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L-Type Calcium Channels Mediate a Slow Excitatory Synaptic Transmission in Rat Midbrain Dopaminergic Neurons

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Patch pipettes were used to record whole-cell synaptic currents under voltage-clamp in dopaminergic neurons in slices of rat substantia nigra pars compacta and ventral tegmental area. We report that dihydropyridines (DHPs), L-type Ca^{2+} channel antagonists, depressed a slow EPSC ($EPSC_{slow}$) evoked by a train of focally delivered electrical stimuli. In fact, the amplitude of the $EPSC_{slow}$ was reduced by the DHP antagonists nifedipine (1–100 μM), nimodipine (1–100 μM), and isradipine (30 nM-100 μM) in a concentration-dependent and reversible manner. On the other hand, Bay-K 8644 (1 μM), an L-type Ca^{2+} channel agonist, increased the $EPSC_{slow}$. The DHPs depressed the $EPSC_{slow}$ only when the high-frequency stimulation that was used to evoke this synaptic current lasted >70 msec. On the other hand, Bay-K 8644 increased the amplitude of the $EPSC_{slow}$ only when it was evoked by a train <70 msec. Moreover, the DHPs did not affect the $EPSC_{fast}$, the $IPSC_{fast}$, and the $IPSC_{slow}$. The inhibition of the $EPSC_{slow}$ caused by the DHPs is attributed to presynaptic mechanisms because (1) the inward current generated by exogenously administered glutamate was not affected and (2) the $EPSC_{slow}$ was reduced to a similar degree even when the activation state of postsynaptic L-type Ca^{2+} channels was changed by holding the neurons at −100, −60, and +30 mV. Finally, a DHP-sensitive component of the $EPSC_{slow}$ could even be detected after the blockade of N-, Q-, and P-type Ca^{2+} channels by the combination of ω-conotoxin GVIA, ω-agatoxin IVA, and ω-conotoxin MVIIC. Taken together, these results indicate that under certain patterns of synaptic activity, L-type Ca^{2+} channels regulate the synaptic release of excitatory amino acids on the dopaminergic neurons of the ventral mesencephalon.

Key words: dopamine neurons; L-type calcium channels; dihydropyridines; excitatory postsynaptic currents; midbrain; electrophysiology

The increase of calcium influx into the presynaptic terminal is generally considered a fundamental event that triggers neurotransmitter release (Katz and Miledi, 1969; Llinàs et al., 1981; Augustine et al., 1987). Several types of presynaptic calcium channels, particularly the N-type and P/Q-type, have been thought to participate in calcium-mediated neurotransmitter secretion (Bean, 1989; Seabrook and Adams, 1989; Mintz et al., 1992; Luebke et al., 1993; Wheeler et al., 1994; Dunlap et al., 1995; Wright and Angus, 1996). Despite observations that L-type calcium channels play an important role in the release of neurotransmitters such as catecholamines from chromaffin cells, dynorphin from the dendrites of rat hippocampal granule cells, neuropeptides from the neurohypophysis, and excitatory amino acids (EAAs) from the retina (Lemos and Nowycky, 1989; Takibana et al., 1993; Lopez et al., 1994; Simmons et al., 1995; Von Gersdorff and Matthews, 1996), these channels are thought to play a minor role in most neuronal excitation–secretion events. In fact, it is generally thought that they are not active during the generation of action potentials in the CNS and the subsequent depolarization of the synaptic terminals (Miller, 1987; Kullman et al., 1992; Dunlap et al., 1995; Reuter, 1996). Consistent with these findings, the inhibition of L-type Ca^{2+} channels has little effect on synaptic transmission in areas such as the frontal cortex, hippocampus, accumbens, cerebellum, and striatum (Kamiya et al., 1988; Llinàs et al., 1989; Horne and Kemp, 1991; Kullman et al., 1992; Mintz et al., 1992; Turner et al., 1992; Zhang et al., 1993; Wheeler et al., 1994). In the present study, we report a specific modulation by L-type Ca^{2+} channels of slow EPSCs ($EPSCs_{slow}$) evoked under voltage-clamp on presumed dopamine-containing neurons of the rat midbrain, by using the whole-cell patch-clamp technique (Wu et al., 1995; Bonci and Williams, 1997; Shen and Johnson, 1997). This slow synaptic excitatory event, generated by the activation of NMDA and metabotropic receptors (Mercuri et al., 1996; Shen and Johnson, 1997), is likely caused by the release of aspartate/glutamate from inputs to the midbrain arising from the cortex, subthalamus, and pedunculopontine nucleus (Christie et al., 1985; Kita and Kitai, 1987; Sesack and Pickel, 1992; Lavoie and Parent, 1994). Given the considerable evidence that the dopaminergic neurons influence various motor and behavioral states (Le Moal 1995), a regulation of their excitatory afferents by L-type Ca^{2+} channels is an important topic that might have physiological and therapeutic implications (Seeman, 1995).

MATERIALS AND METHODS

Preparation of the tissue. Male albino Wistar rats (150–300 gm) were killed under halothane anesthesia. The preparation of the slices has been described previously (Mercuri et al., 1995). Briefly, a single horizontal slice (200–350 μm thick) containing the substantia nigra and the ventral tegmental area was transferred to a recording chamber and continuously perfused at a rate of 2.5 ml/min, with a solution maintained at 35°C and oxygenated with a mixture of 95% O_2/5% CO_2. The standard solution contained (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 11, and $NaHCO_3$ 19, giving a pH of 7.4.

