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Glutamate Receptor Subtypes Mediate Excitatory Synaptic Currents of Dopamine Neurons in Midbrain Slices

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Although dopamine (DA)-containing neurons participate in a number of important cerebral functions, the physiology of their synaptic connections is poorly understood. By using whole-cell patch-clamp recording in thin slices of rat mesencephalon, we have investigated the biophysical properties of synaptic events and the nature of neurotransmitter(s) and receptors involved in the synaptic input to DA neurons in substantia nigra. The histological and electrophysiological characteristics of these cells were consistent with those described by recent *in vivo* and *in vitro* studies, thus allowing their unequivocal identification. Under appropriate experimental conditions, intranigral stimulation produced excitatory synaptic inputs in DA neurons. By voltage-clamp analysis, most of these excitatory postsynaptic currents (EPSCs) had a rise time of about 1.0 msec and a decay phase that could be fit by the sum of two exponential curves so that a fast and a slow component could be distinguished. The slow component was enhanced by glycine, by removing Mg^{2+} from the bath medium, or by membrane depolarization. Moreover, the slow component was consistently decreased by selective antagonists of NMDA receptors, whereas an antagonist for the non-NMDA receptors abolished the fast component slightly affecting the slow component and reduced peak EPSC amplitude. The results indicate that both NMDA-sensitive and non-NMDA-sensitive glutamate receptors contribute to EPSCs of DA neurons. Therefore, it is suggested that these receptors may play a critical role in the physiology (control of excitability, pacemaker firing, and dendritic DA release) as well as pathology (neuronal death in Parkinson's disease, psychosis, and mechanism of action of drugs of abuse, such as ethanol) related to DA neurons.

Mesencephalic dopamine (DA)-containing neurons participate in the complex network of basal ganglia and appear to play an important role in a number of neuronal functions and affective behaviors. For instance, it is well accepted that Parkinson's disease originates from a degenerative process affecting these cells (Hornykyewicz, 1966; Marsden, 1990). Furthermore, DAergic projections have been implicated in the etiology of various psychotic disorders (Snyder, 1973; McKenna, 1987), as well as in the mechanism of action of drugs of abuse (Mereu et al., 1988; Hurd et al., 1989). However, in spite of the fact that pharmacology and electrophysiology of DA neurons have been investigated extensively in the last two decades (see Chiodo and Freeman, 1987), several aspects of their membrane properties and of their synaptic connections remain uncertain. Intracellular recordings from slice preparations have shown that these neurons characteristically exhibit a prominent afterhyperpolarization, followed by a very slow depolarization triggering the spontaneous generation of action potentials, which occur fairly regularly and at a slow rate (Llinas et al., 1984; Kita et al., 1986; Chiodo and Freeman, 1987; Grace and Onn, 1989; Harris et al., 1989; Lacey et al., 1989). Such pacemakerlike activity is maintained in dissociated DA neurons and appears to be controlled by at least five potassium and two calcium conductances (Harris et al., 1989; Silva et al., 1990). Previous *in vivo* and *in vitro* studies have demonstrated that DA, GABA, high concentrations of glycine, and baclofen (an agonist of the $GABA_B$ receptor) inhibit the spontaneous activity of DA neurons (Grace and Bunney, 1985; Chiodo and Freeman, 1987; Lacey et al., 1988, 1989; Mercuri et al., 1990). On the other hand, DA neurons are excited *in vivo* by iontophoretically applied glutamate (Scarnati et al., 1986). However, our goal was to gain information on the synaptic events that regulate DA neuron activity. Indeed, while anatomical and biochemical evidence (see Storm-Mathisen and Ottersen, 1989) indicates that the substantia nigra (SN) does receive corticofugal innervation from glutamate-containing terminals, little is known about the electrophysiology of this input, except that EPSPs (Kang et al., 1989) and orthodromic extracellular spikes can be generated by subthalamic and pedunculopontine nuclei stimulation (Hammond et al., 1983; Scarnati et al., 1986). Moreover, the latter input appears to be suppressed by glutamate antagonists (Scarnati et al., 1986), though the drugs used were not specific for glutamate receptor subtypes.

Therefore, to characterize the synaptic input to DA neurons in the substantia nigra pars compacta (SNC) and to identify the neurotransmitters and receptors involved, we performed low-noise, high-resolution, whole-cell recordings (Hamill et al., 1981) in slices of rat mesencephalon (Edwards et al., 1989).

Materials and Methods

Slice preparation. Rats, at age 15–21 d, were decapitated, and the brains were rapidly removed under ice-cold Ringer's solution. By using a vibrating microtome, 3–4 thin (150–200 μm) coronal slices were obtained from each brain (Edwards et al., 1989). Slices were studied at room

