

Presynaptic dopamine D₂-like receptors inhibit excitatory transmission onto rat ventral tegmental dopaminergic neurones

Eiko Koga and Toshihiko Momiyama

Department of Physiology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

(Received 5 August 1999; accepted after revision 18 November 1999)

1. The effects of dopamine (DA) on non-NMDA glutamatergic transmission onto dopaminergic neurones in the ventral tegmental area (VTA) were examined in rat midbrain slices using the whole-cell patch-clamp technique. EPSCs in dopaminergic neurones evoked by focal stimulation within the VTA were reversibly blocked by 5 μM CNQX in the presence of bicuculline (20 μM), strychnine (0.5 μM) and D-amino-5-phosphonopentanoic acid (D-AP5, 25 μM).
2. Bath application of DA reduced the amplitude of EPSCs up to $65.1 \pm 9.52\%$ in a concentration-dependent manner between 0.3–1000 μM (IC₅₀, 16.0 μM) without affecting the holding current at –60 mV measured using a Cs⁺-filled electrode.
3. The effect of DA on evoked EPSCs was mimicked by the D₂-like receptor agonist quinpirole but not by the D₁-like receptor agonist SKF 81297, and was antagonized by the D₂-like receptor antagonist sulpiride (K_B , 0.96 μM), but not by the D₁-like receptor antagonist SCH 23390 (K_B , 228.6 μM).
4. Dopamine (30 μM) reduced the mean frequency of spontaneous miniature EPSCs (mEPSCs) without affecting their mean amplitude, and the DA-induced effect on the mEPSCs was dependent on the external Ca²⁺ concentration.
5. These results suggest that afferent glutamatergic fibres which terminate on VTA dopaminergic neurones possess presynaptic D₂-like receptors, activation of which inhibits glutamate release by reducing Ca²⁺ influx.

The mesolimbic dopamine (DA) system, which originates in the ventral tegmental area (VTA) and which projects to the nucleus accumbens and prefrontal cortex, participates in various psychological functions such as locomotion (Koob & Swerdlow, 1988) or the reward aspects of food ingestion (Smith & Schneider, 1988), as well as associated disorders including schizophrenia (Nemeroff & Bissette, 1988) or drug abuse (Mereu *et al.* 1988). Although multiple DA receptors have been cloned (for review see Missale *et al.* 1998), functional characterization of DA receptors is still currently limited to the two pharmacologically identifiable families, D₁-like receptors, which activate adenylyl cyclase (AC), and D₂-like receptors, which inhibit or show no effect on AC (Kebabian & Calne, 1979). Previous electrophysiological studies have demonstrated that activation of D₂-like receptors on dopaminergic neurones in the VTA or in the substantia nigra pars compacta (SNc) hyperpolarizes the membrane by increasing inward rectifying potassium conductances (Lacey *et al.* 1987; Kim *et al.* 1995). In addition, synaptic potentials or currents mediated by GABA_A, GABA_B and glutamate receptors have been recorded from

dopaminergic neurones in the VTA or SNc (Mereu *et al.* 1991; Johnson & North, 1992; Cameron & Williams, 1993; Wu *et al.* 1995; Bonci & Williams, 1997; Wigmore & Lacey, 1998; Bonci & Malenka, 1999; Manzoni & Williams, 1999). Activation of D₁-like receptors located on the terminals of GABAergic afferents to VTA dopaminergic neurones augments GABA_B receptor-mediated inhibitory postsynaptic potentials (Cameron & Williams, 1993). In contrast, little information has been available regarding the function of DA in the modulation of excitatory synaptic transmission onto VTA dopaminergic neurones, despite the suggested significance of glutamatergic inputs to midbrain dopaminergic neurones in regulating firing pattern in these neurones (Grace & Bunney, 1984; Johnson *et al.* 1992; Zhang *et al.* 1994). Therefore, the present study was carried out to elucidate the effect of DA on non-NMDA glutamatergic synaptic transmission onto dopaminergic neurones in the VTA using the whole-cell patch-clamp technique in a thin-slice preparation of the rat brain.

Preliminary results have been published previously in an abstract form (Koga & Momiyama, 1998, 1999).

METHODS

Slice preparation and solutions

All experiments were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences of the Physiological Society of Japan (1998). Neonatal rats (7–17 days old) were decapitated under deep ether anaesthesia and the brains were removed. Horizontal brain slices (200 μm) containing the VTA were cut using a microslicer (DTK-2000, Dosaka, Kyoto, Japan) in an ice-cold oxygenated low Ca^{2+} , high Mg^{2+} Krebs solution of the following composition (mM): NaCl, 118; KCl, 3; CaCl_2 , 0.5; MgCl_2 , 6; NaHCO_3 , 25; Hepes, 5;

D-glucose, 11. The slices were then transferred to a holding chamber containing standard Krebs solution of the following composition (mM): NaCl, 118; KCl, 3; CaCl_2 , 2.5; MgCl_2 , 1.2; NaHCO_3 , 25; Hepes, 5; D-glucose, 11; pH 7.4 when bubbled with 95% O_2 –5% CO_2 . Slices were incubated in the holding chamber maintained at room temperature (21–25 $^\circ\text{C}$) for at least 1 h before recording.

Recording and data analysis

For recording, a slice was transferred to the recording chamber, held submerged, and superfused continuously with standard Krebs solution (bubbled with 95% O_2 –5% CO_2) at a rate of 4 ml min^{-1} .

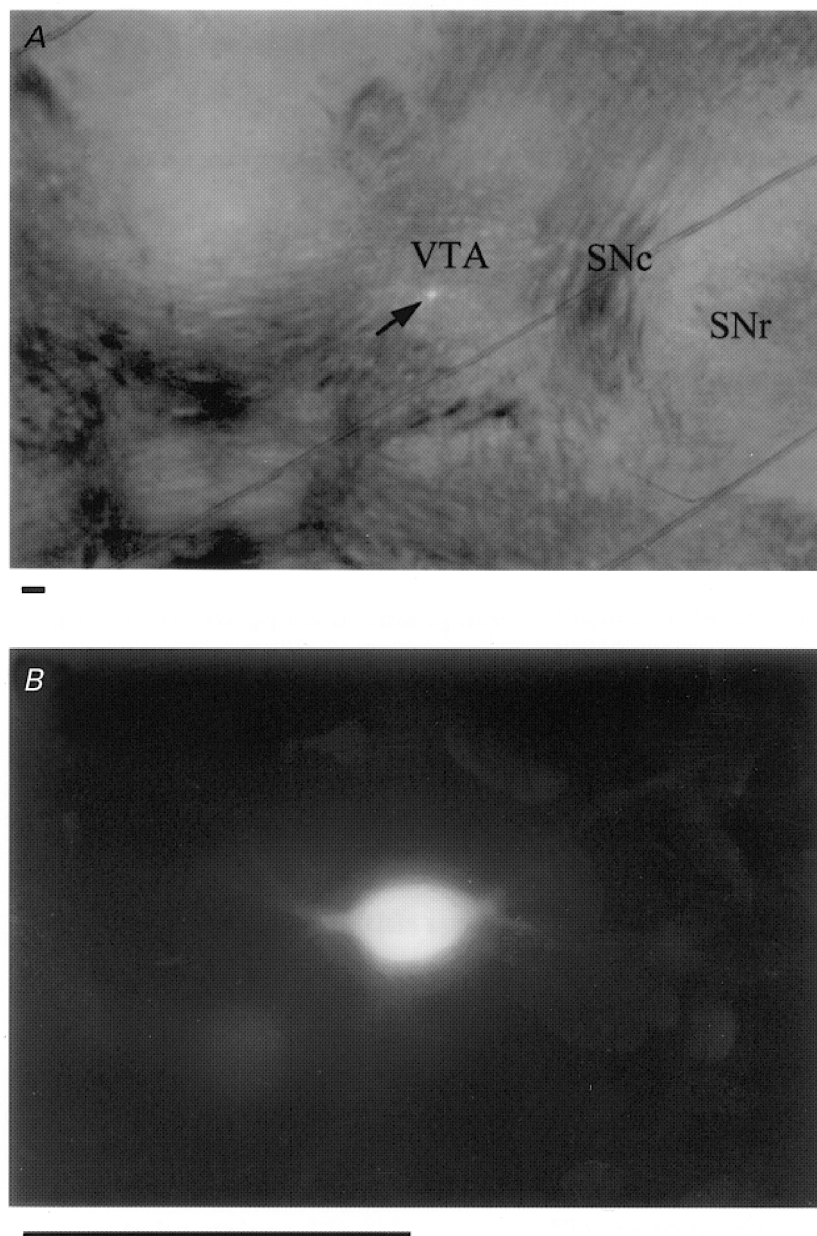


Figure 1. Visual characterization of the recorded neurone in the ventral tegmental area (VTA)