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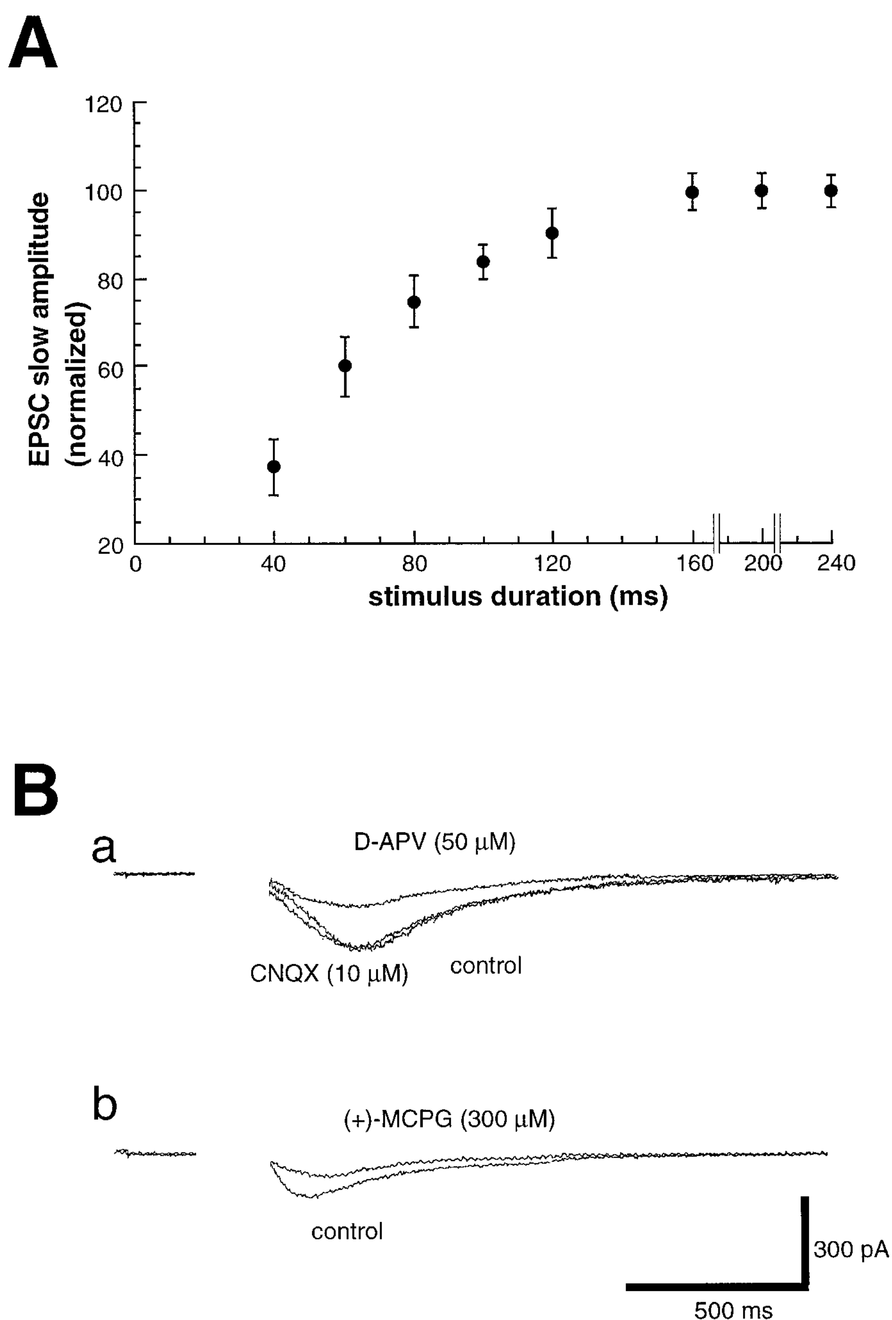

Figure 1. Properties of the $EPSC_{slow}$. *A*, The graph indicates that the amplitude of the $EPSC_{slow}$ is dependent on the duration of the train. The amplitude of the synaptic current was measured after the stimulation. Each point represents at least three experiments. *Ba*, The NMDA antagonist APV depresses the $EPSC_{slow}$ in a reversible manner, whereas the AMPA/kainate antagonist CNQX (10 μM) did not produce any effect on the $EPSC_{slow}$. The stimulus artifacts were blanked (records are average of 4 sweeps). *Bb*, The metabotropic antagonist (+)-MCPG (300 μM) also depresses the $EPSC_{slow}$ in a reversible manner.

Recording and stimulation. The slice was transferred to a recording chamber on the stage of an upright microscope (Axioscope, Carl Zeiss) illuminated with infrared light. The presumed dopaminergic neurons were directly visualized and approached by positive pressure. Whole-cell recordings were made using patch pipettes having a resistance of 4–7 MΩ and containing (in mM): potassium gluconate 144, $CaCl_2$ 0.3, $MgCl_2$ 1.2, HEPES 10, EGTA 1, Mg-ATP 2, and GTP 0.25, pH 7.3. To record the $IPSC_{fast}$, 128 mM KCl and 20 mM NaCl were used instead of potassium gluconate. In the experiments with the neurons clamped at −100 and +30 mV, the intracellular solution contained 120 mM cesium gluconate instead of potassium gluconate, whereas during the recordings at 30 mV the internal solution also contained the sodium channel blocker QX-314 (10 mM). The series resistance compensation was usually set at 80%, and the series resistance (R_s 5–16 MΩ) was monitored during the experiments every time an EPSC was evoked. The neurons were voltage-clamped using an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). The synaptic events were evoked with bipolar tungsten-stimulating electrodes positioned in the ventral mesencephalon (200–400 μm from the recording site). All of the electrical stimuli were locally delivered by a Grass S88 stimulator. The $EPSC_{slow}$ was generated in the dopaminergic neurons by a repetitive electrical stimulation (100–300 Hz, 40–400 msec duration, 1–20 V, delivered at 45 sec intervals). To evoke the $EPSC_{fast}$ and the $GABA_A$ IPSC, single square-wave (0.1–0.3 msec, 1–10 V,) pulses were applied every 20 sec. In experiments examining the $EPSC_{slow}$ and the $EPSC_{fast}$, the superfusion medium contained picrotoxin (100 μM) or bicuculline (30 μM), saclofen (300 μM), and strychnine (1 μM), to block $GABA_A$, $GABA_B$, and glycine receptors, respectively. The $GABA_A$ IPSC was evoked in the presence of DL-2-amino-5-phosphono-pentanoic acid (APV) (30 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) to block NMDA and AMPA/kainate receptors, respectively. A stimulation protocol similar to that used to evoke the $EPSC_{slow}$ was also used to elicit the $IPSC_{slow}$, but the superfusing solution contained picrotoxin (100 μM) or bicuculline (30 μM), APV (30 μM), and CNQX (10 μM). The synaptic currents were captured and stored on a computer by using the Pclamp software 6.0.3 (Axon Instruments) and the analog/digital Maclab Chart software (AD

