ELSEVIER

European Journal of Pharmacology 310 (1996) 1–8

ejp

Inhibition by a putative antipsychotic quinolinone derivative (OPC-14597) of dopaminergic neurons in the ventral tegmental area

Toshihiko Momiyama [a], Taku Amano [b], Naoyuki Todo [a], Masashi Sasa [b,*]

[a] *Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*
[b] *Department of Pharmacology, Hiroshima University School of Medicine, Minami-ku, Hiroshima 734, Japan*

Received 11 January 1996; revised 1 May 1996; accepted 3 May 1996

Abstract

The effects of the newly synthesized quinolinone derivative, OPC-14597 (7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1*H*)-quinolinone), on dopaminergic neuronal activity in the ventral tegmental area were examined using both in vivo microiontophoretic methods in chloral hydrate-anesthetized rats and the tight-seal whole-cell patch-clamp technique in thin-slice preparations of the rat brain. Neurons in the ventral tegmental area were classified as type I or type II according to their responses to antidromic stimulation of the nucleus accumbens, probably corresponding to dopaminergic and non-dopaminergic neurons, respectively. Antidromic spikes elicited by nucleus accumbens stimulation were inhibited by microiontophoretic application of dopamine and OPC-14597 in type I, but not in type II neurons. Although the OPC-14597-induced inhibition was antagonized by simultaneous application of domperidone (5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1*H*-benzimidazo-1-yl)-propy]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one; dopamine D_2 receptor antagonist), SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; dopamine D_1 receptor antagonist) had no such effect. Spontaneous firing of type I neurons was also inhibited by iontophoretically applied OPC-14597 and dopamine, whereas that of type II neurons was unaffected. The inhibitory effect of OPC-14597 on the spontaneous firing of type I neurons was antagonized by domperidone, but not by SCH 23390. In a whole-cell patch-clamp study using a thin-slice preparation of the rat brain, bath application of OPC-14597 induced hyperpolarization accompanied by inhibition of spontaneously occurring action potentials in the large neurons (> 20 μm in diameter) in a concentration-dependent manner. These results suggest that OPC-14597 acts on dopaminergic neurons in the ventral tegmental area as a dopamine D_2 receptor agonist to inhibit neuronal activities, probably by increasing membrane potassium conductance.

Keywords: Ventral tegmental area; OPC-14597; Extracellular recording in vivo; Whole-cell recording; Thin-slice preparation; Dopamine D_2 receptor

1. Introduction

A newly synthesized quinolinone derivative, 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1*H*)-quinolinone (OPC-14597) (Fig. 1), is expected to act as an antipsychotic drug structurally related to OPC-4392, 7-{3-[4-(2,3-dimethylphenyl)-1-piperazinyl]propoxy}-2(1*H*)-quinolinone. OPC-4392 is a dopamine autoreceptor agonist that inhibits dopaminergic neuronal activities of the ventral tegmental area (Momiyama et al., 1990) and dopaminergic nerve terminals in the caudate nucleus (Sasa et al., 1988) as well as synthesis and release of dopamine (Yasuda et al., 1988) and DOPA (L-dihydroxyphenylalanine) formation (Kiuchi et al., 1988). Preliminary clinical trials in schizophrenic patients showed that OPC-4392 is effective to improve the patients' negative symptoms but sometimes aggravates positive symptoms, such as hallucination and delusion. Effectiveness on negative symptoms has been observed with other dopamine D_2 receptor agonists, such as talipexole (6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]-azepinedihydrochloride; B-HT 920) (Ohmori et al., 1993). Therefore, a new drug which acts on dopaminergic neurons as an autoreceptor agonist and simultaneously on postsynaptic neurons as a dopamine D_2 receptor antagonist is expected to be useful for amelioration of negative symptoms in chronic schizophrenic patients.

A recent biochemical study has shown that OPC-14597 inhibits the reserpine and γ-butyrolactone-induced increase in tyrosine hydroxylase activity in the mouse and rat brain, the effect of OPC-14597 being comparable to that of OPC-4392 (Kikuchi et al., 1995). However, OPC-14597

* Corresponding author. Department of Pharmacology, Hiroshima University, School of Medicine, Minami-ku, Hiroshima 734, Japan. Tel.: +81 82 257 5140; fax: +81 82 257 5144.

PII S0014-2999(96)00350-0

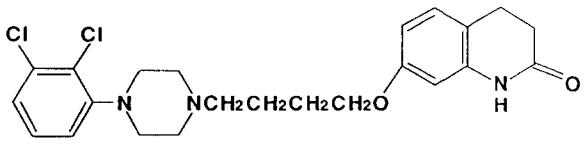

Fig. 1. Structural formula of OPC-14597.

does not induce postsynaptic dopamine receptor-stimulating behavioral changes, but antagonizes apomorphine-induced behaviors, such as hyperlocomotion, stereotypy and rotation, in kainate-lesioned animals (Kikuchi et al., 1995). Although anti-apomorphine effects were also observed with OPC-4392, the effects of OPC-14597 were about 30–140 times greater and were observed at doses that inhibit the increase in tyrosine hydroxylase activity (Kikuchi et al., 1995). These findings suggest the unique pharmacological profile of OPC-14597 as a dopamine autoreceptor agonist and a postsynaptic dopamine receptor antagonist. In addition, our previous electrophysiological study had confirmed that OPC-14597 blocked the effect of dopamine on nucleus accumbens neurons via dopamine D_2 and D_1 receptors (Amano et al., 1995).

Therefore, further electrophysiological studies were performed to directly examine whether OPC-14597 acts on dopaminergic neurons in the ventral tegmental area as a dopamine agonist as does OPC-4392, using both in vivo microiontophoretic methods and the whole-cell patch-clamp technique in thin-slice preparations of the rat brain.