A, photomicrograph of the midbrain region of a horizontal slice obtained from a 15-day-old rat. A putative dopaminergic neurone was filled with Lucifer Yellow (arrow). The caudal part is oriented toward the lower edge of the photograph. SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata. The oblique lines represent a grid of nylon threads glued to a platinum frame to fix the slice on the bottom of the recording chamber. *B*, same neurone as in *A* at higher magnification. Scale bars, 100 μm .

Patch electrodes were pulled from standard-walled borosilicate glass capillaries (1.5 mm outer diameter; Clark Electromedical, Reading, UK) and had resistances of 3–7 M Ω when filled with a caesium chloride-based internal solution of the following composition (mM): CsCl, 140; NaCl, 9; Cs-EGTA, 1; Cs-Hepes, 10; Mg-ATP, 2 (pH adjusted with 1 M CsOH). Whole-cell recordings were made using an Axopatch 1D (Axon Instruments, Foster City, CA, USA) from visually identified large (> 24 μ m in diameter) neurones within the VTA viewed with a microscope fitted with a water-immersion lens (Nikon, Tokyo, Japan). Using a low-magnification lens, VTA was clearly visible as a grey area medial to the SNc in the horizontal slice (Fig. 1A), and was separated from the SNc by the medial terminal nucleus of the accessory optic tract or medial lemniscus depending on the plane (Wu *et al.* 1995; Paxinos & Watson, 1998). The reference electrode was a glass bridge containing 4% agar–saline, one end of which was placed in the recording chamber and the other in the internal solution-containing side chamber connected to the ground via an Ag–AgCl pellet. After establishing the whole-cell configuration, a stimulating electrode made from the same glass capillaries for the whole-cell pipettes and filled with 1 M NaCl was placed within a 50–120 μ m radius of the recorded neurone. A voltage pulse (0.2–0.5 ms in duration) of suprathreshold intensity was delivered extracellularly via the stimulation electrode to evoke synaptic current. The position of the stimulating electrode was varied until a stable response was evoked in the recorded cell. The mean distance between the stimulating electrode and the nearest edge of the recorded cell was 73.6 ± 1.66 μ m ($n = 101$ cells). Experiments were carried out at room temperature (21–25 $^{\circ}$ C).

Data were stored in a DAT recorder (DTR-1204, Biologic, France) (10 kHz). Off-line data acquisition was performed (low-pass filtered at 2–10 kHz with an 8-pole Bessel filter) using pCLAMP 6 software (Axon Instruments) and digitized at 10–20 kHz for computer analysis. Synaptic currents were routinely evoked at 0.2 Hz at a holding potential of –60 mV. For the analysis of mEPSCs, data were digitized at 20 kHz using Axotape software (Axon Instruments) and mEPSCs were detected using software generously provided by Dr Stephan F. Traynelis (Emory University, Atlanta, GA, USA). Curve-fitting was carried out using Origin (Microcal Software, Northampton, MA, USA) software. Data are expressed as means \pm s.e.m. Statistical analysis was carried out using Student's *t* test (two-tailed) or a non-parametrical Mann–Whitney test, where appropriate; $P < 0.05$ was considered statistically significant.

Drugs

Drugs were stored in frozen stock solutions and dissolved in the perfusion solution just prior to each experiment in the final concentration indicated. All drugs were bath-applied. (\pm)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF 81297), *trans*-(-)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo(3,4-*g*) quinoline hydrochloride (quinpirole), *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390), and *S*-(-)-5-amino-sulfonyl-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-2-methoxybenzamide (sulpiride) were purchased from Research Biochemicals International (Natick, MA, USA). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and *D*-(-)-2-amino-5-phosphonopentanoic acid (*D*-AP5) were from Toeris Cookson (Bristol, UK). Dopamine, bicuculline methochloride

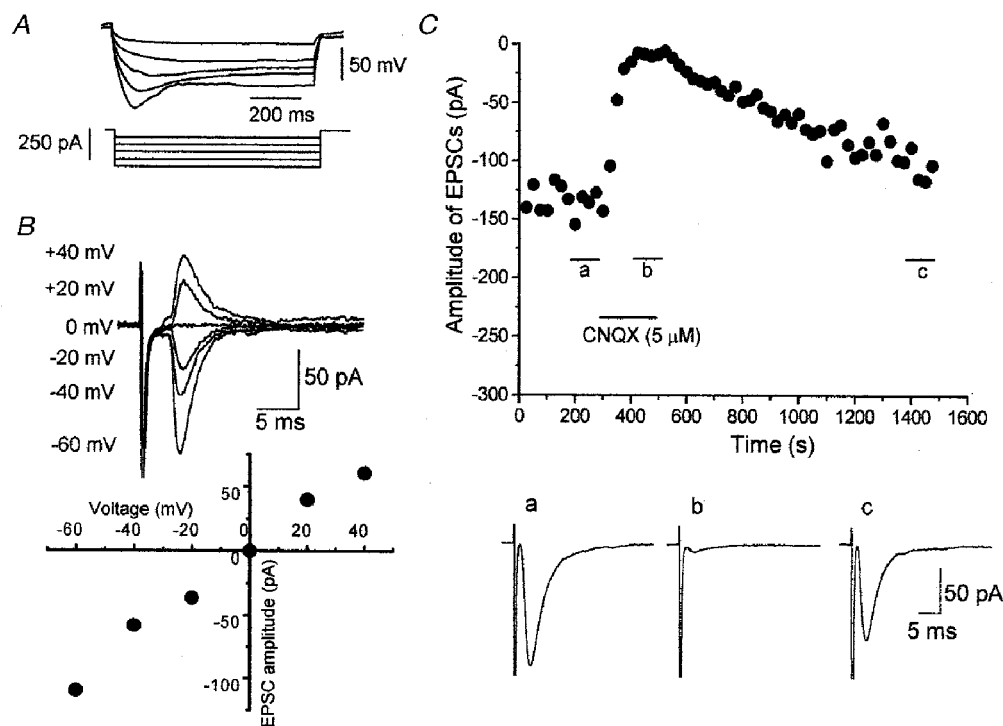


Figure 2. Membrane properties and EPSCs in VTA dopaminergic neurones

A, superimposed traces of membrane potential elicited by hyperpolarizing current pulses (800 ms in duration). Note the characteristic voltage sag during a large hyperpolarizing pulse. B, current–voltage relationship of EPSCs evoked at 0.2 Hz in the presence of bicuculline (20 μ M), strychnine (0.5 μ M) and D-AP5 (25 μ M). Each trace is the average of five responses. C, reversible blocking effect of CNQX (5 μ M) on the EPSCs at the holding potential of –60 mV. Each point represents the mean amplitude of five consecutive responses evoked at 0.2 Hz. Traces a–c are the averaged traces of 20 responses evoked during the periods indicated in the amplitude–time plot.

and strychnine were from Sigma (St Louis, MO, USA). TTX was from Sankyo (Tokyo, Japan).