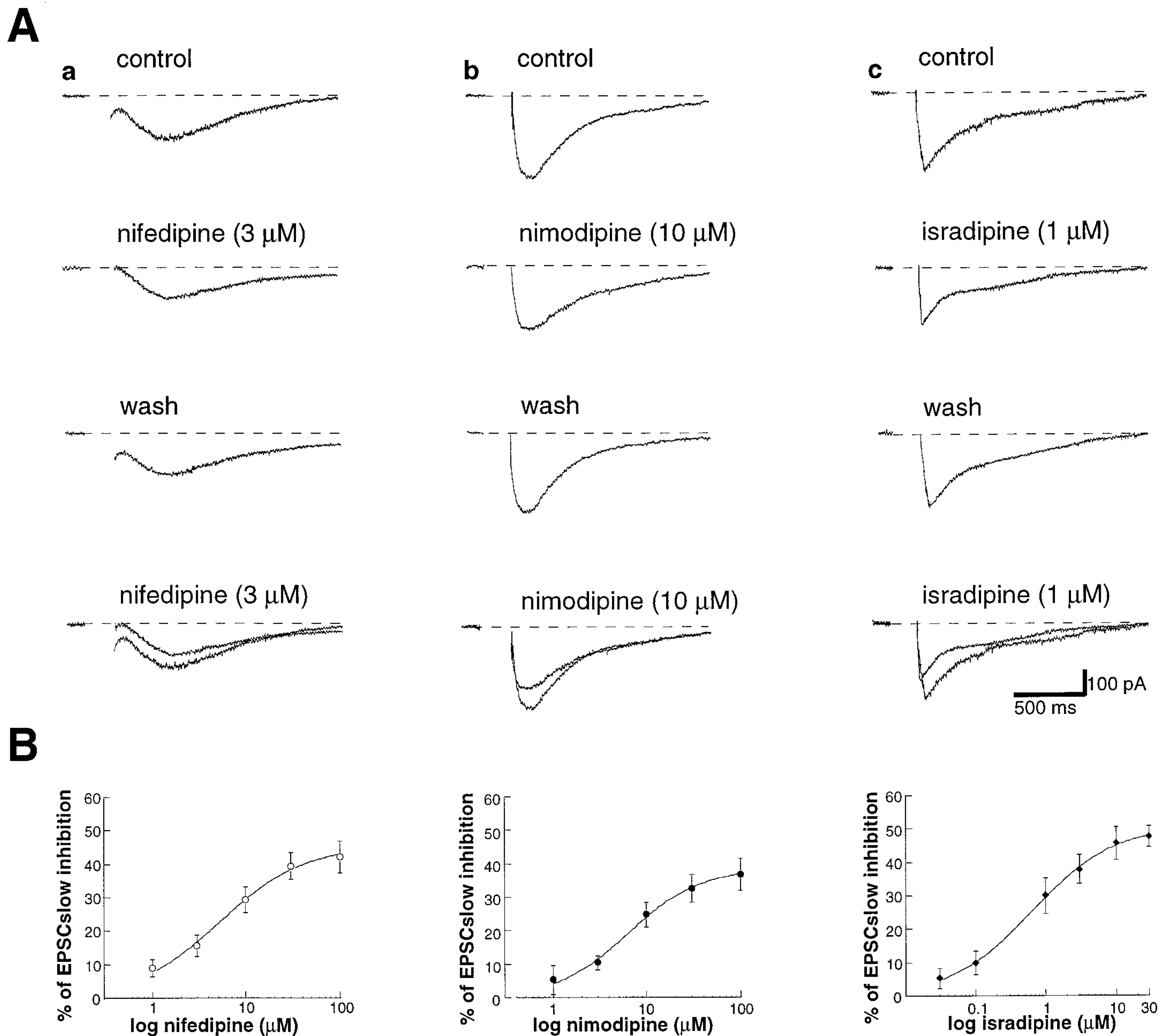

Figure 2. Effects of dihydropyridines on the slow excitatory synaptic transmission. The traces represent the $EPSC_{slow}$ in control, during and after (*wash*) the effect of nifedipine (3 μM), nimodipine (10 μM), and isradipine (1 μM). The *bottom traces* in *Aa*, *Ab*, and *Ac* are superimposed (control vs the effect of the L-type Ca^{2+} antagonists). *B*, Dose–response plots of the inhibition of the $EPSC_{slow}$ caused by nifedipine (*left*), nimodipine (*middle*), and isradipine (*right*). Each point is an average of at least four different experiments. Only one experiment per slice was performed. To calculate the percentage inhibition of the $EPSC_{slow}$, each cell was taken as its own control.

Instruments, Castle Hill, Australia). The $EPSC_{slow}$ amplitude was measured by averaging a period of 10 msec, 400 msec after the end of the train of stimuli. The data were subsequently analyzed with an Axograph 3.0 (Axon Instruments). The dose–response curves of the effects of the drugs and the statistical analyses were performed by using Kaleidograph, Mac Draw, and Stat View 4.1 running on a MacIntosh computer.

Application of drugs. The drugs were bath-applied at a defined concentration. Drug solutions entered the recording chamber no later than 20 sec after a three-way tap was turned. Complete replacement of the medium in the chamber took 90 sec. In some experiments, glutamate (1 mM) was applied via a puffer pipette (20–80 msec, 100–200 kPa) controlled by a Picrospritzer II (General Valve Corporation, Fairfield, NJ) and placed ~50 μm above the recorded cells. The following drugs were used: picrotoxin, bicuculline methiodide, methionine enkephalin, dopamine, nifedipine, and APV [all obtained from Sigma (St. Louis, MO)]. CNQX, (±)-α-methyl-4-carboxyphenylglycine (MCPG), and 2-hydroxy-saclofen (saclofen) were obtained from Tocris Cookson. Nimodipine and Bay-K 8644 were a gift from Bayer Italia. Isradipine was obtained from Sandoz (Basel, Switzerland), and ω-conotoxin GVIA, ω-agatoxin-IVA, and ω-conotoxin MVIIC were obtained from Alomone Labs.

RESULTS

Properties of the dopaminergic neurons and characterization of the slow synaptic currents

The "principal" or presumed dopaminergic neurons of the midbrain were identified by their spontaneous firing, broad (>1.5 msec) action potentials (under current-clamp), a pronounced hyperpolarization-activated inward rectification (I_h), an outward current response to dopamine (10–30 μM), and no outward current to the superfusion of methionine enkephalin