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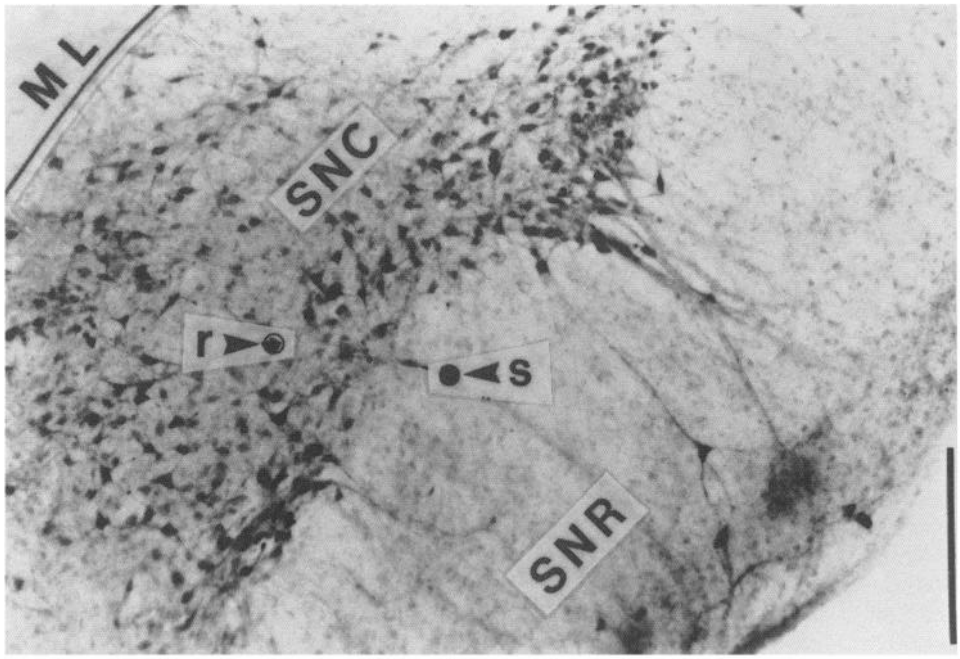

Figure 1. Photomicrograph showing an example of recording (*r*) and stimulating (*s*) sites. DAergic cell bodies and dendritic process are stained with TH antisera (see Materials and Methods). Cells positively identified as DAergic by their electrical membrane properties were always found within SNC. *ML*, medial lemniscus. Scale bar, 500 μm.

temperature (21–24°C) on the stage of an upright microscope (Zeiss UEM, GER) equipped with interferential-contrast Nomarski optics.

Solutions and drugs. Ringer's solution contained (in mM) NaCl (120), KCl (3.1), Na_2HPO_4 (1.25), $NaHCO_3$ (26), dextrose (5.0), $MgCl_2$ (1.0), and $CaCl_2$ (2.0). The solution was maintained at pH 7.4 by bubbling with 5% CO_2, 95% O_2. Patch pipettes were filled with (in mM) K-gluconate (145), $MgCl_2$ (1.0), EGTA (5.0), ATP (2.0), and HEPES-KOH (10) to pH 7.2. CsCl (145 mM) was substituted for K-gluconate in the current to voltage-curve experiments. Drugs were added to the Ringer's solution, which was perfused at a rate of 5 ml/min. All drugs were kept in stock solution in the dark. Glycine (Sigma, St. Louis, MO) was dissolved in the Ringer's solution; 7-chlorokynurenic acid (7-Cl-KA; Tocris, Buckhurst Hill, UK), in dimethyl sulfoxide (final dilution, ≥0.1%); picrotoxin (Sigma), in hot water; mecamylamine (Sigma) and DL-2-amino-5-phosphonovalerate (APV; Tocris), in water; and 6-cyano-7-dinitroquinoxaline-2,3-dione (CNQX; Tocris), in 20 mM Tris.

Electrophysiology. Low-resistance (3–5 MΩ) patch electrodes were pulled (Narishige, PP-83, Tokyo, Japan) from borosilicate glass capillaries of 1.8 mm outer diameter (Drummond, Broomall, PA). No fire polishing or Sylgard coating was used. Series resistance (10 MΩ), in whole cell configuration, was compensated and monitored for constancy throughout the experiment. Current and voltage signals at the headstage of the patch-clamp amplifier (List EPC-7, Darmstad, GER) were filtered at 2.0 kHz (8-pole low-pass Bessel LP902, Frequency Devices, Haverhill, MA) and continuously displayed on an oscilloscope. Events of interest were captured on VCR magnetic tape (VR 10-A, Instrutech Corp., Elmont, NY) for off-line analysis (LSI 11/73 computer, Indec System, Sunny Vale, CA) at a sampling rate of 5 kHz. The amplitudes and decay time constants of synaptically evoked currents were determined by finding the values that gave the best (least-squares) fit to single or double exponential equation of the form

$$I_T(t) = I_f \cdot \exp(-t/\tau_f) + I_s \cdot \exp(-t/\tau_s),$$

where I_T is the EPSC total amplitude, t is time, I_f and I_s are the peak amplitudes, and τ_f and τ_s are the decay time constants of fast (*f*) and slow (*s*) components, respectively. The procedure, which uses a Simplex algorithm to fit the data to the equations, was entirely automated. The procedure also estimated the percent contribution of I_s to I_T as

$$\%I_s = 100 \cdot I_s/I_T.$$

Stimulation. Stimuli (square-wave electric pulses of 100–300-μA intensity, 50-μsec duration, and 0.2-Hz frequency) were delivered into the SN pars reticulata (SNR) through concentric electrodes (D. Kopf, Tujunga, CA) positioned 250–750 μm distally from the recorded cell, along a direction approximately perpendicular to the medial lemniscus (Fig. 1).

Immunohistochemistry. For intracellular staining, the tip of recording pipette was filled with Lucifer yellow CH (Sigma, 1.0 mg/ml in the recording solution). The dye was ejected by pulse (200 msec, 0.5 Hz) of negative current (1.0 nA) for 5 min. For tyrosine hydroxylase (TH) immunoreactivity, slices were placed in fixative (4% paraformaldehyde in 0.1 M phosphate buffer) and then processed for immunocytochemistry using an avidin-biotin immunolabeling procedure (Hsu and Raine, 1981) and a polyclonal antibody (Eugene Tech. Int., Allendale, NJ) against TH.