2. Materials and methods

2.1. In vivo studies

Twelve male Wistar rats weighing 250–350 g, anesthetized with chloral hydrate (400 mg/kg i.p.), underwent trachea canulation. Additional doses of 150 mg/kg i.p. were administered as required. After fixing in a stereotaxic instrument, the animal was immobilized with gallamine triethiodide (80 mg/animal i.p.) which was injected once at the beginning of recording under artificial respiration to avoid sudden movement of the animal during recording. All wound edges and pressure points, including ear bar pressure points, were locally anesthetized with 8% lidocaine spray repeatedly throughout the experiments. Body temperature was maintained at 36.5–37.5°C with a heating pad placed under the animal. The ECG (II leads) was continuously monitored during the experiments to confirm that the animal was free from pain.

After removal of part of the cranium and the dura overlying the ventral tegmental area and the nucleus accumbens, a bipolar stimulating electrode (tip diameter: approximately 0.1 mm) was inserted into the nucleus accumbens (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 and Watson, 1986) ipsilateral to the recording site to activate the ventral tegmental area neurons antidromically. Stimuli composed of a 0.1–0.3 mA pulse of 0.1 ms duration were delivered to the nucleus accumbens every 1.6 s. Single neuronal activities in the ventral tegmental area (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 and Watson, 1986) were extracellularly recorded using a glass-insulated silver wire microelectrode (electrical resistance: approximately 1–2 MΩ) attached along a seven-barreled micropipette (outer diameter: 3–5 μm). The distance between tips of the recording electrode and micropipette was 20–50 μm. Each pipette was filled with 0.2 M dopamine hydrochloride (Sigma, pH 5.5), 5 mM OPC-14597 (Otsuka Pharm.), 30 mM domperidone (Kyowa Hakko), 5 mM SCH 23390 (Schering), 1 M monosodium L-glutamate (Sigma, pH 7.4) and 3 M NaCl. OPC-14597 and domperidone were dissolved in 0.9 and 1% lactate, respectively, and pH was adjusted to 4.5 with 0.1 N NaOH. SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) was dissolved in 0.3% tartrate and the pH was adjusted to 4.0. These chemicals filled in the pipettes were iontophoretically applied to the immediate vicinity of the target neuron, using a microiontophoresis programmer (WP-I, Model 160). Iontophoretic application of 1% lactate (pH 4.5) or 0.3% tartrate (pH 4.0) as vehicles had no effect on the neuronal activity of ventral tegmental area neurons. NaCl solution, 3 M, was used to check the current effects. Retaining currents of approximately +20 nA for glutamate and −20 nA for other drugs were used between application periods. We first screened and identified the cells used for the pharmacological experiments by antidromic stimulation of the nucleus accumbens. Therefore, we recorded only those cells which were activated by antidromic stimulation. Antidromic spikes elicited by stimulation of the nucleus accumbens in the ventral tegmental area were displayed on an oscilloscope (Nihon Kohden, VC-11), and 20–30 successive responses were photographed before and during application of each drug. The mean number and latency of spikes in 20 successive responses elicited by nucleus accumbens stimulation in each neuron were obtained before and after 1 min from the start of the iontophoretic application of each drug. The means of the number and latency of the antidromic spikes calculated from those of each neuron during drug application were compared with those before the treatment. Statistical significance of the data was determined using Student's *t*-test. The spontaneous firing rate was measured for 50 s before and 1 min after the start of drug application, and the mean firing rate was calculated. After the termination of each experiment, recording and stimulating sites were marked by passing a direct current of 20 μA for 2 min and 0.3 mA for 10 s, respectively, and histologically checked with Cresyl violet staining. These procedures have been described in more detail elsewhere (Momiyama et al., 1990, 1991, 1993b).

2.2. In vitro studies using thin-slice preparations

After decapitation of young rats (10–15 days old), a block of tissue containing the midbrain was trimmed and kept in ice-cold Krebs solution bubbled with O_2-CO_2 (95%-5%) as described previously (Momiyama et al., 1993a). Horizontal midbrain slices (140 μm) were cut using a microslicer (Dosaka, DTK-1000) and were incubated at 34°C for 1 h before experiments.

The recording methods were as described previously (Edwards et al., 1989; Takahashi, 1990). Neurons in the ventral tegmental area were viewed under Nomarski optics with a water-immersion objective lens (×40 Nikon). The ionic composition of the Krebs solution for dissection and perfusion was as follows (in mM): NaCl, 113; KCl, 3; NaH_2PO_4, 1; $NaHCO_3$, 25; glucose, 11; $CaCl_2$, 2; $MgCl_2$, 1. The recording chamber (about 1.0 ml) was perfused at 3 ml/min and the pH of the solution was 7.4 when bubbled with O_2-CO_2 (95%-5%). OPC-14597 was diluted from a 10 mM stock solution frozen in aliquots, and was applied into the bath by switching the perfusion line manually. The dead-space time was about 10 s. Experiments were carried out at room temperature (21–25°C).

Patch pipettes were made from thin-walled borosilicate glass capillaries (Clark, o.d. 1.5 mm), and their tips were coated with Sylgard, to reduce stray capacitance across the glass wall, and fire-polished. The pipette had a resistance of 2.5–4.5 MΩ when filled with the internal solution, which had the following ionic composition (in mM): KCl, 140; NaCl, 9; $MgCl_2$, 1; Hepes, 10; EGTA, 0.2 (pH adjusted to 7.4 with 4 mM KOH). The series resistance was 7–12 MΩ. The liquid-junction potential between the pipette and standard Krebs solution was 3 mV with a negative potential inside the pipette (Takahashi, 1990). The membrane potentials reported in the text have been corrected for the liquid-junction potential. All data are expressed as means ± S.E.M.