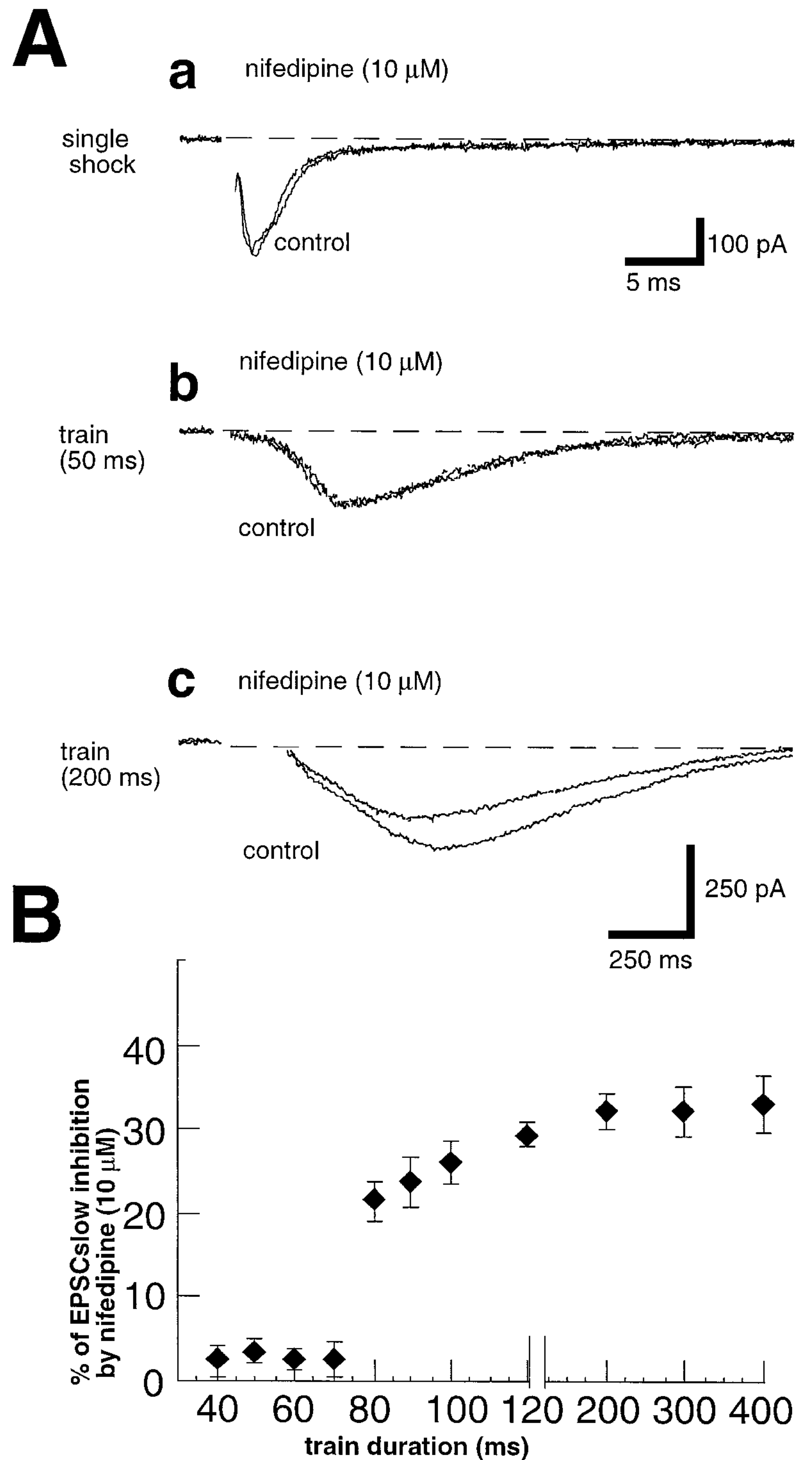

Figure 3. *A*, The percentage of inhibition of the $EPSC_{slow}$ caused by nifedipine is dependent on the duration of the electrical stimulation. *Aa*, Superimposed $EPSC_{fast}$ in control condition and after the application of nifedipine. *Ab, Ac*, A 50 msec and a 200 msec train of stimuli were delivered to evoke $EPSCs_{slow}$. In the presence of nifedipine, the $EPSC_{slow}$ evoked by the longer train (*c*) was depressed, but the EPSC evoked by the short train (*b*) was not affected. The time and current bars in *c* are also valid for *b*. Note that the $EPSC_{fast}$ in *a* and the $EPSC_{slow}$ in *c* were elicited alternately in the same neuron. *B*, The graph shows that the depression of the $EPSC_{slow}$ caused by nifedipine (10 μM) is dependent on the duration of the electrical stimulus.

(10–30 μM) (Kita et al., 1986; Grace and Onn, 1989; Lacey et al., 1989; Yung et al., 1991; Johnson and North, 1992; Mercuri et al., 1995).

The local stimulation of the ventral mesencephalon by a train of electrical stimuli determined an EPSC ($EPSC_{slow}$) in cells patch-clamped at −60 mV. Figure 1*A* shows that the size of the $EPSC_{slow}$ was dependent on the duration of the train. It is clear that 150–200 msec are necessary to fully generate the $EPSC_{slow}$ for a given train of stimuli. The maximal amplitude and the mean duration of the $EPSC_{slow}$ was 371 ± 16.2 pA (n = 60) and 2.12 ± 0.8 sec (n = 20), respectively. The $EPSC_{slow}$ was caused by the coactivation of NMDA and metabotropic EAA receptors (Mercuri et al., 1996; Shen and Johnson, 1997). In fact, the application of the non-NMDA receptor antagonist CNQX (10 μM) did not modify the amplitude of the $EPSC_{slow}$ (n = 6) (Fig. 1*Ba*). In addition, the NMDA receptor antagonist APV (30 μM) depressed this synaptic event by 59 ± 4.1% (n = 11) (Fig. 1*Ba*), whereas the nonspecific metabotropic receptors antagonist MCPG (300 μM) depressed the $EPSC_{slow}$ by 34.9 ± 5.9% (n = 9) (Fig. 1*Bb*).

