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Dopamine selectively reduces $GABA_B$ transmission onto dopaminergic neurones by an unconventional presynaptic action

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The functioning of midbrain dopaminergic neurones is closely involved in mental processes and movement. In particular the modulation of the inhibitory inputs on these cells might be crucial in controlling firing activity and dopamine (DA) release in the brain. Here, we report a concentration-dependent depressant action of dopamine on the $GABA_B$ IPSPs intracellularly recorded from dopaminergic neurones. Such effect was observed in spite of the presence of D_1/D_2 dopamine receptor antagonists. A reduction of the $GABA_B$ IPSPs was also caused by noradrenaline (norepinephrine) and by L-β-3,4-dihydroxyphenylalanine (L-DOPA), which is metabolically transformed into DA. The DA-induced depression of the IPSPs was partially antagonised by the $\alpha 2$ antagonists yohimbine and phentolamine. DA did not change the postsynaptic effects of the $GABA_B$ agonist baclofen, suggesting a presynaptic site of action. Furthermore, DA did not modulate the $GABA_A$-mediated IPSP. The DA-induced depression of the $GABA_B$ IPSP occluded the depression produced by serotonin and was not antagonized by serotonin antagonists. The DA- and 5-HT-induced depression of the $GABA_B$ IPSP persisted when calcium and potassium currents were reduced in to the presynaptic terminals. These results describe an unconventional presynaptic, D_1 and D_2 independent action of DA on the $GABA_B$ IPSP. This might have a principal role in determining therapeutic/side effects of L-DOPA and antipsychotics and could be also involved in drug abuse.

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Different physiological actions of DA on midbrain dopaminergic neurones have been reported so far. These actions are mainly inhibitory and attributed to membrane hyperpolarization, activated by postsynaptic D_2 auto-receptors (Lacey *et al.* 1987; Mercuri *et al.* 1992), and enhancement of the $GABA_B$ IPSPs, mediated by D_1 presynaptic receptors (Cameron & Williams, 1993). In addition, DA might cause presynaptic D_2 or 5-HT receptor-mediated inhibition of excitatory inputs (Koga & Momiyama, 2000; Jones *et al.* 2000) and postsynaptic noradrenergic ($\alpha 1$) receptor-mediated reduction of glutamate metabotropic IPSPs (Paladini *et al.* 2001).

Although it is well established that acute stimulation of D_1 presynaptic receptors enhances the $GABA_B$ IPSP (Cameron & Williams, 1993), after chronic treatment with cocaine and morphine, D_1 receptor activation decreases rather than increases the amplitude of this potential (Bonci & Williams, 1996). This D_1-mediated negative modulation of the $GABA_B$ synaptic inputs, having striatal/accumbal origin (Johnson *et al.* 1992; Sugita *et al.* 1992; Cameron & Williams, 1993), is caused by the co-activation of presynaptic adenosine A_1 receptors. It has been suggested that it is determinant in regulating drug-related phenomena, such as sensitization and withdrawal (Bonci & Williams, 1996; Shoji *et al.* 1999; Fiorillo & Williams, 2000). Interestingly, other abused drugs might exert a modulation of the $GABA_B$ synaptic inputs on the dopaminergic neurones by mechanisms principally involving 5-HT release (Johnson *et al.* 1992; Cameron & Williams, 1994). On the contrary, no clear actions of DA, 5-HT and psychostimulants on the $GABA_A$ IPSPs have yet been reported. Considering, the rather complex regulation of DA of the inhibitory potentials on the dopaminergic neurones, we re-examined the action of this cathecolamine on GABA release. Here, we describe a selective, non D_1/D_2-mediated presynaptic inhibition of GABA release on $GABA_B$ synapses.