RESULTS

General properties of non-NMDA EPSCs in VTA dopaminergic neurones

Whole-cell recordings were made from 561 visually identified neurones within the VTA (Fig. 1*A* and *B*). In the present study, 140 of these 561 neurones recorded in the VTA appeared to be dopaminergic on account of a prominent sag in the membrane potential during a large hyperpolarization (Fig. 2*A*). As reported in a previous study using dopaminergic and non-dopaminergic neurones in the substantia nigra pars reticulata (Radnikow & Misgeld, 1998), the voltage sag was observed even when the recording electrodes were filled with Cs⁺, similar to studies in dopaminergic neurones using K⁺-filled electrodes (Häusser *et al.* 1995; Mercuri *et al.* 1995). The cell capacitance of these neurones was 42.2 ± 0.97 pF ($n = 140$). The diameter of the recorded neurones (as measured from the longest axis) was 34.6 ± 0.31 μ m ($n = 124$).

Of these 140 putative dopaminergic neurones in the VTA, synaptic currents were evoked in 110 neurones by focal stimulation in the presence of bicuculline (20 μ M), strychnine (0.5 μ M) and D-AP5 (25 μ M) to eliminate GABAergic, glycinergic and NMDA glutamatergic components, respectively. The evoked synaptic currents reversed at around 0 mV (Fig. 2*B*), and were reversibly blocked by bath-applied

CNQX (5 μ M) (Fig. 2*C*), suggesting that they are non-NMDA glutamatergic excitatory postsynaptic currents (EPSCs). Access resistance was monitored during the experiments by applying a hyperpolarizing pulse (5 mV, 25 ms) at a holding potential of -60 mV. Some of these neurones were discarded because of the run-down of the evoked EPSCs or the change in access resistance during the control recording, and 77 neurones were used in the analysis of the effects induced by DA, DA receptor agonists, or the effects of DA receptor antagonists on the DA-induced effect. The mean amplitude of these evoked EPSCs was -84.9 ± 4.26 pA ($n = 77$) at a holding potential of -60 mV. The remaining 30 neurones were used in the analysis of the miniature EPSCs.

Effect of DA on evoked EPSCs

Figure 3*A* shows the effect of DA on the amplitude of evoked EPSCs in the presence of bicuculline (20 μ M), strychnine (0.5 μ M) and D-AP5 (25 μ M) at -60 mV. Bath application of DA produced a gradual decline in the amplitude of the evoked EPSCs, reaching a maximum level 2–4 min after the onset of application. The effect of DA was reversible after 3–10 min washout. The DA-induced inhibitory effect on the evoked EPSCs was concentration dependent between 0.3–1000 μ M. Figure 3*B* depicts the concentration–response curve pooled from 58 neurones. The estimated IC₅₀ value, mean maximum effect and Hill slope value were 16.0 μ M, 62.8% and 0.95, respectively. In the present slice preparation and recording condition, no

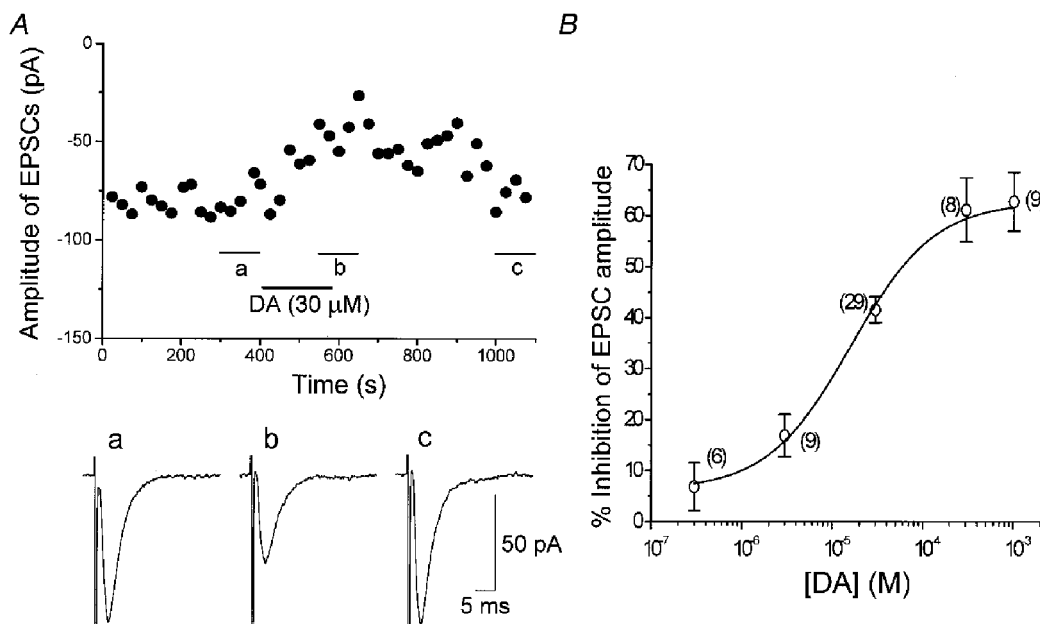


Figure 3. Inhibitory effect of DA on the evoked EPSCs in a VTA dopaminergic neurone

A, effect of DA (30 μ M) on the EPSCs evoked at 0.2 Hz in the presence of bicuculline (20 μ M), strychnine (0.5 μ M) and D-AP5 (25 μ M). The holding potential was -60 mV. Each point represents the mean amplitude of five consecutive responses evoked at 0.2 Hz. Traces *a*–*c* are the averaged traces of 30 responses evoked during the period indicated in the amplitude–time plot. *B*, concentration–response curve of DA-induced effect on the evoked EPSCs. Each point shows the mean \pm s.e.m. obtained from the pooled data. The estimated IC₅₀ value, maximum inhibition and Hill slope value were 16.0 μ M, 62.8% and 0.95, respectively. The numbers of neurones are shown in parentheses.

desensitization was observed when DA was applied repeatedly after at least 10 min washout intervals. Dopamine at concentrations up to 1 mM had no effect on the holding current and access resistance at -60 mV under the present recording conditions with Cs⁺-filled electrodes.

DA receptor pharmacology of DA-induced inhibition of evoked EPSCs

To elucidate the DA receptor subtypes mediating DA-induced inhibition of the evoked EPSCs, the effects of DA receptor agonists on the amplitude of evoked EPSCs (Fig. 4) and the effects of DA receptor antagonists on the DA-induced inhibition of the evoked EPSCs (Fig. 5) were investigated.

Effect of DA receptor agonists

Bath application of the D₁-like receptor agonist SKF 81297 at a concentration of 30 μ M had little or no effect on the amplitude of evoked EPSCs ($8.04 \pm 5.02\%$, $n = 9$) (Fig. 4A and C). On the other hand, the D₂-like receptor agonist quinpirole (30 μ M) inhibited the amplitude of evoked EPSCs (Fig. 4B). The quinpirole (30 μ M)-induced inhibition of the evoked EPSCs was $51.1 \pm 7.10\%$ ($n = 6$) of the control, which was significantly ($P < 0.001$) different from SKF81297-induced effect. The effect of quinpirole (30 μ M) was rather larger than that of DA (30 μ M) ($41.6 \pm 2.57\%$, $n = 29$), but the difference was not significant ($P = 0.15$) (Fig. 4C).