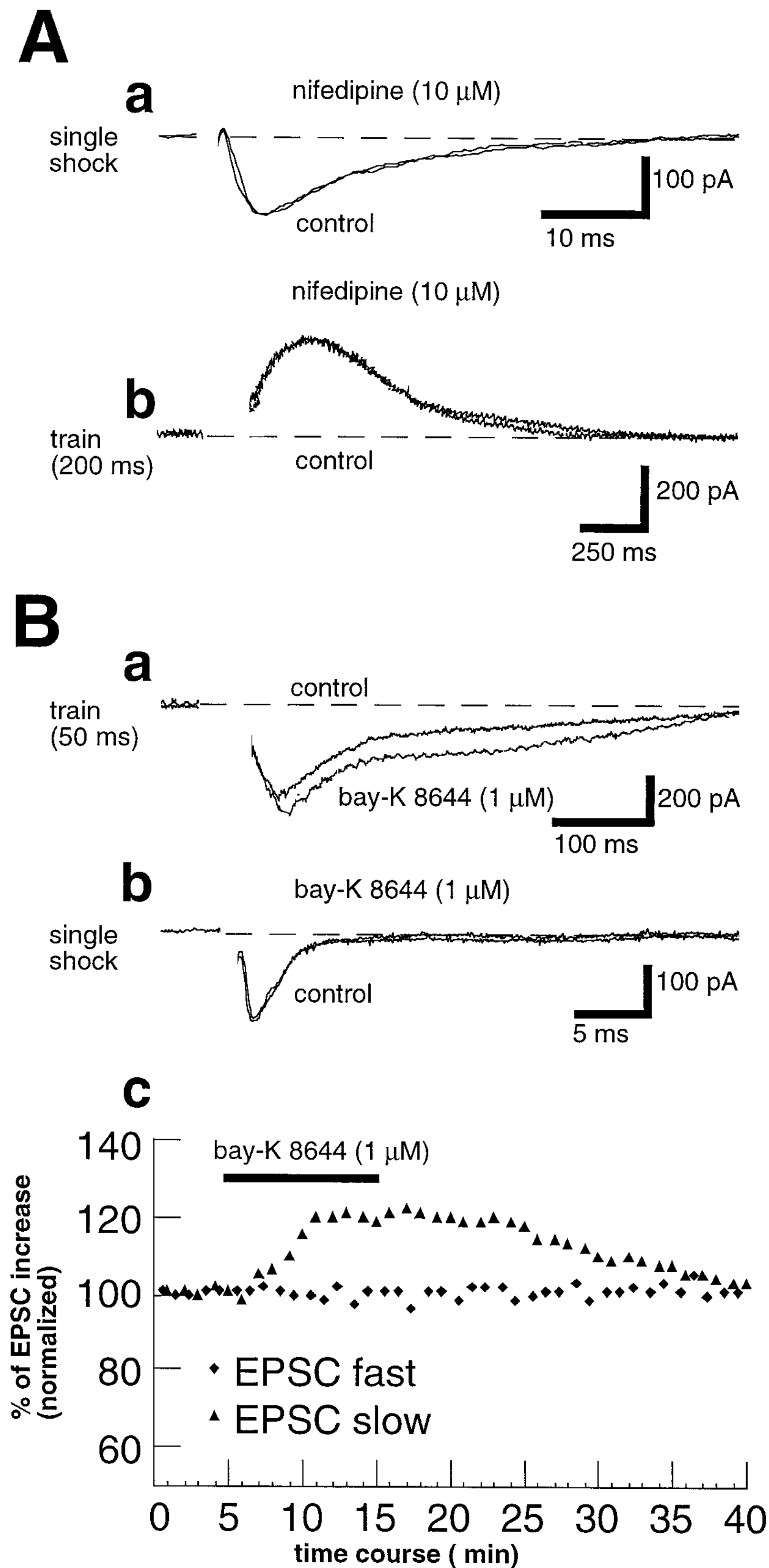

Figure 4. *A*, Nifedipine (10 μM) had no effect on the amplitude of the $GABA_A$ (*a*) and $GABA_B$ (*b*) IPSCs. The $GABA_A$ current is inward because the recording pipette was filled with a solution containing potassium chloride (see Materials and Methods). *Ba, Bb*, Superimposed traces showing that the L-type Ca^{2+} channel agonist Bay-K 8644 (1 μM) increased the $EPSC_{slow}$ evoked by a short train (*a*) but did not augment the $EPSC_{fast}$ (*b*). The $EPSC_{fast}$ and the $EPSC_{slow}$ were elicited alternatively in the same cell. *c*, Plot taken from the same dopamine neuron showing the time course of the enhancing effect of Bay-K 8644 on the $EPSC_{slow}$.

L-type calcium channel antagonists reduce the $EPSC_{slow}$

Bath application of the L-type calcium channel antagonists (Janis and Triggle, 1984) nifedipine (1–100 μM), nimodipine (1–100 μM), and isradipine (30 nM-30 μM) decreased, in a dose-dependent manner, the amplitude of the $EPSC_{slow}$ ($n = 64$) (Fig. 2). The EC_{50} values for the effects of nimodipine, nifedipine, and isradipine were 5.3, 6.3, and 0.63 μM, respectively. The maximum

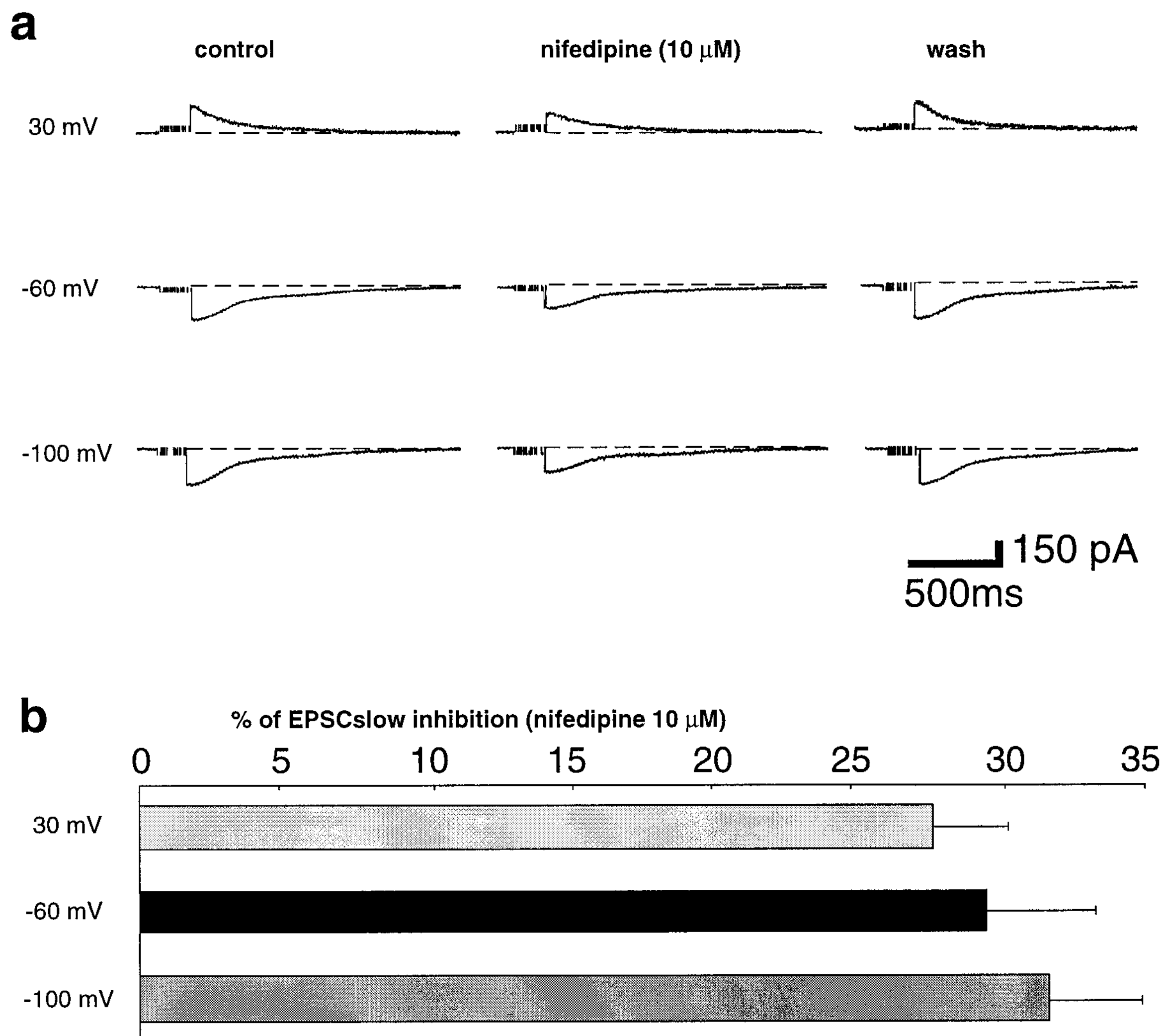

Figure 5. Presynaptic effects of nifedipine. *a*, Changes in the holding potential from −60 mV to 30 mV or −100 mV did not affect the depression of the EPSC produced by nifedipine. Typical traces taken form experiments showing the similar degree of inhibition produced by nifedipine (10 μM). *b*, The horizontal columns show that the percentage of inhibition caused by nifedipine (10 μM) at 30 mV, −60 mV, and −100 mV is not statistically different among the three groups of neurons tested ($p > 0.05$). Each column represents an average of at least five cells. To improve space-clamp between proximal somatic and distal dendritic regions, the I_h current was also reduced by extracellular CsCl (1 mM) during the hyperpolarization.

degree of inhibition was caused by 30 μM isradipine (48.6 ± 3.1%, $n = 5$). Nifedipine (100 μM) and nimodipine (100 μM) reduced the slow synaptic current by 42.2 ± 4.8% and 36.8 ± 4.8%, respectively. The depression of the $EPSC_{slow}$ induced by the DHPs reached a steady-state in 5–12 min and was washed out in ~15–25 min.