Results

Electrophysiological and immunohistological identification

Nigral neurons were localized under microscopic vision (total magnification, 500×), and those within the SNC (Fig. 1) were identified as DAergic versus non-DAergic on the basis of their respective *in vitro* electrophysiological properties (Llinas et al., 1984; Kita et al., 1986; Chiodo and Freeman, 1987; Grace and Onn, 1989; Harris et al., 1989; Lacey et al., 1989; Silva et al., 1990). Briefly, it was observed that putative DAergic neurons very often displayed spontaneous activity at a constant rate of 0.5–3.0 Hz. This activity became apparent upon suction to obtain the cell-attached gigaseal (>10 GΩ) configuration (Hamill et al., 1981), and it was maintained, after disruption of patch membrane, in whole-cell current-clamp mode (Fig. 2*A*). Indeed, at their resting potential of −58 ± 2.5 mV (mean ± SEM), these cells exhibited a characteristic pacemaker firing pattern consisting of isolated, wide action potentials of 2.5–5.0 msec duration (measured at the threshold level of −48 ± 4 mV) and spike amplitudes of 70–80 mV from threshold, which were followed by a pronounced and long afterhyperpolarization. Application of DA (5 μM) resulted in a rapid termination of activity associated with membrane hyperpolarization (Fig. 2*A*). Under hyperpolarizing current pulses, the membrane voltage deflections showed inward (anomalous) rectification (not shown).

On the contrary, non-DA cells were rarely spontaneously active at their resting potentials of −64 ± 4.2 mV. However, after

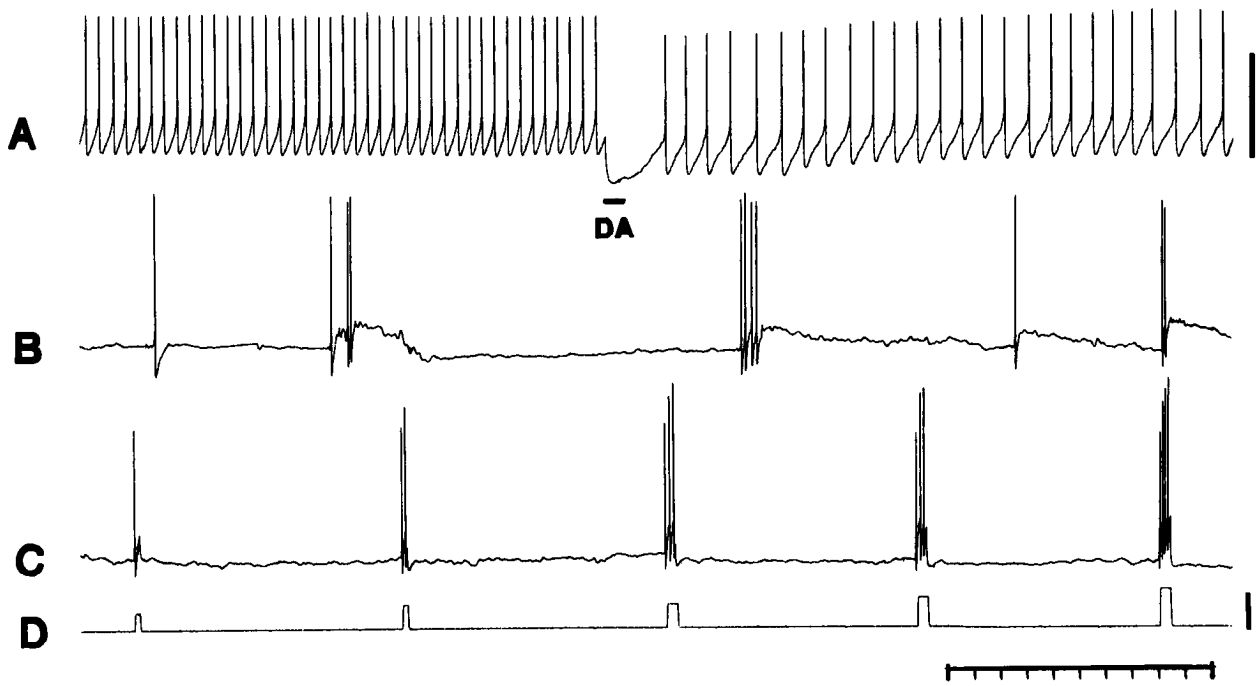

Figure 2. Whole-cell current-clamp recording from DA and non-DA neurons with electrodes filled with K-gluconate as major current carrier (see Materials and Methods). *A*, This DA neuron was spontaneously active, had resting potential of −56 mV, and exhibited the characteristically slow pacemaker firing (2 Hz) with wide action potentials (about 3.5 msec duration in this neuron) followed by long afterhyperpolarization. Application of DA (5 μM) resulted in the termination of spiking activity and membrane hyperpolarization. *B*, Example of a non-DA neuron (quiescent at its resting potential of −62 mV) depolarized to the threshold voltage (−50 mV) by current injection. Action potentials (1.8 msec) occurred singularly or in short bursts. *C*, In the same cell as in *B*, a similar firing pattern was elicited by depolarizing pulses (*D*; 100–200 pA, 400 msec) from resting potential. Calibration: 50 mV, 10 sec for *A–C*; 200 pA, 10 sec for *D*.

depolarization to the threshold voltage (−51 ± 4.8 mV), action potentials (≤2.2 msec) occurred singularly or packed in short bursts (Fig. 2*B*). Similar irregular activity was elicited in these non-DA cells by depolarizing pulses (100–200 pA), as shown in Figure 2*C*. The spontaneous or evoked activity of non-DA neurons was relatively insensitive to DA. Moreover, these neurons did not display inward rectification.