Effect of DA receptor antagonists on DA-induced inhibition of evoked EPSCs

The effects of the D₁-like receptor antagonist SCH 23390, and the D₂-like receptor antagonist sulpiride on the DA-induced inhibition of evoked EPSCs were examined. The D₁- or D₂-like antagonist was perfused for at least 5–10 min after the recovery of evoked EPSCs from DA-induced inhibition, then DA was applied again in the continuing presence of the antagonist. Bath application of SCH 23390 (10 μ M) or sulpiride (5 μ M) alone had no effect on the amplitude of evoked EPSCs or the holding current at -60 mV. In the presence of SCH 23390, DA showed similar inhibition of the EPSCs to that observed with DA alone (Fig. 5A). The concentration–response curve for DA remained unchanged in the presence of SCH 23390, with an estimated IC₅₀ value of 16.7 μ M (16.0 μ M in the case of DA alone) (Fig. 5A). On the other hand, DA-induced inhibition of the evoked EPSCs was reduced in the presence of sulpiride (Fig. 5B). The concentration–response curve for DA was shifted to the right in the presence of sulpiride, with an IC₅₀ value of 99.7 μ M (Fig. 5B). Assuming that both SCH 23390 and sulpiride behave as competitive antagonists, apparent K_B values of 228.6 μ M (pK_B 3.64) and 0.96 μ M (pK_B 6.02), respectively, were calculated using the equation $K_B = [B]/(DR - 1)$, where [B] is the concentration of the antagonist and DR (dose ratio) is the ratio of the IC₅₀ values for pooled data observed in the presence and absence of antagonist.

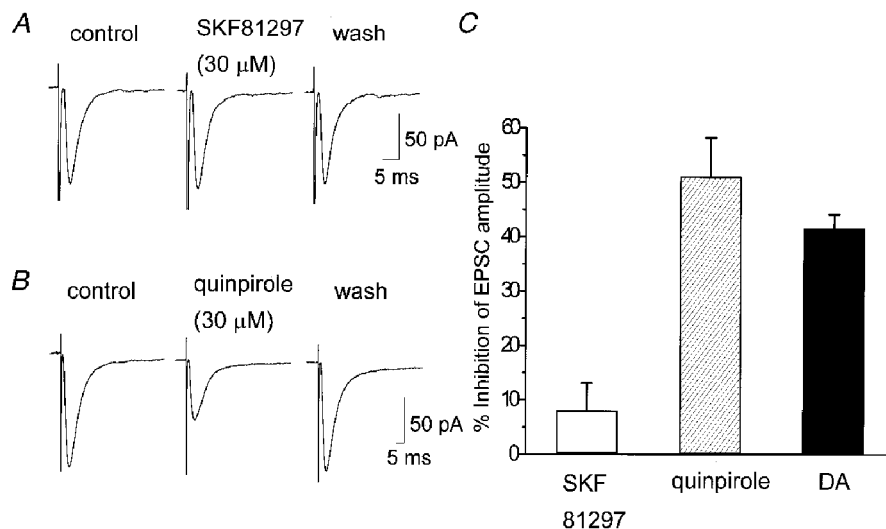


Figure 4. Effect of DA receptor agonists on the EPSCs

EPSCs were evoked at 0.2 Hz in the presence of bicuculline (20 μ M), strychnine (0.5 μ M) and D-AP5 (25 μ M). The holding potential was -60 mV. *A*, lack of effect of the D₁-like receptor agonist SKF 81297 (30 μ M) on the evoked EPSCs. The traces are the averaged traces of 20 consecutive responses in control, 4 min after the onset of drug application, and 4 min after washout of the drug, respectively. *B*, inhibitory effect of the D₂-like receptor agonist quinpirole (30 μ M) on the evoked EPSCs. The traces are the averaged traces of 20 consecutive responses in control, 2 min after the onset of drug application, and 4 min after washout of the drug, respectively. *C*, summarized histograms showing the means \pm s.e.m. of the inhibitory effects of SKF 81297, quinpirole and DA. All agonists were applied at 30 μ M. The value of DA was derived from the concentration–response curve in Fig. 3. Values for SKF 81297, quinpirole and DA were $8.04 \pm 5.02\%$ ($n = 9$), $51.1 \pm 7.10\%$ ($n = 6$) and $41.6 \pm 2.57\%$ ($n = 29$), respectively. The difference in the effects between SKF 81297 and quinpirole or SKF 81297 and DA was significant ($P < 0.001$), whereas the difference between quinpirole and DA was not significant ($P = 0.15$).

Effect of DA on miniature EPSCs

In order to determine whether the DA-induced inhibition of EPSCs is mediated by a presynaptic mechanism, the effect of DA on miniature EPSCs (mEPSCs) was analysed. dopaminergic neurones in the VTA exhibited spontaneous EPSCs in the presence of TTX ($0.3 \mu\text{M}$) in addition to bicuculline ($20 \mu\text{M}$), strychnine ($0.5 \mu\text{M}$) and D-AP5 ($25 \mu\text{M}$), and these spontaneous EPSCs were abolished by CNQX ($5 \mu\text{M}$) (Fig. 6A), so they were mEPSCs mediated by non-NMDA glutamate receptors. The mean frequency of mEPSCs in standard external Ca^{2+} concentration (2.5 mM) was $0.29 \pm 0.05 \text{ Hz}$ ($n = 8$). The frequency was increased to $0.80 \pm 0.14 \text{ Hz}$ ($n = 13$) when the external Ca^{2+} concentration was raised to 7.5 mM , and was decreased to $0.17 \pm 0.06 \text{ Hz}$ ($n = 5$) upon replacement of Ca^{2+} with Mg^{2+} (5 mM), suggesting that Ca^{2+} influx contributes to spontaneous glutamate release. Changing the external Ca^{2+} concentration had little or no effect on the amplitude of

mEPSCs. The effect of DA on the mEPSCs was analysed in the external solution containing 7.5 mM Ca^{2+} , 2.5 mM Ca^{2+} or Ca^{2+} -free solution. When the external Ca^{2+} concentration was changed, the control recording was started after at least 5 min monitoring of the mEPSCs.

In 7.5 mM Ca^{2+} solution, the frequency of mEPSCs was reduced by bath application of DA ($30 \mu\text{M}$), and reached a steady-state level within 3 min (Fig. 6B). The amplitude histogram shows a reduction in the total number of events but no change in the mean amplitude of mEPSCs (Fig. 6B). Pooled data from twelve VTA neurones showed that DA ($30 \mu\text{M}$) reduced the frequency of mEPSCs by $48.1 \pm 4.60\%$ (Fig. 7B), with no change in mean amplitude ($102.3 \pm 5.02\%$ of their respective control values). The inhibitory effect of DA on the frequency of mEPSCs was decreased when the external Ca^{2+} concentration was reduced. In 2.5 mM Ca^{2+} solution, DA ($30 \mu\text{M}$) reduced the frequency of mEPSCs by $30.2 \pm 6.79\%$ ($n = 7$) (Fig. 7B) without