Interestingly, there was a direct correlation between the degree of inhibition of the slow synaptic current and the duration of the stimulation necessary to produce the $EPSC_{slow}$. In fact, the reduction of DHP's $EPSC_{slow}$ was not observed when it was generated by a train of stimuli having a duration <70 msec ($n = 11$) (Fig. 3). However, when the train of stimuli lasted >80 msec, the DHPs clearly depressed the $EPSC_{slow}$ ($n = 53$) (Fig. 3). In another series of experiments, a single stimulus and a train of stimuli (200 msec duration) were delivered alternately on the same presumed dopaminergic neuron to compare the effect of nifedipine on the fast and slow EPSC (Fig. 3*Aa,c*). Nifedipine (10 μM) reduced the $EPSC_{slow}$ by 30.1 ± 2.1% ($n = 4$), whereas it did not affect the $EPSC_{fast}$.

The superfusion of the slices with nifedipine (10–100 μM) changed neither the amplitude of the $IPSC_{fast}$ ($GABA_A$ mediated) (Hausser and Yung, 1994) evoked by a single shock ($n = 5$) nor the $IPSC_{slow}$ ($GABA_B$ mediated) evoked by a train of stimuli of various durations, ranging from 50 to 400 msec ($n = 7$) (Fig. 4*Aa,b*) (Wu et al., 1995). It is also worth noting that the $IPSC_{slow}$ was evoked (in the presence of APV and CNQX) by the same stimulation protocol that evoked the $EPSC_{slow}$ (see Materials and Methods).

Bay-K 8644 enhances the $EPSC_{slow}$

The application of the L-type Ca^{2+} channels agonist Bay-K 8644 (1 μM) (Nowycky et al., 1985) increased the amplitude of the $EPSC_{slow}$ evoked by a short train of stimuli (50–70 msec duration) by 21.8 ± 2.1% ($n = 5$) but did not affect the $EPSC_{fast}$ (Fig. 4*B*). In four experiments, a single stimulus and a train of stimuli (50 msec duration) were also delivered alternately on the same dopaminergic neuron (Fig. 4*B*). However, when the train of stimuli lasted >200 msec, Bay-K 8644 did not produce any detectable effect on the $EPSC_{slow}$ ($n = 5$) (data not shown). Thus, when a strong stimulation activated maximally the presynaptic L-type

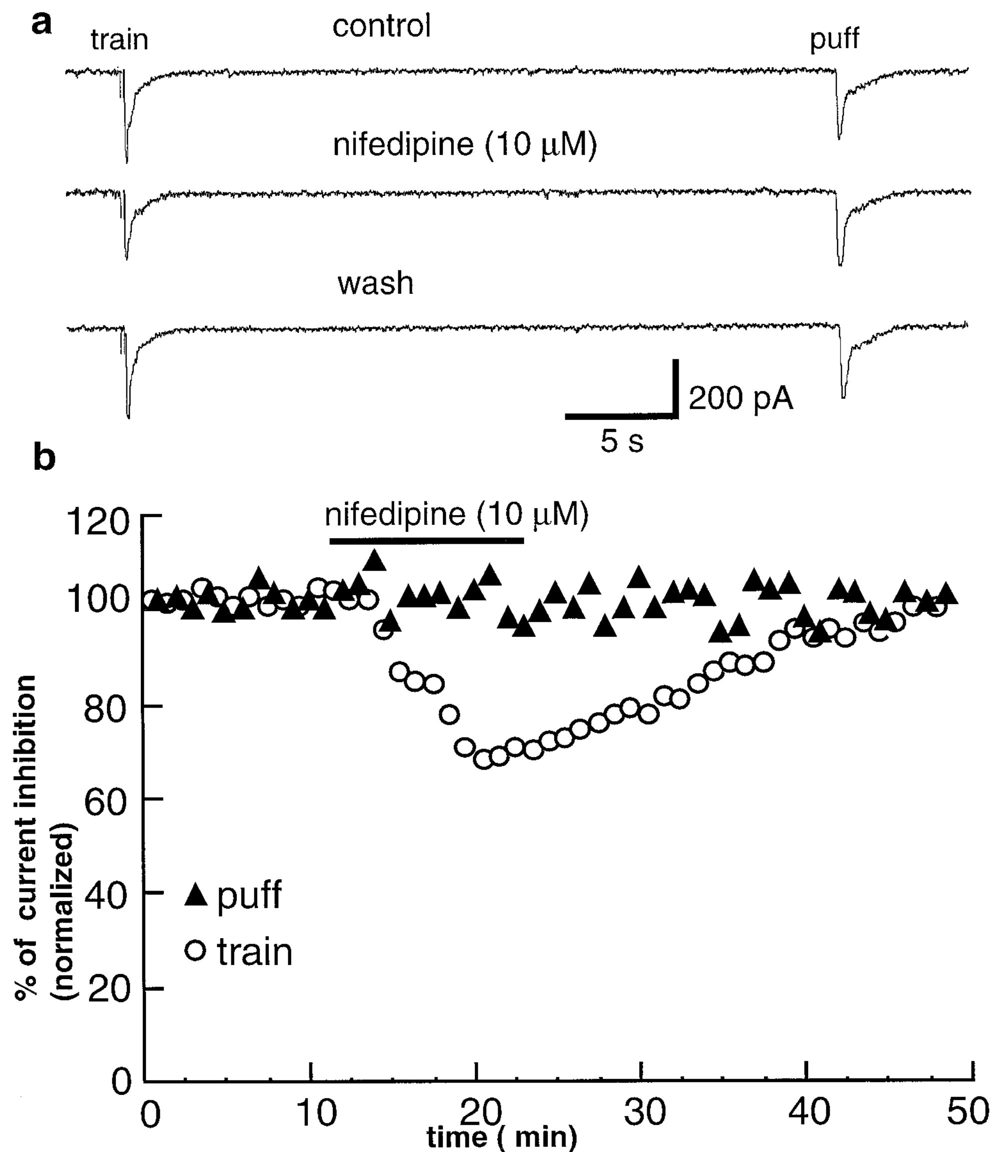

Figure 6. *a*, Traces from an experiment in which a 200 msec train was given alternately with a 50 msec extracellular (*puff*) application of glutamate (1 mM). Nifedipine (10 μM) decreased the amplitude of the $EPSC_{slow}$ but not the inward current evoked by the local application of glutamate. *b*, The plot represents the time course of the experiment shown in *a*.