With the use of CsCl-filled electrodes, we measured the membrane input resistance to be 200–700 MΩ in both cell populations, a value much higher than that reported by conventional intracellular recording in slices (Grace and Onn, 1989), but comparable to that observed by Silva et al. (1990) using whole-cell recording of dissociated nigral DA neurons. The Lucifer yellow dye was injected into 12 neurons, positively identified as DAergic by their electrical properties. Cell bodies of DA neurons were of medium-size diameter (20–30 μm), had fusiform or multipolar shape, and generated two to five major dendritic processes extending medially or laterally within SNC or ventrally to SNR. Morphology of DA neurons is not shown because it was consistent with that already described in detail by Grace and Onn (1989) and Juraska et al. (1977).

Synaptic currents in SN neurons

Stimuli were delivered at low frequency (0.2 Hz) in the edge between SNC and SNR (Fig. 1), where the dense dendritic projections of DA neurons are located (Juraska et al., 1977). In DA neurons, voltage clamped at −50 mV, stimulation elicited pure excitatory postsynaptic currents (EPSCs) in 79% (26/33) of neurons in the presence of 50 μM picrotoxin (an antagonist of the $GABA_A$ receptor), as well as in 56% (18/32) of neurons in the absence of the toxin. The remaining DA neurons showed either no response, inhibitory postsynaptic currents (IPSCs), or mixed EPSCs–IPSCs. the majority of neurons (13/20) with membrane properties corresponding to those of non-DA cells located within the SNC also showed EPSCs. In a number of DA (n = 16) and non-DA (n = 9) neurons, spontaneous miniature EPSCs were present. However, for the purpose of the present study, we analyzed only pure EPSCs generated in identified DA neurons. Unless otherwise stated, evoked EPSCs were studied with perfusion solution containing glycine (1.0 μM; Johnson and Ascher, 1987), Mg^{2+} (1.0 mM; Mayer et al., 1984; Nowak et al., 1984), and picrotoxin (50 μM).

At holding potential (HP) close to the membrane resting potential (−60 to −50 mV), evoked EPSCs had peak amplitudes ranging from 10 to 300 pA, depending on the stimulus strength. At fixed stimulus intensity, amplitudes fluctuated between discrete values (Fig. 3*A*), as would be expected for either quantal release or variability in the number of presynaptic inputs activated. Most EPSC rise times were quite fast (about 1.0 msec), and we rejected those with a rise time >3.0 msec because, as suggested by Hestrin et al. (1990), they might reflect a poor spatial voltage clamp of the thin and extended distal dendrites of DA neurons (Juraska et al., 1977). In the experimental condition chosen, the decay phase could be fit by either a single exponential curve or by the sum of two exponentials. In 126 EPSCs recorded from five neurons, the decay phase had a single component with a time constant, τ, of 4.2 ± 0.61 msec, with a range of 2.1–8.3 msec. However, 935 EPSCs from 26 other cells were fit by two exponentials (Fig. 3*B*). The time constant of the initial fast component, τ_f, was 4.4 ± 0.32 msec, with a range of

