 761–769.

MOMIYAMA, T., SASA, M. & TAKAORI, S. (1991). Inhibition by talipexole, a thiazolo-azepine derivative, of dopaminergic neurons in the ventral tegmental area. *Life Sci.*, **49**, 535–543.

OHNO, Y., SASA, M. & TAKAORI, S. (1987). Coexistence of inhibitory dopamine D-1 and excitatory D-2 receptors on the same caudate nucleus neurons. *Life Sci.*, **40**, 1937–1945.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. 2nd Edition, Sydney: Academic Press.

SEUTIN, V., MOREAU, J.S., GIESBERS, I., MASSOTTE, L. & DRESSE, A. (1990). Effects of B-HT 920 on monoaminergic neurons of the rat brain: an electrophysiological in vivo and in vitro study. *Naunyn-Schmied. Arch. Pharmacol.*, **242**, 502–507.

SIMON, H., LE MOAL, M. & CALAS, A. (1979). Efferents and afferents of the ventral tegmental A10 region studied after local injection of [^{3}H]leucine and horseradish peroxidase. *Brain Res.*, **178**, 17–40.

SUNAHARA, R.K., NIZNIK, H.B., WEINER, D.M., STORMANN, T.M., BRANN, M.R., KENNEDY, J.M., GELERNTER, J.E., ROZMAHEL, R., YANG, Y., ISRAEL, Y., SEEMAN, P. & O'DOWD, B.F. (1990). Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. *Nature*, **347**, 80–83.

TIBERI, J., JARVIE, K.R., SILVIA, C., FALARDEAU, P., GINGRICH, J.A., GODINOT, N., BERTRAND, L., YANG-FENG, T.L., FREMEAU, Jr. R.T. & CARON, M.G. (1991). Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D_1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D_{1A} receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 7491–7495.

WACHTEL, S.R., HU, X.T., GALLOWAY, M.P. & WHITE, F.J. (1989). D_1 dopamine receptor stimulation enables the postsynaptic, but not autoreceptor, effects of D_2 dopamine agonists in nigrostriatal and mesoaccumbens dopamine systems. *Synapse*, **4**, 327–346.

WALTERS, J.R., BERGSTROM, D.A., CARLSON, J.H., CHASE, T.N. & BRAUN, A.R. (1987). D_1 dopamine receptor activation required for postsynaptic expression of D_2 agonist effects. *Science*, **236**, 719–722.

WANG, R.Y. (1981a). Dopaminergic neurons in the ventral tegmental area. I. Identification and characterization. *Brain Res. Rev.*, **3**, 123–140.

WANG, R.Y. (1981b). Dopaminergic neurons in the ventral tegmental area. II. Evidence for autoregulation. *Brain Res. Rev.*, **3**, 141–151.

WEINER, D.M., LEVEY, A.I., SUNAHARA, R.K., NIZNIK, H.B., O'DOWD, B.F., SEEMAN, P. & BRANN, M.R. (1991). D_1 and D_2 dopamine receptor mRNA in rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 1859–1863.

WHITE, F.J. & WANG, R.Y. (1984a). A 10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. *Life Sci.*, **34**, 1161–1170.

WHITE, F.J. & WANG, R.Y. (1984b). Pharmacological characterization of dopamine autoreceptors in the rat ventral tegmental area: microiontophoretic studies.[1] *J. Pharmacol. Exp. Ther.*, **231**, 275–280.

(Received November 23, 1992
Revised April 24, 1993
Accepted June 16, 1993)