Ca^{2+} channels, no further increase of the slow synaptic current was caused by this L-type channel agonist. Furthermore, neither the $IPSC_{fast}$ nor $IPSC_{slow}$ were affected by Bay-K 8644 (1 μM) (three cells for each condition; data not shown).

Site of action

To exclude a possible involvement of postsynaptic L-type calcium channels on the dihydropyridine-mediated depression of the $EPSC_{slow}$, additional experiments were performed by holding the potential of the dopaminergic neurons at +30 ($n = 6$) and −100 mV ($n = 5$). Nifedipine (10 μM) reduced the $EPSC_{slow}$ at 30 mV, −60 mV, and −100 mV to a similar degree (Fig. 5). The percentage reduction was 27.6 ± 2.7% ($n = 4$), 29.4 ± 3.2% ($n = 4$), and 31.8 ± 2.9% ($n = 4$) at + 30 mV, −60 mV, and −100 mV, respectively ($p > 0.05$) (Fig. 5*b*). Furthermore, a train of stimuli lasting 200 msec and pressure-ejected glutamate (1 mM) were delivered alternately on the same dopaminergic neuron. Nifedipine (10 μM) did not modify the inward current caused by locally applied glutamate; however, it did reduce significantly the synaptic current ($p < 0.05$) (Fig. 6), consistent with a presynaptic site of action.

Effects of the blockade of calcium channels subtypes on the EPSC

When the N- and P/Q-type Ca^{2+} channels were inhibited by the application of ω-conotoxin GVIA (1 μM), ω-AGA-IVA (200 nM), and ω-conotoxin MVIIC (5 μM) (Olivera et al., 1985; Williams et al., 1992; Takahashi and Momiyama, 1993; Boland et al., 1994; Randall and Tsien, 1995), the $EPSC_{fast}$ was almost completely blocked ($n = 4$; data not shown). The $EPSC_{slow}$ evoked by a short train of stimuli (50 msec duration) was reduced by 88.2 ± 6.2% ($n = 3$) (Fig. 7*A*). The subsequent application of nifedipine (30 μM) did not produce any further effect (91.3 ± 2.7%; $n = 3$) (Fig. 7*A*). The next experiments examined the contribution of L-type calcium channels to the $EPSC_{slow}$ elicited by a long train of stimuli. The amplitude of the $EPSC_{slow}$ evoked by a 80–200 msec

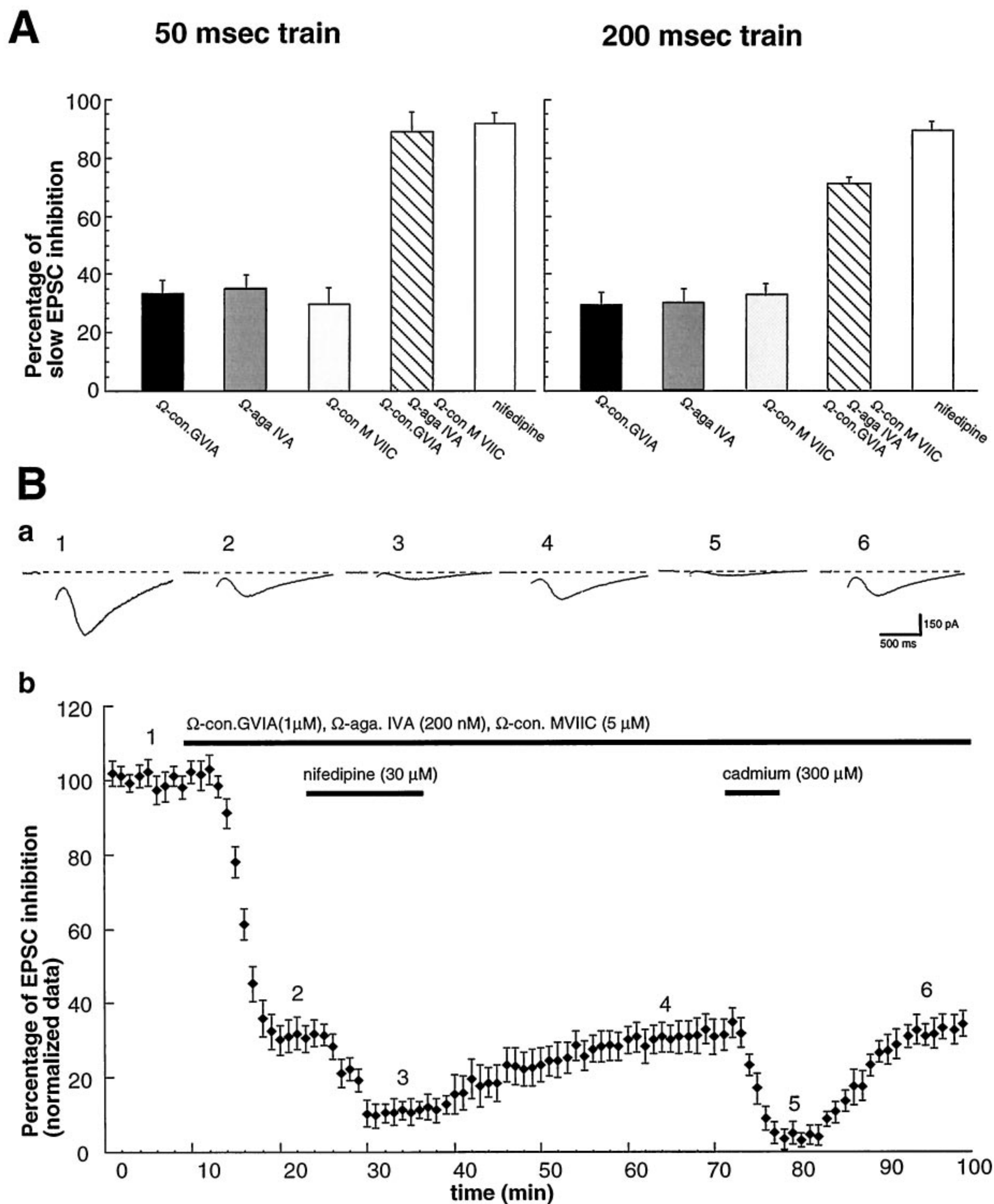

Figure 7. *A*, The graph shows the depressant effects of ω-conotoxin GVIA (1 μM), ω-AGA-IVA (200 nM), and ω-conotoxin MVIIC (5 μM) on the $EPSC_{slow}$ evoked by a short train of stimuli. Note that nifedipine (30 μM) added to the three toxins did not depress the synaptic current. *A, B*, In the presence of ω-conotoxin GVIA (1 μM), ω-AGA-IVA (200 nM), and ω-conotoxin MVIIC (5 μM), nifedipine (30 μM) reversibly depressed the residual $EPSC_{slow}$. The residual synaptic current recorded after the treatment with the toxins was blocked by cadmium (300 μM). *Ba*, Sample records of a cell obtained at the times indicated by the numbers *1–6* in the graph. *Bb*, Graph of the amplitude of the EPSC $_{slow}$ during the application of the natural toxins nifedipine (30 μM) and cadmium (300 μM). Each point is an average of four experiments performed on four different slices.