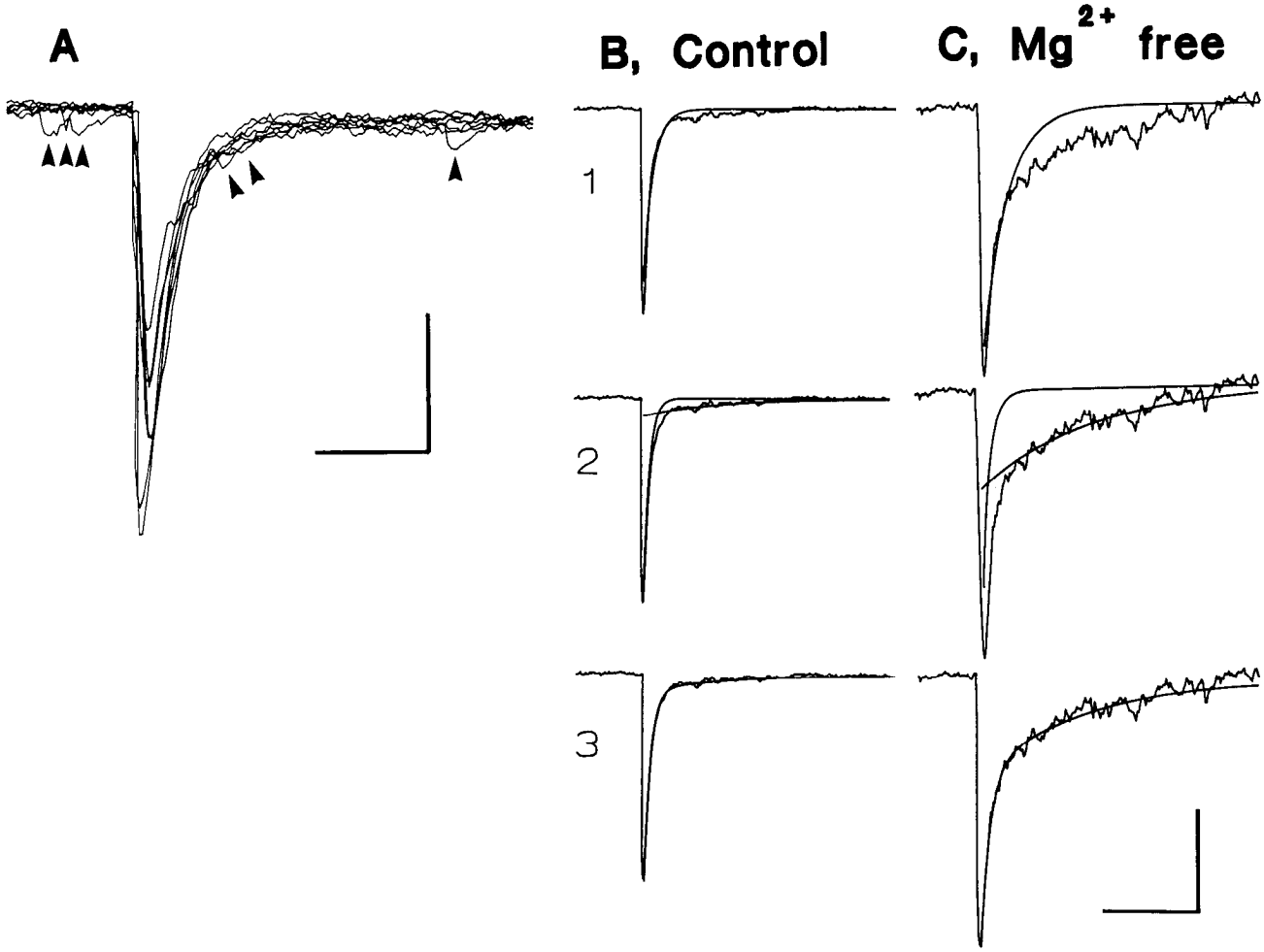

Figure 3. Properties of evoked EPSCs in DA cells. *A,* Seven evoked EPSCs have been superimposed to observe the fluctuation of amplitudes at a fixed stimulus intensity (150 μA). Note the occurrence of spontaneous miniature EPSCs (*arrowheads*). *B,* Fitting analysis of EPSC decay phases in control condition. The matching degree of one (superimposed) exponential curve (*1*), two distinct exponential curves (*2*), and the sum of them (*3*) is illustrated. *C,* Same analysis after omission of Mg^{2+} ions from Ringer's solution. Both control and Mg^{2+}-free EPSCs are the averages of five distinct events. In the presence of Mg^{2+} (1.0 mM), the slow exponential component, I_s, of this cell was 5% of the total current amplitude. It increased to 35% in the absence of Mg^{2+}. Calibration: 50 pA, 10 msec for *A*; 50 pA, 20 msec for *B*.

2.5–6.8 msec, whereas that of the slow component, τ_s, was 39 ± 3.1 msec, with a range of 14–65 msec (see Table 1). The contribution of the slow component, I_s, to the total peak amplitude, I_T, was 16 ± 4.2%, with a range of 0.5–38%. The slow component of the synaptic current decay phase was enhanced either by removing Mg^{2+} (Fig. 3*C*) or by membrane depolarization to values more positive than −30 mV (Fig. 4*A,B*). Conversely, in the presence of 1.0 mM Mg^{2+}, the slow component was reduced or abolished by membrane hyperpolarization greater than −60 mV (Fig. 4*A,B*) or by omitting glycine from the perfusion solution, as described by Forsythe and Westbrook (1988). The voltage dependence of I_T and I_s amplitudes is shown in Figure 4*B*. While the I_T/V relation was linear, with the exclusion of a notch at −60 mV, the relation between voltage and I_s was J-shaped, with a region of a negative slope between −70 and −30 mV. The reversal potential was close to 0 mV for both currents.

Pharmacology of EPSCs

The biophysical properties of EPSCs generated in SNC neurons were similar to those mediated by glutamate receptor activation in other neuronal populations (Forsythe and Westbrook, 1988), most notably in CA1 hippocampal neurons (Hestrin et al., 1990). However, with respect to these studies, EPSC kinetics of DA neurons appear to be faster. A possible cholinergic contribution was considered unlikely because application of mecamylamine (25 μM), a nicotinic ACh receptor antagonist, failed to affect EPSCs from DA neurons (n = 3; not shown), and also because the EPSCs mediated by muscarinic receptors have been reported (Cole and Nicoll, 1983) to be much slower. Therefore to determine whether EPSCs in DA neurons were actually mediated by glutamate and, if so, to characterize the subtypes of receptor involved, we studied EPSC kinetics in the presence of different and specific glutamate receptor antagonists. Indeed, it is known that, though both NMDA-sensitive and kainate- or quisqualate-sensitive (i.e., non-NMDA) glutamate receptors are activated during excitatory synaptic input, the slow (NMDA) and fast (non-NMDA) components of EPSCs are pharmacologically separable (Forsythe and Westbrook, 1988; Hestrin et al., 1990).

As shown in Figure 5 and Table 1, APV (50 μM), an NMDA receptor antagonist (Monaghan et al., 1989), reduced the peak-amplitude I_T of EPSCs by 40%, mainly by affecting the percent