3. Results

3.1. Neurons in ventral tegmental area in vivo

Histological examination revealed that all 37 neurons studied were properly located within the ventral tegmental area. As reported previously (Momiyama et al., 1990, 1991, 1993b), neurons were classified into two types, I and II, according to their responses to nucleus accumbens stimulation. Briefly, type I neurons had a long latency of more than 7 ms (10.41 ± 0.36 ms, $n = 22$) and long spike duration of 2.5 ms, while type II neurons showed a short latency of less than 7 ms (1.97 ± 0.17 ms, $n = 15$) and short spike duration of less than 2.5 ms. In addition, the spontaneous firing rate was significantly ($P < 0.01$) lower in type I (4.57 ± 0.58/s, $n = 22$) than in type II neurons (21.71 ± 2.04/s, $n = 15$).

3.2. Effects of dopamine and OPC-14597 on antidromic spikes of ventral tegmental area neurons in vivo

Effects of iontophoretically applied dopamine and OPC-14597 on antidromic spikes elicited by nucleus accumbens stimulation were examined in 12 type I and 8 type II neurons which were electrophysiologically identified with the criteria mentioned above. Iontophoretic application of dopamine at a current of 40 nA resulted in significant ($P < 0.01$) inhibition of the nucleus accumbens stimulation-induced antidromic spike in all 12 type I neurons examined (Fig. 2). Similarly, iontophoretically applied OPC-14597 at a current of 40 nA also inhibited the antidromic spikes of neurons in the ventral tegmental area (Fig. 2). Significant ($P < 0.01$ for 11 and $P < 0.05$ for the remaining one) inhibition of the antidromic spikes was observed in all 12 type I neurons tested with OPC-14597 at a current of 40 nA. The mean number of nucleus

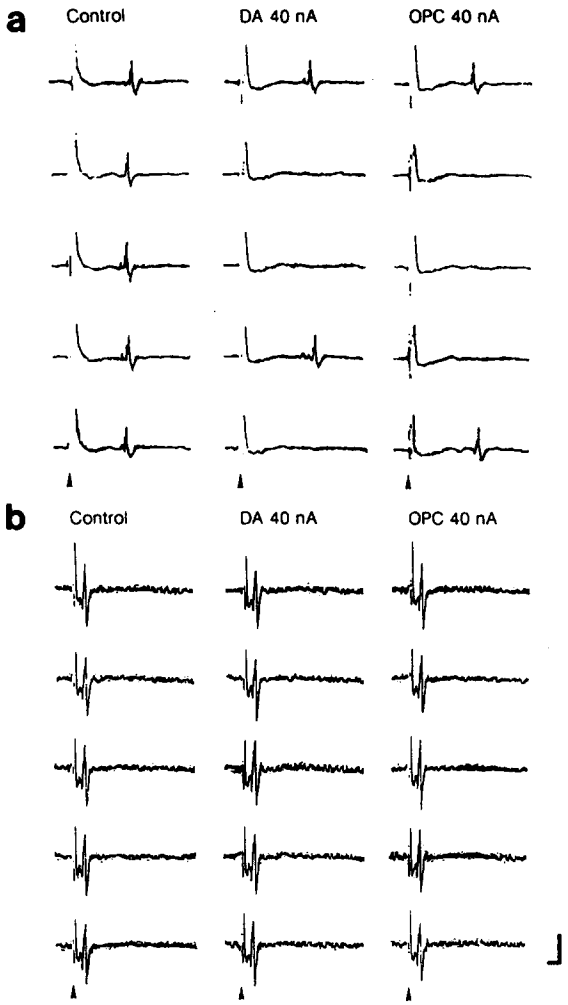

Fig. 2. Effects of dopamine (DA) and OPC-14597 (OPC) on antidromic spikes elicited by nucleus accumbens stimulation in type I (a) and type II (b) neurons in the ventral tegmental area. The recordings show the responses 60 s after iontophoretic application of DA and OPC at a current of 40 nA. Solid triangles indicate stimulus artifacts. Calibration: 2 ms, 1 mV.

accumbens stimulation-induced spikes in the type I neurons was significantly ($P < 0.01$) decreased by iontophoretic application of dopamine and OPC-14597 at currents of 40 nA (Table 1). On the other hand, antidromic spikes elicited by nucleus accumbens stimulation in type II neurons were unaffected by either dopamine or OPC-14597 at a current of 40 nA in any of the 8 type II neurons tested (Fig. 2, Table 1). Neither dopamine nor OPC-14597 had any effects on latency of antidromic spikes produced by nucleus accumbens stimulation in type I or type II neurons.

The effects of a selective dopamine D_2 receptor antagonist, domperidone, and a selective dopamine D_1 receptor antagonist, SCH 23390, were examined in 12 type I neurons in which nucleus accumbens stimulation-induced antidromic spikes were significantly inhibited by iontophoretic application of OPC-14597. The OPC-14597-induced inhibition was antagonized by simultaneous application of domperidone (Fig. 3). When domperidone at a current of 40 nA was applied 30 s prior to application of OPC-14597 and then simultaneously with OPC-14597 (40 nA) for a further 60 s, the OPC-14597-induced inhibition of antidromic spikes was significantly ($P < 0.01$ for 4 and $P < 0.05$ for 7 neurons) antagonized, although the antagonizing effect of domperidone was not significant in the one remaining neuron. The mean number of antidromic spikes of the 12 type I neurons increased again during simultaneous application of domperidone (40 nA) with OPC-14597 (40 nA) compared with OPC-14597 (40 nA) alone (Table 1). However, SCH 23390 (40 nA) did not affect the OPC-14597-induced inhibition of antidromic spikes in any of the 12 type I neurons tested (Fig. 3). The mean spike number of type I neurons was not significantly changed by simultaneous application of SCH 23390, compared with the value obtained during application of OPC-14597 alone (Table 1).