METHODS

Preparation and recordings

Intracellular recordings with sharp microelectrodes were made from midbrain dopaminergic neurones in horizontal slices (250–300 μm thick) prepared from male Wistar rats (150–300 g) (Mercuri *et al.* 1995). The animals were anaesthetized with halothane and decapitated. The Comitato Etico of Tor Vergata University approved the experimental procedures. The brain was

rapidly removed from the skull and horizontal slices of the ventral midbrain were cut using a vibratome. A single slice containing the substantia nigra (SN) and the ventral tegmental area (VTA) was transferred to a recording chamber, immobilized with titanium mesh and perfused at a rate of 2.5 ml min^{-1}, with a solution maintained at 35 °C and equilibrated with a mixture of 95 % O_2–5 % CO_2. The standard solution contained (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 10 and $NaHCO_3$ 19, (pH 7.4). The dopaminergic neurones of the VTA and substantia nigra pars compacta were identified by their electrical properties (Lacey *et al.* 1987; 1989; Grace & Onn, 1989; Johnson *et al.* 1992; Johnson & North, 1992; Mercuri *et al.* 1995; Liss *et al.* 1999), which included the presence of a regular spontaneous firing activity, relaxation of hyperpolarizing electrotonic potentials mediated by the activation of I_h and the $GABA_B$ IPSP. No differences were observed between neurones of the VTA and SN, the data were pooled. To prevent spontaneous action potentials the membrane potential was adjusted to between −65 and −70 mV by hyperpolarizing current injection. The recording electrodes were filled with 2 M KCl and had a tip resistance of 30–80 MΩ.

Synaptic potentials

$GABA_B$ inhibitory synaptic potentials were evoked in dopaminergic cells using bipolar tungsten stimulating electrodes with a tip separation of 300–700 μm (Johnson *et al.* 1992; Johnson & North, 1992; Sugita *et al.* 1992; Cameron & Williams, 1993; Wu *et al.* 1995; Bonci & Williams, 1996; Shoji *et al.* 1999; Fiorillo & Williams, 2000). A train of four to eight stimuli of 70 μs at 8–20 V was delivered at 70 Hz every 30 s. Stimulating electrodes were placed within 500–700 μm rostral or caudal of the recording electrode. The amplitude of the evoked synaptic potential was measured from recordings that represent the average of four responses. To isolate these potentials pharmacologically, experiments were done in the presence of the dopamine D_2 receptor antagonist sulpiride (3–30 μM), the D_1 receptor antagonist SCH 23390 (1–10 μM), bicuculline methiodide (30–50 μM, $GABA_A$ and mGluR IPSP), strychnine (1 μM, glycine) and prazosin (300 nM, α1 noradrenaline). In some experiments we also perfused either apamin (100 nM, small-conductance calcium-activated potassium (SK) channel antagonist; Ishii *et al.* 1997; $n = 4$) or MCPG (500 μM, mGluR antagonist; Conn & Pin, 1997; $n = 3$).

In addition, the ionotropic glutamate AMPA and NMDA receptors were blocked by using 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and 2-amino-5-phosphonopentanoic acid (AP-5, 50 μM), respectively. The $GABA_B$ receptor antagonists CGP 55845 (300 nM) and 2-hydroxy-saclofen (100–300 μM) were perfused to block the $GABA_B$ IPSP.

Drugs

All drugs were bath applied at a known concentration. Only baclofen (300 μM) was delivered by pressure ejection (20–30 p.s.i.) from a micropipette positioned a few micrometres above the slice. The following drugs were used: cyanopindolol, pindolol, methysergide, CNQX, clozapine, MAP4, prazosin, yohimbine, sulpiride, SKF 38393, SCH 23390, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), UK 14304, quinpirole hydrochloride, alpha-methyl-4-carboxyphenylglycine (MCPG), from Tocris Cookson, 4-aminopiridine (4-AP), dopamine hydrochloride, noradrenaline (norepinephrine) (NA), AP-5, L-β-3,4-dihydroxyphenylalanine (L-DOPA), carbidopa, 5-hydroxytryptamine hydrochloride (serotonin, 5-HT), bicuculline methiodide, nifedipine, strychnine, tolbutamide, spiperone hydrochloride, chlorpromazine, atropine, phentolamine from Sigma, apamin and ω-conotoxin MVIIC (ω-CgTx), from Alomone Labs, baclofen from Roche. CGP 55845 was a gift from Novartis.