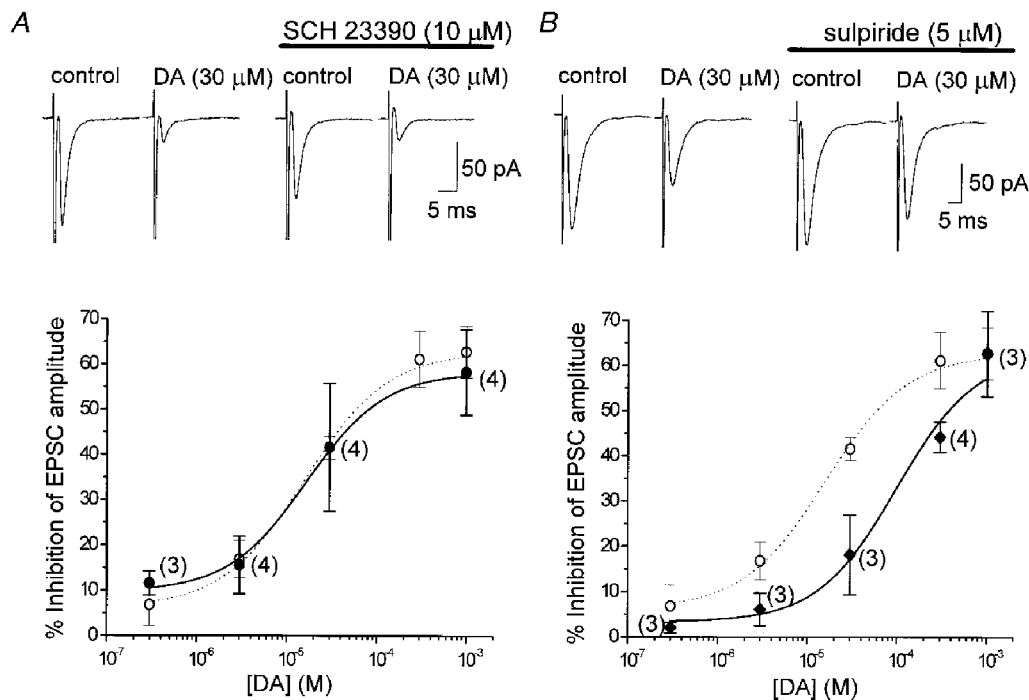


Figure 5. Effects of DA receptor antagonists on DA-induced inhibition of EPSCs

EPSCs were evoked at 0.2 Hz in the presence of bicuculline ($20 \mu\text{M}$), strychnine ($0.5 \mu\text{M}$) and D-AP5 ($25 \mu\text{M}$). Current traces in A and B are the averaged traces of 20 consecutive responses. The holding potential was -60 mV . All points represent means \pm s.e.m. The numbers of neurones examined in the presence of each antagonist are given in parentheses. Note that the concentration-response curves of DA alone (O) are the same as in Fig. 3B. All of the neurones examined in the presence of either antagonist are included in the concentration-response curve of DA alone (O). A, lack of effect of the D_1 -like receptor antagonist SCH 23390 ($10 \mu\text{M}$) on DA ($30 \mu\text{M}$)-induced inhibition. SCH 23390 was perfused for 6 min after EPSCs recovered from DA-induced inhibition. Then DA was applied again in the continuous presence of SCH 23390. The concentration-response curve for EPSC inhibition remained unchanged in the presence of SCH 23390 (●) compared with the curve in the absence of SCH 23390 (O). The estimated IC_{50} value, maximum inhibition and Hill slope were $16.7 \mu\text{M}$, 58.3% and 1.00 , respectively. B, antagonizing effect of the D_2 -like receptor antagonist sulpiride ($5 \mu\text{M}$) on the DA ($30 \mu\text{M}$)-induced inhibition of EPSCs. Sulpiride was perfused for 6 min before the second application of DA after the EPSCs recovered from DA-induced inhibition. The concentration-response curve was shifted to the right in the presence of sulpiride (◆) from the curve without sulpiride (O), with the estimated IC_{50} value, maximum effect and Hill slope of $99.7 \mu\text{M}$, 62.7% and 1.00 , respectively.

affecting mean amplitude ($96.2 \pm 2.69\%$, $n = 7$). In Ca^{2+} -free solution, DA ($30 \mu\text{M}$) had little or no effect on the frequency of mEPSCs (Fig. 7A), the mean reduction being $7.68 \pm 3.63\%$ ($n = 5$) (Fig. 7B). The mean amplitude of mEPSCs was also unaffected by DA ($97.9 \pm 3.20\%$, $n = 5$).

DISCUSSION

The present results have demonstrated that afferent glutamatergic fibres terminating onto VTA dopaminergic neurones possess presynaptic D_2 -like receptors, activation of which inhibits glutamate release. They also suggest that this presynaptic action of DA may be mediated mainly by blocking Ca^{2+} influx, an ionic mechanism different from that underlying D_2 -like receptor-mediated postsynaptic inhibition of neuronal activity of dopaminergic neurones in the VTA.

A limitation of the approach taken in the present study is that the source of the excitatory afferents cannot be

definitively identified. Glutamatergic innervation of the VTA has been reported to arise from three potential sources, the medial prefrontal cortex, pedunculo-pontine nucleus and subthalamic nucleus (for review see Kalivas, 1993). In addition, an electrophysiological study has recorded glutamatergic excitatory postsynaptic potentials in VTA dopaminergic neurones by stimulation of the habenula efferent fibres of the rat (Matsuda & Fujimura, 1992). Therefore, the EPSCs recorded in the present study may be evoked by stimulation of these fibres. Furthermore, a recent study has demonstrated that dopaminergic neurones in microculture show autaptic glutamatergic EPSCs, which are inhibited by activation of presynaptic D_2 -like receptors (Sulzer *et al.* 1998). Therefore, it might be possible that EPSCs evoked in the present study also include those mediated by glutamate released by the stimulation of nearby dopaminergic neurones. Actually, the idea that monoaminergic neurones as a class might release glutamate has been suggested by a morphological study using intact