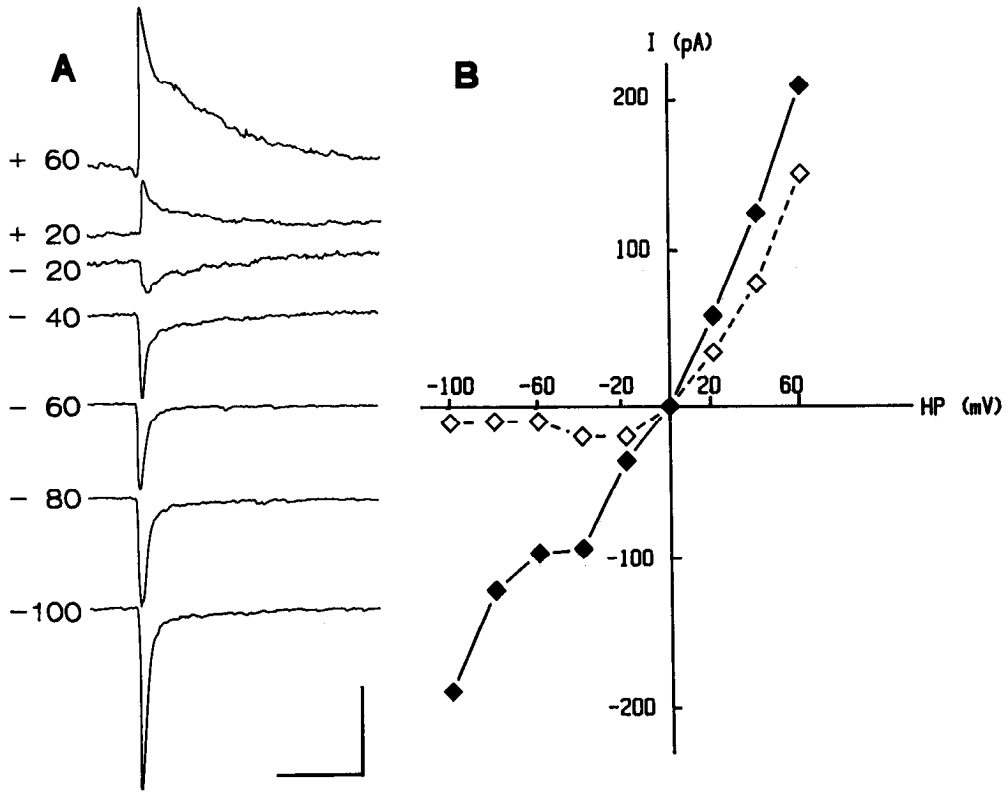

Figure 4. Voltage dependence of EPSCs in DA neurons. *A*, Evoked EPSCs recorded at different HPs (mV) as indicated. Each *trace* is the average of 5–10 events. Calibration, 50 pA, 20 msec. *B*, Current versus voltage relation of the EPSC averages, shown in *A*, for I_T (◆) and I_s (◇).

contribution of I_s to I_T, which was reduced by 53%. Conversely, the non-NMDA receptor antagonist CNQX (10 μM; Forsythe and Westbrook, 1988; Hestrin et al., 1990), completely eliminated the fast component; as a consequence, I_T was reduced to 15% of baseline and became equal to I_s.

A concentration of 50 μM of 7-Cl-KA preferentially antagonizes NMDA-sensitive receptors through the blockade of their coupled glycine-sensitive allosteric modulatory site (Kemp et al., 1988), with some antagonistic action also on the non-NMDA receptors, however. When 50 μM 7-Cl-KA was added in the perfusion solution, both I_T and the percentage contribution of I_s to I_T were inhibited by approximately 50% and 80% of control values, respectively. After removal of 7-Cl-KA from the perfusion solution, complete recovery of I_T occurred in about 10 min. However, in the case of neurons (n = 6) in which the percentage contribution of I_s to I_T was appreciable (≥5%) before 7-Cl-KA treatment, I_s component gradually reappeared in about 5–8 min of washout. Coapplication of CNQX (10 μM) and 7-Cl-KA (50 μM), or CNQX perfusion soon after 7-Cl-KA discontinuation, completely abolished EPSCs (not shown). In five cells, perfusion with either APV (10 μM) or 7-Cl-KA (50 μM) was associated with a long-lasting outward current of up to 400 pA and a decrease in membrane conductance. A comparable effect (not shown) was also produced (n = 3) by 1.0 μM 7-Cl-KA. Figure 6 illustrates a case of 7-Cl-KA (50 μM) application.

Effect of drugs on EPSC kinetics

As stated, under control conditions, the rise time of EPSCs ranged from 0.8 to 1.5 msec, while the fast (τ_f) and slow (τ_s) time constant decays were 4.4 and 39 msec, respectively. As reported in Table 1, drug application failed to affect time constant decays significantly. It should be observed, however, that after CNQX, τ_f was undetectable while the rise time was enhanced two to three times (Fig. 5). Rise time was unchanged by APV or 7-Cl-KA (Fig. 5).

Table 1. Pharmacology of EPSCs in DA cells

Drugs	(n)	I_T (pA)	I_s (pA)	Percent I_s	τ_f (msec)	τ_s (msec)
Control	(26)	95 ± 8.7	15 ± 3.3	16 ± 4.2	4.4 ± 0.32	39 ± 3.1
APV	(10)	56 ± 6.2	4.8 ± 0.44	8.6 ± 2.3	5.4 ± 0.81	38 ± 2.2
CNQX	(8)	10.5 ± 0.42	10.5 ± 0.42	100 ± 20	ND	29 ± 4.3
7-Cl-KA	(9)	53 ± 4.5	1.5 ± 0.23	2.8 ± 0.35	4.6 ± 0.52	45 ± 5.5

Each value represents a mean ± SEM obtained from the reported number, (n), of cells. From each cell under study, a minimum of 20 EPSCs were sampled during control period and drug application. Because control values were homogeneous, they have been pooled. In the case of cells tested with various agents, a period of washing of at least 15 min was observed. Series resistance was monitored for constancy during this period. The increase in the percent contribution of I_s to I_T (percent I_s) in the presence of CNQX was due to the disappearance of the I_f. Drug concentrations were as in Figure 5. ND, not detectable.