train of stimuli was reduced by the application of the three toxins by 71.1 ± 2.3% ($n = 4$) (Fig. 7*A*). In contrast to the cells stimulated with a short train, the addition of nifedipine (30 μM) produced a further reduction to 89.2 ± 2.8%; $n = 4$) (Fig. 7*A,B*). Cd^{2+} (300 μM) had a similar but more prominent effect on the residual $EPSC_{slow}$ ($n = 4$). In three experiments, the concentration of ω-conotoxin GVIA, ω-AGA IVA, and ω-conotoxin MVIIC was raised from 1 to 1.5 μM, from 200 to 300 nM, and from 5 to 7 μM, respectively. However, the depression of the residual $EPSC_{slow}$ caused by nifedipine (30 μM) was similar

(93.1 ± 2.9%; $p > 0.05$) to that observed with the lower concentrations of toxins.

DISCUSSION

Our results indicate that high frequency stimulation of the ventral mesencephalon reveals a specific role for L-type Ca^{2+} channels in the release of excitatory amino acids on dopaminergic neurons. The observation that the $EPSC_{slow}$ was reduced but not blocked by ω-conotoxin-GVIA, ω-agatoxin-IVA, and ω-conotoxin MVIIC not only demonstrates the importance of the N- and P/Q-type Ca^{2+} channels in the release of EAAs, but it also confirms previous biochemical and electrophysiological findings showing that a part of the release of glutamate in the brain is resistant to N- and P/Q-type Ca^{2+} channel antagonists (Brown et al., 1986; Turner et al., 1992; Huston et al., 1995). Thus, in contrast to a single or a short train, a prolonged high-frequency stimulation may activate a sufficient number of L-type Ca^{2+} channels by producing a prolonged depolarization of the presynaptic terminals. Consequently, the influx of Ca^{2+} ions through them could significantly participate in the exocytosis of EAAs on the dopaminergic cells. The fact that the DHPs do not reduce the excitatory transmission mediated either by single electrical stimuli or by a short-stimulus train supports a more specific function for L-type calcium channels in controlling the excitatory synaptic events caused by a long sequence of action potentials. In fact, as observed in other parts of the CNS, the influx of calcium during a weak stimulation of presynaptic terminals is certainly controlled by the cooperative activation of N- and P/Q-type channels (Luebke et al., 1993; Mintz et al., 1995) but not by L-type channels that require a stronger depolarization to open.

It is conceivable that the naturally occurring release of excitatory neurotransmitters on the dopaminergic neurons is not only related to the invasion of the synaptic terminals by a single action potential but also by a sequence of spikes. Indeed, the sustained release of EAAs from cortical cells has an important role in regulating the type of firing of the dopaminergic cells (bursting vs pacemaker) (Grenhoff et al., 1988; Overton and Clark, 1992), the extracellular level of dopamine (Taber and Fibiger, 1995a,b), and the activity of dopaminergic neurons to salient stimuli (Schultz, 1992). The cortical and subthalamic neurons that release EAAs on the dopaminergic cells are certainly able to maintain a high rate of repetitive firing (Connors et al., 1982; Stafstrom et al., 1985; Smith and Grace, 1992; Overton and Greenfield, 1995; Kreiss et al., 1997) and to depolarize their excitatory terminals for a longer time. The biophysical properties of L-type calcium channels (time- and voltage-dependence) allow them to be active under conditions of repetitive stimulation. Thus, the time-, voltage-, and use-dependent properties of DHPs on L-type calcium channels (Sanguinetti and Kass, 1984) could account for the depression of the $EPSC_{slow}$. The DHP-sensitive Ca^{2+} channels might be localized on all the different sources of EAAs that project to the ventral mesencephalon (Christie et al., 1985; Kita and Kitai, 1987; Sesack and Pickel, 1992; Lavoie and Parent, 1994). Alternatively, only selected nerve terminals of a more heterogeneous population of afferents might bear L-type channels that regulate EAA exocytosis. It is worth mentioning that other neurons from various regions of the CNS are able to fire in a burst or in a sustained manner. For this reason, it could be postulated that the action of L-type Ca^{2+} channels on the slow synaptic event observed on the dopaminergic neurons might occur in other areas of the brain.

All three DHP antagonists used in this study depressed in a reversible manner the $EPSC_{slow}$ in a micromolar range. This is a rather peculiar effect, because micromolar doses of DHP antagonists do not usually change fast synaptic transmission in the dopaminergic cells and in various central neurons in *in vitro* conditions (Takayashi and Momiyama, 1993; Wheeler et al., 1994; Dunlap et al., 1995; Sim and Griffith, 1996; Poncer, 1997). It is also interesting to note that, as observed in other areas of the brain, the fast and slow inhibitory synaptic currents were not affected by the DHP antagonists. This suggests that the exocytosis of glutamate/aspartate but not that of GABA is controlled by L-type Ca^{2+} channels during a sustained stimulation. Because the DHPs reduce only the long $EPSC_{slow}$ but leave unaffected the amplitude and duration of the short $EPSC_{slow}$ and the $IPSC_{slow}$, it is unlikely that a nonspecific depression of excitability of presynaptic fibers could account for the reduced release of transmitter after a train of stimuli. Thus, L-type calcium channels could, during high frequency stimulation, modulate excitatory transmission to dopaminergic cells. The manipulation of the holding potential of the dopaminergic cells from −100 to +30 mV indicates that postsynaptic voltage-dependent dihydropyridine-sensitive calcium channels are not required for nifedipine action on the $EPSC_{slow}$. Although at −100 mV the L-type calcium channels should be closed, at 30 mV the contribution of postsynaptic L-type channels to the synaptic event should be minimal (Cardozo and Bean, 1995). The reversal of the $EPSC_{slow}$ indicates that adequate voltage-clamp was maintained. These sets of experiments together with those in which glutamate was locally applied imply that L-type presynaptic calcium channels are involved.

Conclusions

In conclusion, the experiments described in the present paper demonstrate that L-type calcium channels play an important role in triggering the release of excitatory neurotransmitters on the dopaminergic neurons of the ventral mesencephalon. Thus, the activation of presynaptic boutons by a relatively long burst of action potentials could open not only the N- and P/Q-type but also the L-type Ca^{2+}channels that participate in controlling the strength of excitation of the dopaminergic neurons.

Our data also suggest a rationale for the pharmacological manipulation of the EAA inputs to the dopaminergic neurons by drugs that modulate presynaptic L-type calcium channels in neurological and psychiatric disorders involving the dopamine system.

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