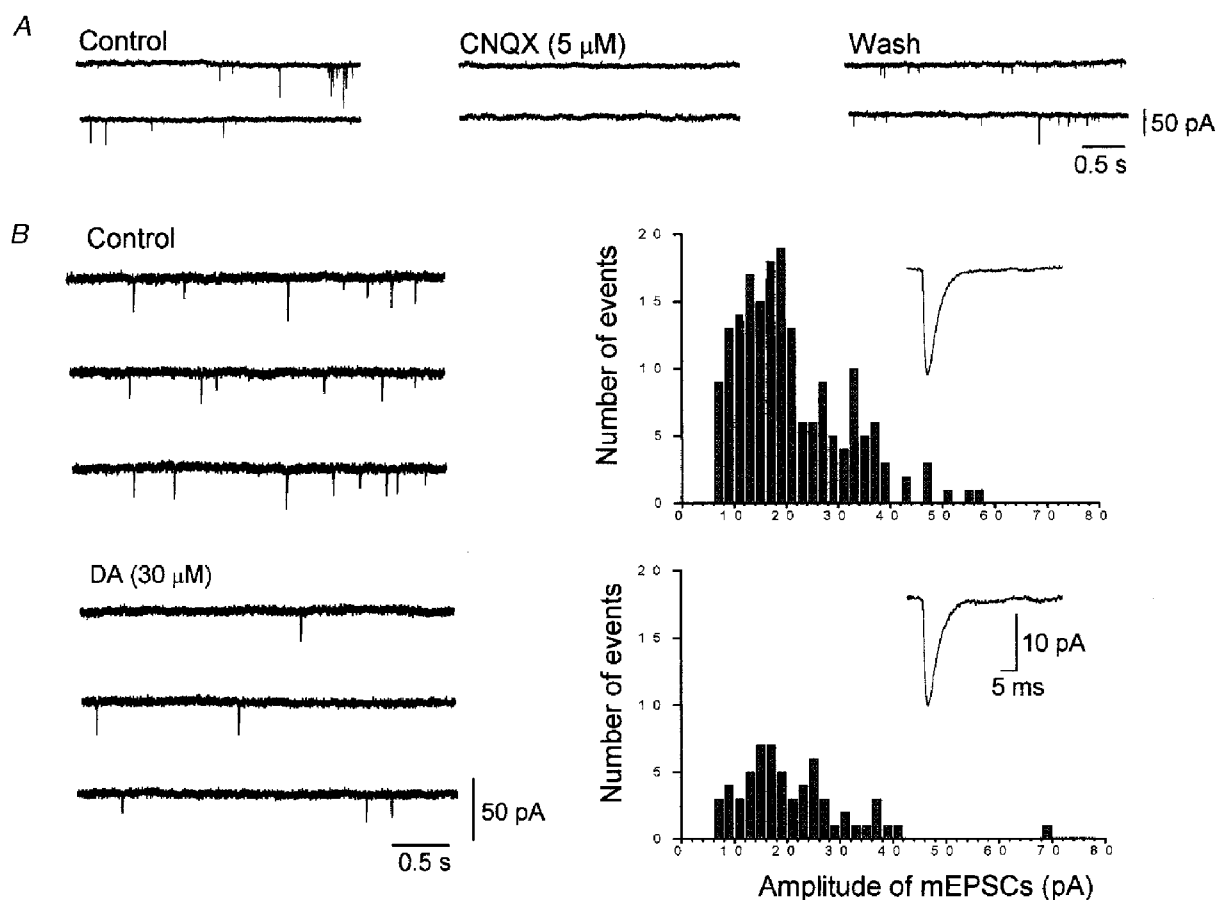


Figure 6. Effect of DA ($30 \mu\text{M}$) on spontaneous miniature EPSCs (mEPSCs)

The mEPSCs were recorded in 7.5 mM external CaCl_2 Krebs solution containing TTX ($0.3 \mu\text{M}$), bicuculline ($20 \mu\text{M}$), strychnine ($0.5 \mu\text{M}$) and D-AP5 ($25 \mu\text{M}$). The holding potential was -60 mV . *A*, consecutive traces taken in control, 2 min after application of CNQX ($5 \mu\text{M}$), and after 15 min washout of CNQX. The mEPSCs were reversibly abolished by $5 \mu\text{M}$ CNQX. *B*, consecutive traces were taken in control and 3 min after application of $30 \mu\text{M}$ DA. The amplitude histograms were derived from 5 min stretches in control and 2–7 min after the onset of DA application. These histograms show the reduction in frequency from 180 in control to 61 after application of DA. The inset traces in the histograms are the averaged traces of 180 events in control and 61 events during application of DA, respectively. The mean amplitudes of mEPSCs in control and in the presence of DA were 20.9 and 20.9 pA , respectively.

animals (Kaneko *et al.* 1990). Despite the indefinite source of glutamatergic afferents, the present approach enabled us to demonstrate robust and consistent inhibition of the EPSCs by DA.

The presynaptic locus of DA action on non-NMDA glutamatergic transmission onto dopaminergic neurones in the VTA was indicated by the present finding that DA or quinpirole, the D₂-like receptor agonist, did not induce an

outward current or had no effect on the access resistance with the caesium chloride internal solution which blocks potassium conductances, and a presynaptic mechanism was confirmed by the finding that DA reduced the frequency of mEPSCs without affecting their mean amplitude.

The inhibitory effect of DA on the frequency of mEPSCs was dependent on the external Ca²⁺ concentration, suggesting that DA reduced mEPSC frequency mainly by reducing

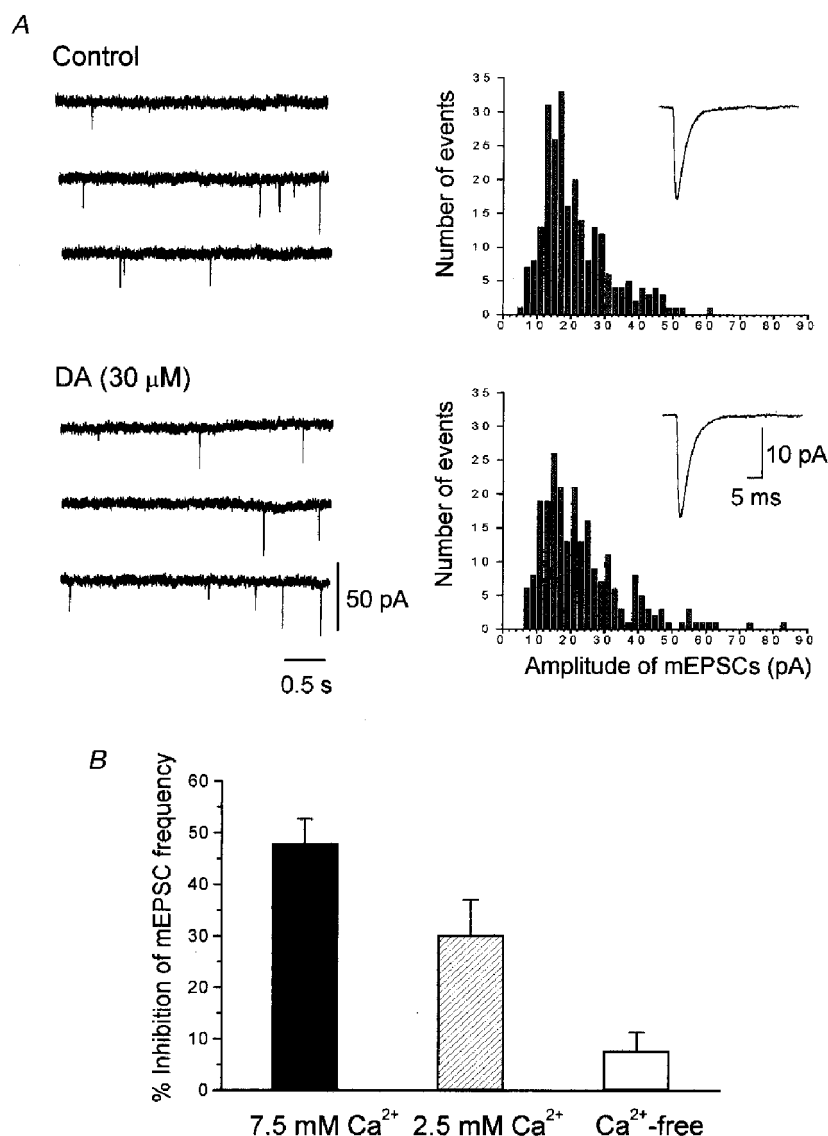


Figure 7. Lack of DA-induced effect on mEPSCs in Ca²⁺-free solution

A, the mEPSCs were recorded in Ca²⁺-free Krebs solution containing TTX (0.3 μM), bicuculline (20 μM), strychnine (0.5 μM) and D-AP5 (25 μM). The holding potential was -60 mV. The consecutive traces were taken from control and 3 min after application of DA (30 μM). The amplitude histograms were derived from 10 min stretches in control (241 events) and 2–12 min after the onset of DA application (231 events). The inset traces in the histograms are the averaged traces of 241 events in control and 231 events during DA application, respectively. The mean amplitudes of mEPSCs in control and during DA application were 21.2 and 23.2 pA, respectively. *B*, summarized histograms showing the inhibitory effect of DA (30 μM) on the frequency of mEPSCs in Ca²⁺ concentrations of 7.5, 2.5 and 0 mM. Values for 7.5 mM Ca²⁺, 2.5 mM Ca²⁺ and Ca²⁺-free solutions were 48.1 ± 4.60% (*n* = 12), 30.2 ± 6.79% (*n* = 7) and 7.68 ± 3.63% (*n* = 5), respectively. The differences in the inhibitory effects between 7.5 mM Ca²⁺ and 2.5 mM Ca²⁺, 2.5 mM Ca²⁺ and Ca²⁺-free, and 7.5 mM Ca²⁺ and Ca²⁺-free are all significant (*P* < 0.04, *P* < 0.03, and *P* < 0.0001, respectively).