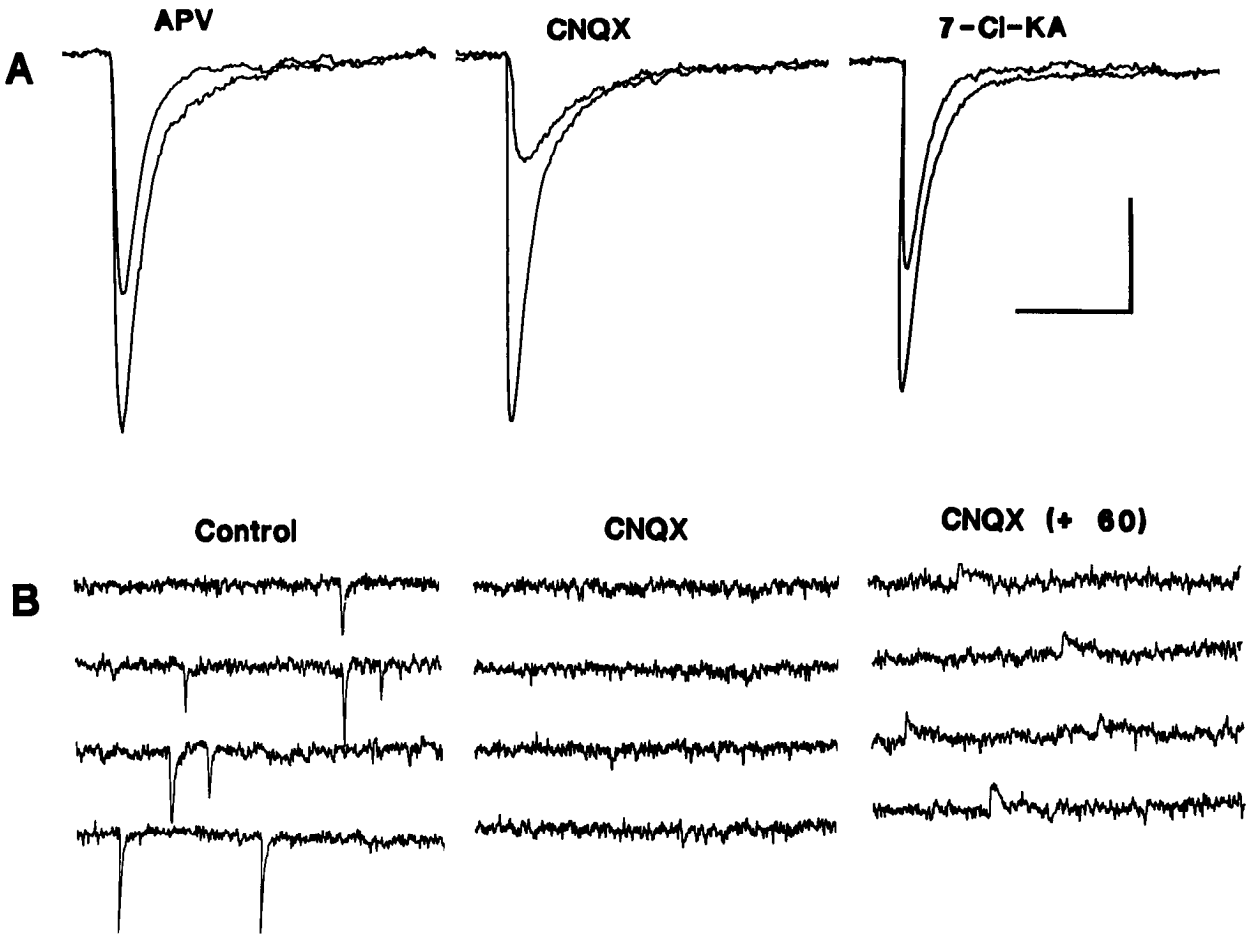

Figure 5. Pharmacology of EPSCs and miniature EPSCs. *A*, Representative traces showing the modification produced by various glutamate receptor antagonists, such as APV (50 μM), CNQX (10 μM), and 7-Cl-KA (50 μM), on the amplitude and kinetics of EPSCs. Each *trace* is the average of five distinct events sampled during control period (large EPSC) and drug application (*smaller superimposed traces*) recorded at resting potential (−50 mV). *B*, Example of spontaneous miniature EPSCs occurring at resting potential (−55 mV), in control condition, after their suppression by CNQX (10 μM), and their partial recovery upon membrane depolarization at +60 mV. Calibration: 50 pA, 20 msec for *A*; 25 pA, 40 msec for *B*.

Miniature EPSCs

In a number (n = 16) of DA neurons, we observed spontaneous miniature EPSCs of 5–40 pA (Figs. 3*A*, 5*B*). At resting potential (−55 mV), they were completely eliminated by application of CNQX (10 μM; Fig. 5*B*), but not by APV (50 μM; not shown). However, upon membrane depolarization to HPs of +30 to +60 mV, slow miniature EPSCs were still observed in the presence of CNQX (Fig. 5*B*).