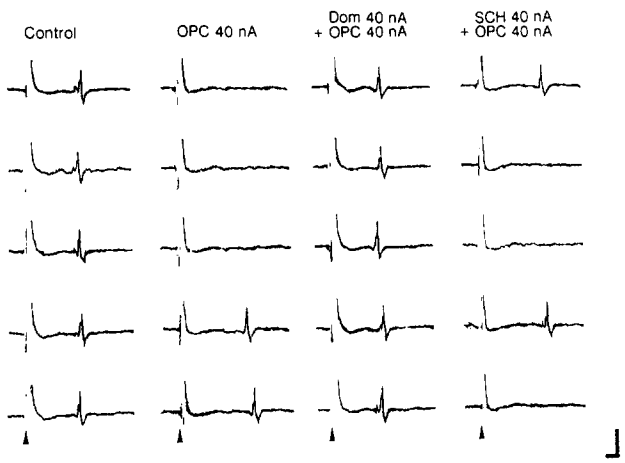

Fig. 3. Antagonism by domperidone (DOM) of OPC-14597 (OPC)-induced inhibition of antidromic spikes elicited by nucleus accumbens stimulation in a type I ventral tegmental area neuron. The responses are shown 60 s after iontophoretic application of OPC alone and simultaneous application of DOM or SCH 23390 (SCH) with OPC. Calibration: 2 ms, 1 mV.

3.3. Effects of dopamine and OPC-14597 on spontaneous firing of ventral tegmental area neurons in vivo

The effects of iontophoretically applied dopamine and OPC-14597 on spontaneous firing of neurons in the ventral tegmental area were examined in 10 type I and 7 type II neurons. Spontaneous firing was inhibited by dopamine (60 nA) in all 10 type I neurons tested (Fig. 4a), whereas no such effect was seen in any of the 7 type II neurons examined (Fig. 4b). The mean spontaneous firing rate during application of dopamine (60 nA) in type I and type II neurons was 22.97 ± 3.98 ($n = 10$) and 99.65 ± 0.63 ($n = 7$)% of the respective controls. Similarly to dopamine, OPC-14597 inhibited spontaneous firing of type I neurons (Fig. 4a). The mean spontaneous firing rate was reduced current dependently to 78.34 ± 5.81 ($n = 6$) and 26.10 ± 2.89 ($n = 10$)% of the control by OPC-14597 at currents of 20 and 40 nA, respectively. On the other hand, the spontaneous firing of type II neurons was unaffected by OPC-14597 (Fig. 4b). The mean spontaneous rates of type II neurons during application of OPC-14597 at currents of 20, 40, 60 nA were 100.40 ± 1.43 ($n = 6$), 97.36 ± 1.27 ($n = 6$) and 98.76 ± 1.13 ($n = 7$)% of the control value, respectively.

Furthermore, the OPC-14597-induced inhibition of

Table 1
Inhibitory effects of dopamine (DA) and OPC-14597 (OPC) on antidromic spikes elicited by nucleus accumbens stimulation in ventral tegmental area neurons and antagonism by domperidone (DOM) but not SCH 23390 (SCH) of the OPC-induced inhibition

	Type I neurons ($n = 12$)		Type II neurons ($n = 8$)	
	Number of spikes	Latency (ms)	Number of spikes	Latency (ms)
Control	1.18 ± 0.08	11.02 ± 0.55	1.04 ± 0.02	2.20 ± 0.27
DA	0.52 ± 0.06 [a]	11.05 ± 0.05	1.05 ± 0.03	2.23 ± 0.27
OPC	0.53 ± 0.06 [a]	11.05 ± 0.52	1.06 ± 0.03	2.20 ± 0.27
DOM + OPC	1.00 ± 0.07 [b]	11.02 ± 0.52		
SCH + OPC	0.50 ± 0.04	11.11 ± 0.54		

Each value represents the mean ± S.E. DA, OPC, DOM and SCH (each 40 nA) were iontophoretically applied for 60 s. DOM + OPC and SCH + OPC: either DOM or SCH was applied for 30 s and then either DOM and OPC or SCH and OPC were simultaneously applied for 60 s. [a] $P < 0.01$, significantly different from the control. [b] $P < 0.01$, significantly different from the value with OPC alone.

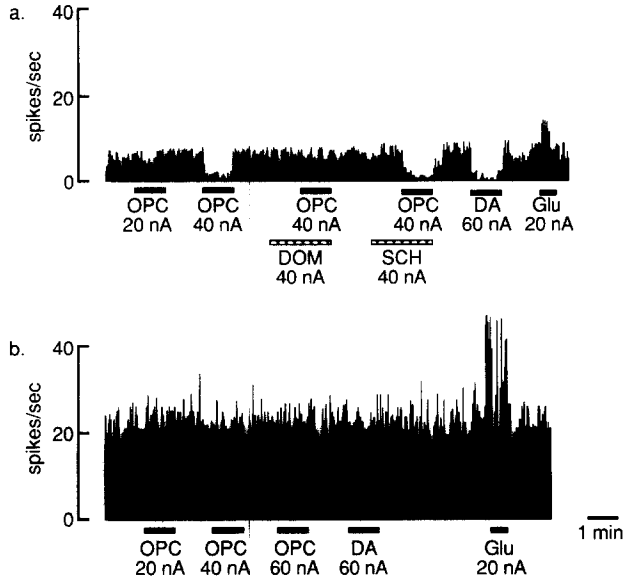

Fig. 4. Effects of iontophoretically applied dopamine (DA), OPC-14597 (OPC) and glutamate (Glu) on spontaneous firing of type I (a) and type II (b) neurons in the ventral tegmental area, and antagonism by domperidone (DOM) and SCH 23390 (SCH) of the effects of OPC in the type I same neuron. Periods during drug application are indicated by horizontal bars, under which the current amplitude is shown by numbers.

spontaneous firing was antagonized by simultaneous application of domperidone, whereas it remained unaffected by SCH 23390 (Fig. 4a). Neither domperidone nor SCH 23390 at a current of 40 nA had any effect on spontaneous firing when applied alone (Fig. 4a). The mean spontaneous firing rate increased to 90.01 ± 3.56 ($n = 10$)% of the control value during simultaneous application of domperidone with OPC-14597 from 26.10 ± 2.89 ($n = 10$)%, which was the value during application of OPC-14597 alone. On the other hand, the mean spontaneous firing rate during simultaneous application of SCH 23390 with OPC-14597 was 33.80 ± 2.43 ($n = 10$)% of the control value.