Ca²⁺ influx, as previously reported in the case of D₁-like receptor-mediated presynaptic inhibition of glutamatergic transmission onto magnocellular basal forebrain neurones (Momiya *et al.* 1996) or GABAergic transmission onto nucleus accumbens neurones (Nicola & Malenka, 1997), as well as being responsible for the effects of enkephalin or the metabotropic glutamate receptor agonist *trans*-ACPD at other synapses (Hori *et al.* 1992; Sladeczek *et al.* 1993).

The present pharmacological results obtained with DA receptor agonists and antagonists suggest that the inhibitory effect of DA on the EPSCs is mediated by D₂-like receptors. Presynaptic D₂-like receptor-mediated inhibition of excitatory transmission is not itself a unique mechanism, since previous electrophysiological studies using intracellular recording techniques have reported the involvement of D₂-like receptors in the inhibition of excitatory postsynaptic potentials evoked in the nucleus accumbens (O'Donnell & Grace, 1994), striatum (Hsu *et al.* 1995) and hippocampus (Hsu, 1996). In addition, recent studies using whole-cell patch-clamp technique in brain slices have demonstrated the involvement of presynaptic D₁-like receptors in the modulation of excitatory synaptic transmission in several brain regions receiving dopaminergic fibres, including the nucleus accumbens (Harvey & Lacey, 1996; Nicola *et al.* 1996), basal forebrain nuclei (Momiya *et al.* 1996) and striatum (Umemiya & Raymond, 1997). These studies have shown that presynaptic D₁- or D₂-like receptors located on the terminals of glutamatergic afferents could modulate excitability of neurones receiving dopaminergic projections. The present study has demonstrated that presynaptic D₂-like receptors could also modulate dopaminergic neurone excitability. Although no desensitization was observed in the present study after at least 10 min washout intervals, a further detailed analysis will be necessary to elucidate the time course of the desensitization of the present presynaptic D₂-like receptors and recovery from it. In apparent contrast to the present results, a previous neurochemical study has reported D₁-like receptor-mediated facilitation of glutamate release in the VTA (Kalivas & Duffy, 1995). However, as the authors themselves have speculated in their discussion, the D₁-like receptor stimulation may selectively release glutamate from terminals synapsing on GABAergic neurones, which have been reported to inhibit dopaminergic neurones (Johnson & North, 1992). Analysis of DA-induced modulation of glutamatergic transmission onto non-dopaminergic neurones in the VTA will be necessary to clarify the issue. Another apparent discrepancy between the present findings and previous autoradiographic studies with 6-hydroxydopamine (6-OHDA) lesions of the dopaminergic pathway is that D₂-like receptors in the VTA or SNc are suggested to be located exclusively on dopaminergic neurones (Araki *et al.* 1998; for review Missale *et al.* 1998). One possibility might be that D₂-like receptors on the terminals of glutamatergic afferents terminating onto dopaminergic neurones could be down-regulated after 6-OHDA lesions.

Activation of D₂-like receptors on midbrain dopaminergic neurones hyperpolarizes the membrane, thereby inhibiting the neuronal activity (Lacey *et al.* 1987). The apparent K_B value of sulpiride, a D₂-like receptor antagonist, calculated from the present data (0.96 μ M) is higher than that calculated for postsynaptic D₂-like receptors on midbrain dopaminergic neurones (11.0–47.3 nM, Lacey *et al.* 1987). Such a difference, as well as the above-mentioned discrepancies, raises another possibility that the pharmacological and/or molecular biological profiles of D₂-like receptors in the glutamatergic terminals terminating onto dopaminergic neurones might be different from those on the dopaminergic neurones themselves. However, this possibility cannot be examined at present, since the pharmacological identification of the receptor is limited due to the lack of available ligands selective for each of the cloned subtypes of D₂-like receptors.

It has been shown that glutamatergic innervation to the VTA or SNc plays a crucial role in the switch from the pacemaker-like firing in dopaminergic neurones to burst firing (Grace & Bunney, 1984; Johnson *et al.* 1992; Zhang *et al.* 1994; Kang & Futami, 1999). To our knowledge, the exact site of contact of glutamatergic terminals with dopaminergic neurones in the rat VTA remains unclear. However, an ultrastructural study using squirrel monkey brain has shown that most glutamate-enriched terminals contact with dendrites of VTA dopaminergic neurones (Smith *et al.* 1996). In addition, a potential site for DA release has been demonstrated to be the dendrites of VTA dopaminergic neurones (Nirenberg *et al.* 1996). Therefore, under physiological conditions, DA released from the dendrites of VTA dopaminergic neurones may act on the D₂-like receptors in the nearby terminals of glutamatergic fibres, thereby regulating the firing pattern of VTA dopaminergic neurones by converting the burst firing induced by glutamate to pacemaker-like firing. Dopaminergic neurones in the VTA are thought to play roles in psychopathological behaviours (Nemeroff & Bissette, 1988), and it has been hypothesized that altered glutamatergic transmission contributes to the brain structures and the clinical symptoms of schizophrenia (cf. Tamminga, 1998). Changes in AMPA receptor response in VTA neurones have been demonstrated following adaptation to psychostimulants (Zhang *et al.* 1997; Bonci & Malenka, 1999). The actions of DA described here might participate in such alterations and provide clues for the elucidation of the underlying mechanisms of these brain functions, as well as for the pharmacological therapeutics of the associated disorders.

ARAKI, T., TANJI, H., KATO, H. & ITOYAMA, Y. (1998). Sequential changes of dopaminergic receptors in the rat brain after 6-hydroxydopamine lesions of the medial forebrain bundle. *Journal of the Neurological Sciences* **160**, 121–127.

BONCI, A. & MALENKA, R. C. (1999). Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *Journal of Neuroscience* **19**, 3723–3730.