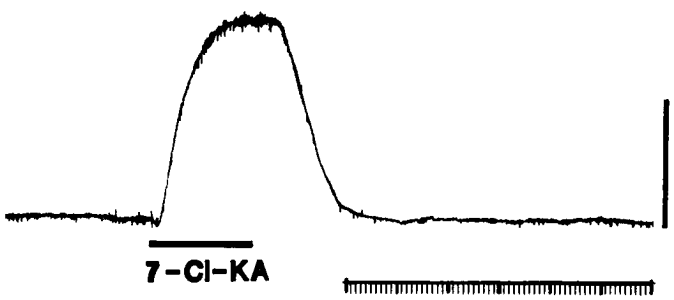

Figure 6. Record of the outward current produced by the application of 7-Cl-KA (50 μM) for 25 sec in the bath solution, as indicated by the *bar*. Cell membrane potential was held at −50 mV. Intracellular solution contained CsCl (145 mM; see Materials and Methods). Calibration, 200 pA, 1 min.

Discussion

Glutamate is the most abundant excitatory neurotransmitter in the CNS (see Monaghan et al., 1989; Storm-Mathisen and Ottersen, 1989). By activating specific ionotropic receptor subtypes, glutamate controls neuronal excitability and mediates synaptic plasticity (Monaghan et al., 1989). Moreover, a persistent and paroxysmal stimulation of glutamate receptors causes neuronal excitotoxicity (Choi, 1988; Manev et al., 1990). It has been thought that local nigral circuits and nigro-striato-nigral loops were regulated mainly by neurotransmitters, such as DA, GABA, and ACh (Grace and Bunney, 1985; Chiodo and Freeman, 1987; Lacey et al., 1988, 1989). Our results now provide conclusive evidence that SNC-DA neurons in the rat receive a glutamatergic excitatory input that can be activated by intranigral stimulation. The midbrain coronal thin-slice preparation does not allow determination of the origin of these excitatory afferents. However, on the basis of previous studies, we can hypothesize that they arise from subthalamic (Hammond et al., 1983) and pedunculopontine (Scarnati et al., 1986) nuclei, as well as from cortex (Storm-Mathisen and Ottersen, 1989),

and perhaps also from excitatory interneurons located within the confines of the slices.

The biophysical and pharmacological properties of evoked EPSCs in DA neurons of SNC indicate that both NMDA and non-NMDA receptors are involved. However, because the *I/V* relation of total EPSC peak amplitudes of DA neurons was not linear in the region of negative voltages (Fig. 3*B*), where the Mg^{2+} blockade of NMDA receptor channels is more effective (Mayer et al., 1984; Nowak et al., 1984; Hestrin et al., 1990), and because both APV and 7-Cl-KA consistently reduced I_T, one can surmise a faster onset of the NMDA component of synaptic current amplitude in SNC-DA neurons than in pyramidal neurons of hippocampus (Hestrin et al., 1990). Although spontaneous miniature EPSCs were mainly CNQX sensitive, the presence of slow miniature EPSCs at positive potentials indicates a role for both NMDA and non-NMDA receptors in their generation. Moreover, a steady outward current was elicited in some SNC-DA neurons by APV as well as by either low (1.0 μM) or high (50 μM) concentrations of 7-Cl-KA, indicating that, as in the hippocampus (Sah et al., 1989), NMDA-selective glutamate receptors might be tonically activated. Because it has been inferred that this tonic activation could be present also *in vivo* (Errington et al., 1987), it is likely that it is influenced not only by "ambient" glutamate (Sah et al., 1989) active in the synaptic cleft, but also by the positive modulation of NMDA receptors by the endogenous glycine (Johnson and Ascher, 1987) present in the interstitial fluid of SNC. Such a possibility is consistent with the different mechanism of action exerted by APV and 7-Cl-KA in inhibiting the I_T and I_s of EPSCs. Indeed, as shown in Table 1, both drugs reduce I_T by a similar extent, whereas I_s is inhibited about 65% and 90% by APV and 7-Cl-KA, respectively. These observations indicate that, while APV isosterically antagonizes the action of glutamate on NMDA-selective glutamate receptors (Monaghan et al., 1989), 7-Cl-KA produces its antagonistic action through the blockade of the binding site of glycine located on the glutamate receptor domain (Kemp et al., 1988; Costa, 1989).

Whatever the role played by ambient glycine in regulating the slow component of EPSCs, our finding of an excitatory, NMDA- and non-NMDA-mediated, input to SNC-DA neurons might have a number of relevant implications, for instance, (1) the excitability of DA neurons may be controlled, in part, by phasic (i.e., synaptic; Hammond et al., 1983; Scarnati et al., 1986; Kang et al., 1989) as well as tonic (Sah et al., 1989) activation of glutamate receptors. (2) As in lamprey spinal cord neurons (Wallen and Grillner, 1987), the tetrodotoxin-insensitive oscillations in membrane potential of DA neurons (Kita et al., 1986; Grace and Onn, 1989; Harris et al., 1989) could be due to Ca^{2+} and other voltage-dependent conductances that are finely tuned by voltage-sensitive NMDA receptor-operated channels. (3) NMDA receptor-activated current appears to be responsible for Ca^{2+}-dependent dendritic release of DA (Cheramy et al., 1981; Llinas et al., 1984; Araneda and Bustos, 1989). (4) A persistent and paroxysmal activation of NMDA receptors might result in excitotoxicity (Choi, 1988; Manev et al., 1990). Thus, it is conceivable that the glutamatergic innervation of SNC might be responsible, at least in part, for the death of DA neurons that occurs with age (Marsden, 1990) and in Parkinson's disease. This possibility suggests novel strategies in search for Parkinson's disease etiology, diagnosis, and therapy. (5) Finally, because it has been demonstrated (Lovinger et al., 1989) that ethanol inhibits NMDA-activated currents in cultured hippocampal neurons, it is possible that the reduction of DAergic firing produced by local application of ethanol (Mereu et al., 1988) is due to a similar mechanism.

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