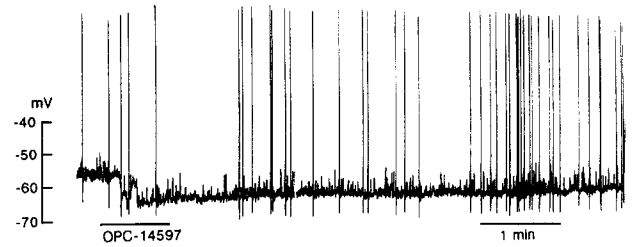

Fig. 6. Effect of bath application of OPC-14597 (OPC) at a concentration of 10^{-5} M on the membrane potential and spontaneously occurring action potentials of a large ventral tegmental area neuron. The period during drug application is indicated by the horizontal bar.

3.4. Effect of OPC-14597 on large ventral tegmental area neurons in a thin-slice preparation

Whole-cell recordings were made from 20 visually identified large neurons in the ventral tegmental area, the diameter of which was more than 20 μm (Fig. 5). The electrophysiological properties of these neurons were consistent with those found in previous studies on dopaminergic neurons in the substantia nigra or ventral tegmental area using conventional intracellular recording techniques (Grace and Bunney, 1980; Lacey et al., 1987, 1990; Grace and Onn, 1989, Mueller and Brodie, 1989; Johnson and North, 1992; Momiyama et al., 1993a,b). Briefly, action potentials with long duration followed by a large after hyperpolarization and low-frequency (0–5 Hz) spontaneous firing rate were seen. The mean resting membrane potential and cell capacitance of neurons recorded in the present study were -56.7 ± 0.8 mV ($n = 18$) and 37.0 ± 3.3 pF ($n = 15$), respectively.

Under current-clamp conditions, bath application of dopamine at a concentration of 10^{-5} M hyperpolarized the membrane of large neurons in the ventral tegmental area. The mean amplitude (with S.E.) of hyperpolarization induced by dopamine (10^{-5} M) was 8.4 ± 0.7 mV ($n = 5$).

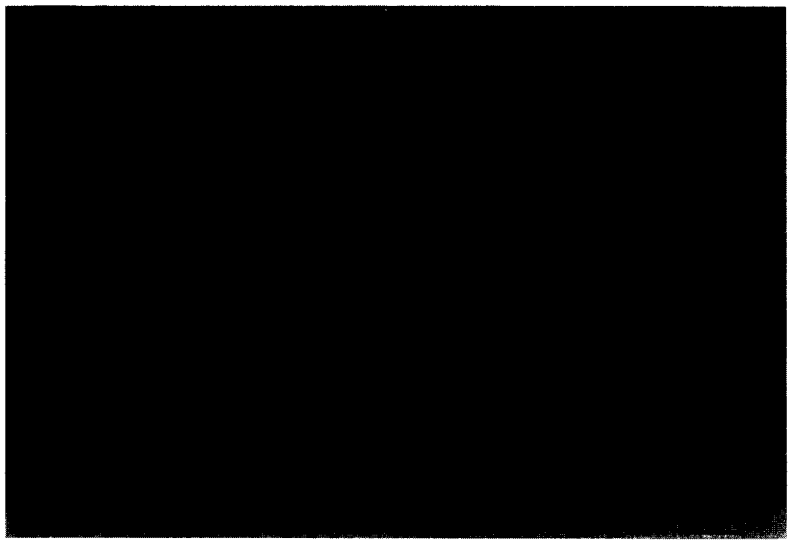

Fig. 5. Large neurons in the ventral tegmental area viewed under Nomarski optics. Calibration: 20 μm.

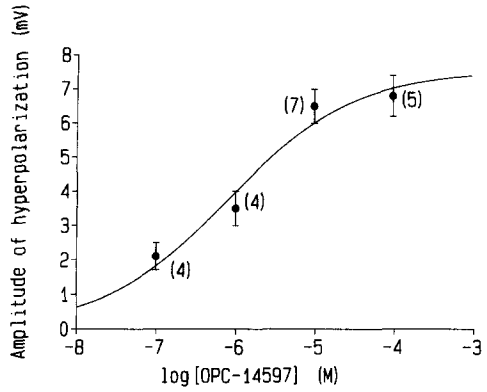

Fig. 7. Relationship between the concentration of bath-applied OPC-14597 and amplitude of hyperpolarization of the membrane potential in large ventral tegmental area neurons (points are means ± S.E.). Numbers of neurons tested are shown in parentheses. The data points were fitted by a sigmoid curve. The estimated EC_{50} value, maximum amplitude of hyperpolarization and Hill slopes were 0.83 ± 0.32 μM, 7.6 ± 1.5 mV and 0.54 ± 0.28, respectively.