- BONCI, A. & WILLIAMS, J. T. (1997). Increased probability of GABA release during withdrawal from morphine. *Journal of Neuroscience* **17**, 796–803.
- CAMERON, D. L. & WILLIAMS, J. T. (1993). Dopamine D1 receptors facilitate transmitter release. *Nature* **366**, 344–347.
- GRACE, A. A. & BUNNEY, B. S. (1984). The control of firing pattern in nigral dopamine neurons: Burst firing. *Journal of Neuroscience* **4**, 2877–2890.
- HARVEY, J. & LACEY, M. G. (1996). Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens *in vitro* via D₁ receptor activation. *Journal of Physiology* **492**, 143–154.
- HÄUSSER, M., STUART, G., RACCA, C. & SAKMANN, B. (1995). Axonal initiation and active dendrite propagation of action potentials in substantia nigra neurons. *Neuron* **15**, 637–647.
- HORI, Y., ENDO, K. & TAKAHASHI, T. (1992). Presynaptic inhibitory action of enkephalin on excitatory transmission in superficial dorsal horn of rat spinal cord. *Journal of Physiology* **450**, 673–685.
- Hsu, K.-S. (1996). Characterization of dopamine receptors mediating inhibition of excitatory synaptic transmission in the rat hippocampal slice. *Journal of Neurophysiology* **76**, 1887–1895.
- Hsu, K.-S., HUANG, C.-C., YANG, C.-H. & GEAN, P.-W. (1995). Presynaptic D₂ dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. *Brain Research* **690**, 264–268.
- JOHNSON, S. W. & NORTH, R. A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *Journal of Physiology* **450**, 455–468.
- JOHNSON, S. W., SEUTIN, V. & NORTH, R. A. (1992). Burst firing in dopamine neurons induced by N-methyl-D-aspartate: Role of electrogenic sodium pump. *Science* **258**, 665–667.
- KALIVAS, P. W. (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Research Reviews* **18**, 75–113.
- KALIVAS, P. W. & DUFFY, P. (1995). D₁ receptors modulate glutamate transmission in the ventral tegmental area. *Journal of Neuroscience* **15**, 5379–5388.
- KANEKO, T., AKIYAMA, H., NAGATSU, I. & MIZUNO, N. (1990). Immunohistochemical demonstration of glutaminase in catecholaminergic and serotonergic neurons of rat brain. *Brain Research* **507**, 151–154.
- KANG, Y. & FUTAMI, T. (1999). Arrhythmic firing in dopamine neurons of rat substantia nigra evoked by activation of subthalamic neurons. *Journal of Neurophysiology* **82**, 1632–1637.
- KEBABIAN J. W. & CALNE, D. B. (1979). Multiple receptors for dopamine. *Nature* **277**, 93–96.
- KIM, K.-M., NAKAJIMA, Y. & NAKAJIMA, S. (1995). G protein-coupled inward rectifier modulated by dopamine agonists in cultured substantia nigra neurons. *Neuroscience* **69**, 1145–1158.
- KOGA, E. & MOMIYAMA, T. (1998). Dopamine D₂-like receptor-mediated inhibition of glutamatergic synaptic transmission onto dopaminergic neurons in the rat ventral tegmental area. *Neuroscience Research Supplement* **22**, S100.
- KOGA, E. & MOMIYAMA, T. (1999). Presynaptic inhibition of excitatory transmission onto rat ventral tegmental dopaminergic neurons by dopamine. *Neuroscience Research Supplement* **23**, S76.
- KOOB, G. F. & SWERDLOW, N. R. (1988). The functional output of the mesolimbic dopamine system. *Annals of New York Academy of Sciences* **537**, 216–227.
- LACEY, M. G., MERCURI, N. B. & NORTH, R. A. (1987). Dopamine acts on D₂ receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. *Journal of Physiology* **392**, 397–416.
- MANZONI, O. J. & WILLIAMS, J. T. (1999). Presynaptic regulation of glutamate release in the ventral tegmental area during morphine withdrawal. *Journal of Neuroscience* **19**, 6629–6636.
- MATSUDA, Y. & FUJIMURA, K. (1992). Action of habenular efferents on ventral tegmental area neurons studied *in vitro*. *Brain Research Bulletin* **28**, 743–749.
- MERCURI, N. B., BONDI, A., CALABRESI, P., STEFANI, A. & BERNARDI, G. (1995). Properties of the hyperpolarization-activated cation current I_h in rat midbrain dopaminergic neurons. *European Journal of Neuroscience* **7**, 462–469.
- MEREU, G., COSTA, E., ARMSTRONG, D. M. & VICINI, S. (1991). Glutamate receptor subtypes mediate excitatory synaptic currents of dopamine neurons in midbrain slice. *Journal of Neuroscience* **11**, 1359–1366.
- MEREU, G., PASSINO, N., CAECANGIU, G. & GESSA, G. L. (1988). Electrophysiological evidences for the primary role of dopamine in the central effects of alcohol and other drugs of abuse. In *Neurodegenerative Disorders*, ed. NAPPI, G., HRNYKIEWICZ, O., AGNOLI, A., FARIELLO, R. G. & CLAVAZZA, A., pp. 287–301. Raven Press, New York.
- MISSALE, C., NASH, S. R., ROBINSON, S. W., JABER, M. & CARON, M. G. (1998). Dopamine receptors: from structure to function. *Physiological Reviews* **78**, 189–225.
- MOMIYAMA, T., SIM, J. A. & BROWN, D. A. (1996). Dopamine D₁-like receptor-mediated presynaptic inhibition of excitatory transmission onto rat magnocellular basal forebrain neurones. *Journal of Physiology* **495**, 97–106.
- NEMEROFF, C. B. & BISSETTE, G. (1988). Neuropeptides, dopamine, and schizophrenia. *Annals of the New York Academy of Sciences* **537**, 273–291.
- NICOLA, S. M., KOMBIAN, S. B. & MALENKA, R. C. (1996). Psychostimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D₁-like dopamine receptors. *Journal of Neuroscience* **16**, 1591–1604.
- NICOLA, S. M. & MALENKA, R. C. (1997). Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. *Journal of Neuroscience* **17**, 5697–5710.
- NIRENBERG, N. J., CHAN, J., LIU, Y., EDWARDS, R. H. & PICKEL, V. M. (1996). Ultrastructural localization of the vesicular monoamine transporter-2 in midbrain dopaminergic neurons: potential sites for somatodendritic storage and release of dopamine. *Journal of Neuroscience* **16**, 4135–4145.
- O'DONNELL, P. & GRACE, A. A. (1994). Tonic D₂-mediated attenuation of cortical excitation in nucleus accumbens neurons recorded *in vitro*. *Brain Research* **634**, 105–112.
- PAXINOS, G. & WATSON, C. (1998). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.
- RADNIKOW, G. & MISGELD, U. (1998). Dopamine D₁ receptors facilitate GABA_A synaptic currents in the rat substantia nigra pars reticulata. *Journal of Neuroscience* **18**, 2009–2016.
- SLADACEK, F., MOMIYAMA, A. & TAKAHASHI, T. (1993). Presynaptic inhibitory action of a metabotropic glutamate receptor agonist on excitatory transmission in visual cortical neurons. *Proceedings of the Royal Society B* **253**, 297–303.
- SMITH, G. P. & SCHNEIDER, L. H. (1988). Relationship between mesolimbic dopamine function and eating behavior. *Annals of the New York Academy of Sciences* **537**, 254–261.
- SMITH, Y., CHARARA, A. & PARENT, A. (1996). Synaptic innervation of midbrain dopaminergic neurons by glutamate-enriched terminals in the squirrel monkey. *Journal of Comparative Neurology* **364**, 231–253.

- SULZER, D., JOYCE, M. P., LIN, L., GELDWERT, D., HABER, S. N., HATTORI, T. & RAYPORT, S. (1998). Dopamine neurons make glutamatergic synapses *in vitro*. *Journal of Neuroscience* **18**, 4588–4602.
- TAMMINGA, C. A. (1998). Schizophrenia and glutamatergic transmission. *Critical Review of Neurobiology* **12**, 21–36.
- UMEMIYA, M. & RAYMOND, L. A. (1997). Dopaminergic modulation of excitatory postsynaptic currents in rat neostriatal neurons. *Journal of Neurophysiology* **78**, 1248–1255.
- WIGMORE, M. A. & LACEY, M. G. (1998). Metabotropic glutamate receptors depress glutamate-mediated synaptic input to rat midbrain dopamine neurones *in vitro*. *British Journal of Pharmacology* **123**, 667–674.
- WU, Y.-N., MERCURI, N. B. & JOHNSON, S. W. (1995). Presynaptic inhibition of γ -aminobutyric acid_B-mediated synaptic current by adenosine recorded *in vitro* in midbrain dopamine neurons. *Journal of Pharmacology and Experimental Therapeutics* **273**, 576–581.
- ZHANG, J., CHIOD, L. A. & FREEMAN, A. S. (1994). Influence of excitatory amino acid receptor subtypes on the electrophysiological activity of dopaminergic and nondopaminergic neurons in the substantia nigra. *Journal of Pharmacology and Experimental Therapeutics* **269**, 313–321.
- ZHANG, X.-F., HU, X.-T., WHITE, F. J. & WOLF, M. E. (1997). Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *Journal of Pharmacology and Experimental Therapeutics* **281**, 699–706.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and the grant from Yamanouchi Foundation for Research on Metabolic Disorders to T.M. The authors are grateful to Professor Y. Matsuda for useful comments and continual encouragement. We are also grateful to Dr Stephen F. Traynelis (Emory University) for the software to analyse miniature EPSCs.

Corresponding author

T. Momiyama: Laboratory of Cerebral Structure, National Institute for Physiological Sciences, Myodaiji, Okazaki 444-8585, Japan.

Email: tmomi@nips.ac.jp