Similarly to dopamine, OPC-14597 both hyperpolarized the membrane of large neurons in the ventral tegmental area, the hyperpolarizing effect being concentration-dependent between 10^{-7} and 10^{-4} M and inhibited spontaneously occurring action potentials (Fig. 6, Fig. 7). The mean amplitude of hyperpolarization induced by 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M OPC-14597 was 2.1 ± 0.4 (n = 4), 3.5 ± 0.5 (n = 4), 6.5 ± 0.5 (n = 7) and 6.8 ± 0.6 (n = 5) mV, respectively (Fig. 7). The data points were best fitted by a sigmoidal function. The estimated EC_{50} value, maximum amplitude of hyperpolarization and Hill slope were 0.83 ± 0.32 μM, 7.6 ± 1.5 mV and 0.54 ± 0.28, respectively. When OPC-14597 was applied repeatedly to the same neuron, it was applied at least 10 min after the termination of the previous application. Under such conditions, no desensitization was observed.

4. Discussion

The present study confirmed previous reports which classified the ventral tegmental area neurons projecting to the nucleus accumbens into two types; type I and type II, probably corresponding to dopaminergic and non-dopaminergic neurons, respectively (Bunney et al., 1973; Wang, 1981; Momiyama et al., 1990, 1991, 1993b). Wang (1981) reported that only 20% of non-dopaminergic neurons could be activated by nucleus accumbens stimulation. However, in the present study we recorded and analyzed only those neurons which were activated by nucleus accumbens stimulation. Therefore, it is possible that there are considerable populations of non-dopaminergic neurons in the ventral tegmental area which do not project to the nucleus accumbens as reported by Wang (1981).

Microiontophoretic application of dopamine inhibited both the antidromic spikes elicited by nucleus accumbens stimulation and spontaneous firing in dopaminergic type I neurons without affecting those in non-dopaminergic type II neurons, in agreement with previous reports (Wang, 1981; White and Wang, 1984a,b; Momiyama et al., 1990, 1991, 1993b). Similarly, OPC-14597 also inhibited both the antidromic spikes elicited by nucleus accumbens stimulation and spontaneous firing in the same type I neurons that were inhibited by dopamine, but did not affect those of type II neurons. In addition, the OPC-14597-induced inhibition of both antidromic spike generation and spontaneous firing in type I neurons was antagonized by simultaneous application of domperidone but not of SCH 23390. These results suggest that OPC-14597 inhibits neuronal activities of dopaminergic neurons in the ventral tegmental area via dopamine D_2-like receptors, without affecting the activity of non-dopaminergic neurons. Dopamine receptors are classified as D_1-like receptors composed of D_1 (or D_{1A}) and D_5 (or D_{1B}) receptors or D_2-like receptors composed of D_2 (D_{2S} and D_{2L}), D_3 and D_4 receptors. Recently, Sibley et al. (1994) have reported that OPC-14597 has the highest affinity for dopamine D_2 (D_{2S} and D_{2L}) receptors and 1/10 that of D_2 (D_{2S} and D_{2L}) receptors for dopamine D_3 receptors. Therefore, the effects of OPC-14597 are probably mediated by dopamine D_2 (D_{2S} and D_{2L}) and/or D_3 receptors; D_4 receptors could be excluded as OPC-14597 affinity is 1/400 less for the latter than for dopamine D_2 receptors. Furthermore, the observation that OPC-14597 and dopamine inhibited antidromic spikes induced by nucleus accumbens stimulation in type I neurons of the ventral tegmental area, implies that these drugs act on the receptors located in the dopaminergic neurons, including soma and dendrites.

In the present study, the inhibitory effect of OPC-14597 on dopaminergic neurons in the ventral tegmental area was confirmed using thin-slice preparations of the rat brain. The electrophysiological properties of the large neurons (> 20 μm) in the ventral tegmental area recorded in the present study were similar to those observed in the previous studies on dopaminergic neurons in the substantia nigra or in the ventral tegmental area (Grace and Bunney, 1980; Lacey et al., 1987, 1990; Grace and Onn, 1989; Mueller and Brodie, 1989; Johnson and North, 1992; Momiyama et al., 1993a). Therefore, it is suggested that the visually identified large neurons in the ventral tegmental area are included in the same population as those reported upon previously.

In the whole-cell current-clamp mode, hyperpolarization with concomitant inhibition of action potentials was observed in the large neurons of the ventral tegmental area on bath application of OPC-14597 as well as dopamine. This effect is consistent with the previously reported hyperpolarizing effect of dopamine or quinpirole, where the affinity for D_3 receptors is 40-fold higher than that for D_2 receptors (Sokoloff et al., 1992) on dopaminergic neurons in the substantia nigra (Lacey et al., 1987, 1990) or ventral tegmental area (Mueller and Brodie, 1989; Johnson and

North, 1992; Momiyama et al., 1993a). These findings suggest that OPC-14597 hyperpolarizes dopaminergic neurons in the ventral tegmental area via D_2 and/or D_3 receptors, probably by increasing membrane potassium conductance, thereby inhibiting neuronal activity in the ventral tegmental area.

Our recent in vivo electrophysiological study demonstrated that OPC-14597 antagonized the dopaminergic inhibition of the nucleus accumbens neurons receiving an input from the parafascicular nucleus of the thalamus by acting on both dopamine D_1- and D_2-like receptors located on postsynaptic sites without any agonistic effects when applied microiontophoretically (Amano et al., 1995). The findings that OPC-14597 acted as a dopamine D_2-like receptor agonist on putative dopaminergic neurons in the ventral tegmental area in the present study and that it acted as a dopamine D_1/D_2-like receptor antagonist on nucleus accumbens neurons in our previous investigation (Amano et al., 1995) are consistent with results of other recent studies: OPC-14597 was reported to inhibit the reserpine-induced increase in DOPA accumulation in the mouse and rat forebrain without inducing hyperlocomotion in reserpinized animals, indicating that the drug acts as an agonist on the presynaptic dopaminergic neurons in ventral tegmental area. In addition, the drug inhibited the apomorphine-induced stereotypy, hyperlocomotion and ipsilateral rotation which resulted from stimulation of contralateral dopamine receptors in kainate-lesioned rats, and the ipsilateral rotation which was due to an effect on postsynaptic intact dopamine receptors in kainic acid-lesioned animals (Kikuchi et al., 1995).

One explanation for such a unique profile of OPC-14597 might be receptor reserve. The pharmacological properties of the dopamine autoreceptors located on the dopamine cells on the ventral tegmental area and the substantia nigra pars compacta are not distinct from those of postsynaptic dopamine D_2 receptors in the nucleus accumbens or the caudate nucleus, but the former receptors are about 10-fold more sensitive to dopamine or dopamine receptor agonists than the latter (Roth, 1979). A large receptor reserve exists for the dopamine autoreceptors, which may explain in part why many dopamine receptor agonists are more potent for presynaptic than postsynaptic receptors (Meller et al., 1986, 1987). In addition, there are lower levels of receptor reserve for partial agonists than for full agonists (Cox and Waszczak, 1989). It is possible that OPC-14597 has postsynaptic agonist effects in animals in which the dopamine pathway has been denervated, since an apparent receptor reserve for a dopamine receptor agonist has been reported in the denervated striatum, despite the absence of receptor reserve in the intact striatum (Enz et al., 1990). However, this possibility could be excluded since OPC-14597 did not induce rotation in the rats with 6-hydroxydopamine-induced lesions of the dopamine pathway but inhibited the apomorphine-induced rotation (Kikuchi et al., 1995).

It has been reported that other dopaminergic agents, such as 3-PPP (*R*(+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine) and CI-1007 (*R*(+)-1,2,3,6,-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexen-1-yl)methyl]pyridine maleate), have a dopamine autoreceptor agonist and postsynaptic antagonist profiles similar to those of OPC-14597 (Clark et al., 1985; Ackerman et al., 1993; Meltzer et al., 1995). Although most of the effects induced by iontophoretic application of dopamine autoreceptor agonists, such as (+)-3-PPP, EMD 23448 (3-[4-(4-phenyl-1,2,3,6-tetrahydropyridine-1-yl) butyl]indole hydrochloride) or talipexole, are consistent with the results obtained after their systemic administration (Chiodo and Bunney, 1983; Clark et al., 1985; Todo et al., 1994; Matsubayashi et al., 1995), it has been reported that (−)-3-PPP shows an antagonist effect when applied intravenously, whereas when applied iontophoretically this agent acts as an agonist in the caudate nucleus (Clark et al., 1985). Since, in the present study, we focused on the effects of locally applied OPC-14597, the present data should be interpreted with caution. However, the above-mentioned findings of behavioral and electrophysiological studies in which OPC-14597 applied systemically and microiontophoretically inhibited the apomorphine-induced behavior and the dopamine-induced depression of nucleus accumbens neurons, respectively, could exclude the possibility that systemic application induces antagonistic effects and local application has agonistic effects.

In conclusion, OPC-14597 is a unique compound which acts as a dopamine D_2 and/or D_3 receptor agonist on dopaminergic neurons in the ventral tegmental area as well as a dopamine receptor antagonist on nucleus accumbens neurons (Amano et al., 1995).

Acknowledgements

The authors are grateful to Otsuka Pharmaceutical Company (Tokushima, Japan) for the gift of OPC-14597. This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 05770062) to T.M., and (No. 05304026) to M.S. and by Suzuken Memorial Foundation to M.S.

References

Ackerman, J.M., P.A. Johansen, D. Clark and F.J. White, 1993, Electrophysiological effects of putative autoreceptor-selective dopamine agonists on A10 dopamine neurons, J. Pharmacol. Exp. Ther. 265, 963.

Amano, T., H. Matsubayashi, T. Momiyama, K. Ishihara, N. Todo and M. Sasa, 1995, Antagonizing effects of a novel antipsychotic quinolinone derivative (OPC-14597) on dopaminergic inhibition of neuronal activities in the nucleus accumbens, Prog. Neuro-psychopharmacol. Biol. Psychiat. 19, 105.

Bunney, B.S., J.R. Walters, R.H. Roth and G.K. Aghajanian, 1973,

Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity, J. Pharmacol. Exp. Ther. 185, 560.

Chiodo, L.A. and B.S. Bunney, 1983, Electrophysiological studies on EMD 23448, an indolyl-3-butylamine, in the rat: a putative selective dopamine autoreceptor agonist, Neuropharmacol. 22, 1087.

Clark, D., G. Engberg, E. Pileblad, T.H. Svensson, A. Carlson, A.S. Freeman and B.S. Bunney, 1985, An electrophysiological analysis of the actions of the 3-PPP enantiomers on the nigrostriatal dopamine system, Naunyn-Schmiedebergs Arch. Pharmacol. 329, 344.

Cox, R.F. and B.L. Waszczak, 1989, Differences in dopamine receptor reserve for *N*-*n*-propylnorapomorphine enantiomers: single unit recording studies after partial inactivation of receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, Mol. Pharmacol. 35, 125.

Edwards, F.A., A. Konnerth, B. Sakmann and T. Takahashi, 1989, A thin-slice preparation for patch clamp recordings from neurons of the mammalian central nervous system, Pflügers Arch. 414, 600.

Enz, A., M. Goldstein and E. Meller, 1990, Dopamine agonist-induced elevation of striatal acetylcholine: relationship between receptor occupancy an response in normal and denervated rat striatum, Mol. Pharmacol. 37, 560.

Grace, A.A. and B.S. Bunney, 1980, Nigral dopamine neurons: intracellular recording and identification with L-dopa injection and histofluorescence, Science 210, 654.

Grace, A.A. and S.P. Onn, 1989, Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro, J. Neurosci. 9, 3463.

Johnson, S.W. and R.A. North, 1992, Two types of neuron in the rat ventral tegmental area and their synaptic inputs, J. Physiol. 450, 455.

Kikuchi, T., K. Tottori, Y. Uwahodo, T. Hirose, T. Miwa, Y. Oshiro and S. Morita, 1995, 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1*H*)-quinolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D2 receptor antagonistic activity, J. Pharmacol. Exp. Ther. 274, 329.

Kiuchi, K., Y. Hirata, M. Minami and T. Nagatsu, 1988, Effect of 7-{3-[4-(2,3-dimethylphenyl)piperazinyl]propoxy}-2(1*H*)-quinolinone (OPC-4392), a newly synthesized agonist for presynaptic dopamine D_2 receptor, on tyrosine hydroxylation in rat striatal slices, Life Sci. 42, 343.

Lacey, M.G., N.B. Mercuri and R.A. North, 1987, Dopamine acts on D_2 receptors to increase potassium conductance in neurons of the substantia nigra zona compacta, J. Physiol. 392, 397.

Lacey, M.G., N.B. Mercuri and R.A. North, 1990, Actions of cocaine on rat dopaminergic neurons in vitro, Br. J. Pharmacol. 99, 731.

Matsubayashi, H., T. Amano, Y. Honging, Y. Kohno and M. Sasa, 1995, Action of intravenously administered talipexole on the rat striatal neurons receiving excitatory input from nigral dopamine neurons, Psychopharmacology 120, 369.

Meller, E., E. Helmer-Matyjec, K. Bohmaker, C.H. Adler, A.J. Friedhoff and M. Goldstein, 1986, Receptor reserve at striatal dopamine autoreceptors: implications for selectivity of dopamine agonists, Eur. J. Pharmacol. 123, 311.

Meller, E., K. Bohmaker, Y. Namba, A.J. Friedhoff and M. Goldstein, 1987, Relationship between receptor occupancy and responses at striatal dopamine autoreceptors, Mol. Pharmacol. 31, 592.

Meltzer, L.T., C.L. Christoffersen, A.E. Corbin, F.W. Ninteman, K.A. Serpa, J.N. Wiley. L.D. Wise and T.G. Heffner, 1995, CI-1007, a dopamine partial agonist and potential antipsychotic agent. II. neurophysiological and behavioral effects, J. Pharmacol. Exp. Ther. 274, 912.

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

Momiyama, T., M. Sasa and S. Takaori, 1991, Inhibition by talipexole, a thiazolo-azepine derivative, of dopaminergic neurons in the ventral tegmental area, Life Sci. 49, 535.

Momiyama, T., N. Todo and M. Sasa, 1993a, A mechanism underlying dopamine D_1 and D_2 receptor-mediated inhibition of dopaminergic neurons in the ventral tegmental area in vitro, Br. J. Pharmacol. 109, 933.

Momiyama, T., M. Sasa and S. Takaori, 1993b, Enhancement of D_2 receptor agonist-induced inhibition by D_1 receptor agonist in the ventral tegmental area, Br. J. Pharmacol. 110, 713.

Mueller, A.L. and M.S. Brodie, 1989, Intracellular recording from putative dopamine-containing neurons in the ventral tegmental area of Tsai in a brain slice preparation, J. Neurosci. Methods 28, 15.

Ohmori, T., T. Koyama, T. Inoue, S. Matsubara and I. Yamashita, 1993, B-HT 920, a dopamine D_2 agonist, in the treatment of negative symptoms of chronic schizophrenia, Biol. Psychiat. 33, 687.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates, 2nd Ed., Academic Press, Sydney.

Roth, R.H., 1979, Dopamine autoreceptors: pharmacology, function and comparison with post-synaptic dopamine receptors, Commun. Psychopharmacol. 3, 429.

Sasa, M., Y. Ohno and S. Takaori, 1988, Presynaptic inhibition of excitatory input from the substantia nigra to caudate nucleus neurons by a substituted quinolinone derivative, 7-{3-[4-(2,3-dimethylphenyl)piperazinyl]propoxy}-2(1*H*)-quinolinone (OPC-4392), Life Sci. 43, 263.

Sibley, D.R., A.M. Gonzalez, L.H. Burgess, S.I. Max, B.N. Atkinson and L.-J. Zhang, 1994, Pharmacological characterization of OPC-14597, a novel antipsychotic agent, using cloned dopamine and serotonin receptors, Neuropsychopharmacology 10, Suppl. II, 252S.

Sokoloff, P., M. Andrieux, R. Besancon, C. Pilon, M.P. Martres, B. Giros and J.C. Schwartz, 1992, Pharmacology of human dopamine D_3 receptor expressed in a mammalian cell line: comparison with D_2 receptor, Eur. J. Pharmacol. 225, 331.

Takahashi, T., 1990, Membrane currents in visually identified motoneurons, J. Physiol. 423, 27.

Todo, N., T. Momiyama, T. Amano, Y. Kohno and M. Sasa, 1994, Excitation by talipexole, a dopamine D_2 agonist, of caudate nucleus neurons activated by nigral stimulation, Life Sci. 54, 957.

Wang, R.Y., 1981, Dopaminergic neurons in the ventral tegmental area. I. Identification and characterization, Brain Res. Rev. 3, 123.

White, F.J. and R.Y. Wang, 1984a, A 10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists, Life Sci. 34, 1161.

White, F.J. and R.Y. Wang, 1984b, Pharmacological characterization of dopamine autoreceptors in the rat ventral tegmental area: microiontophoretic studies, J. Pharmacol. Exp. Ther. 231, 275.

Yasuda, Y., T. Kikuchi, S. Suzuki, M. Tsutsui, K. Yamada and T. Hiyama, 1988, 7-{3-[4-(2,3-dimethylphenyl)piperazinyl]propoxy}-2(1*H*)-quinolinone (OPC-4392), a presynaptic dopamine autoreceptor agonist and postsynaptic D_2 receptor antagonist, Life Sci. 42, 1941.