Data analysis

Numerical data were expressed as means ± standard error of the mean (S.E.M.). Student's *t* test for paired observations was used to compare data. A $P < 0.05$ was considered significant. The percentage change produced by a drug was calculated from mean amplitude of four responses before and after equilibrium had been reached. To estimate the IC_{50} and maximal response, concentration–response curves were fitted with a least squares regression using a logistic equation: $y = ax/(x + b)$. Where y is the magnitude of effect, a is maximum effect, x is drug concentration and b is the concentration that inhibits the effects by 50 %.

RESULTS

Dopamine reduces the $GABA_B$ IPSP in a concentration-dependent manner

Intracellular recordings were made from dopaminergic neurones in slices of the rat substantia nigra and ventral tegmental area maintained *in vitro* (Lacey *et al.* 1989; Johnson & North, 1992; Mercuri *et al.* 1995). $GABA_B$ IPSPs (10–20 mV) (Johnson *et al.* 1992; Sugita *et al.* 1992; Cameron & Williams, 1993; Wu *et al.* 1995; Bonci & Williams, 1996; Shoji *et al.* 1999; Fiorillo & Williams, 2000) were evoked by a local short train of stimuli (holding potential, −65 to −70 mV), in the presence of a cocktail of ionotropic and metabotropic glutamate-, $GABA_A$-, glycine-, noradrenaline α1-receptor antagonists and SK channel antagonists. These IPSPs were reduced or abolished by either saclofen (300 μM) or CGP 55845 (300 nM), $GABA_B$ receptor antagonists (Fig. 1).

Even in the presence of D_1- and D_2-selective antagonists (SCH 23390 (1–10 μM) and sulpiride (3–30 μM), respectively), dopamine (3–300 μM) reduced the amplitude of the $GABA_B$ IPSP (115 of 121 neurones) (Fig. 1). This action had a slow onset of 2–3 min, peaked in 4–5 min, and recovered in 7–10 min of washing (Fig. 1*B*) and was reproducible. The depressant effect of dopamine was concentration dependent (IC_{50}, 54.1 ± 1.3 μM, $n = 25$) (Fig. 1*C*). The decrease in IPSP amplitude was 54.8 ± 15.3 % ($n = 25$) when a concentration of 100 μM DA was superfused while no changes in membrane input resistance and IPSP time course were observed. During the effects of DA a small depolarization of the membrane potential (2.1 ± 0.8 mV, $n = 22$) was observed in 22 of 35 cells. This depolarization required the injection of 10–30 pA hyperpolarizing current to hold the membrane potential constant (at approximately −65 to −70 mV) throughout the experimental session. No current was required in the remaining cells. In some experiments we perfused either apamin (100 nM, SK channel antagonist; $n = 4$) or MCPG (500 μM, mGluR antagonist; $n = 3$) to reduce further the possible metabotropic glutamate component in the slow IPSP. Under these conditions the depressant effects of DA on the $GABA_B$ IPSPs were not affected (not shown).

In the absence of the D_1 antagonist SCH 23390 and in the presence of the DA-uptake inhibitor nomifensine (10 μM), DA (3 μM) slightly increased the amplitude of the IPSPs by $2.2 \pm 1\%$ ($P > 0.05$, $n = 4$), while in three neurones decreased it by $4 \pm 0.8\%$ ($P < 0.05$). In the attempt to counteract the depressant effect of DA we used other DA antagonists besides sulpiride and SCH 23390. High concentrations of haloperidol (10 μM), clozapine (10 μM), chlorpromazine (10–30 μM) and spiperone (10 μM) (DA and also 5-HT_{1A}/5-HT_2 antagonist) did not significantly

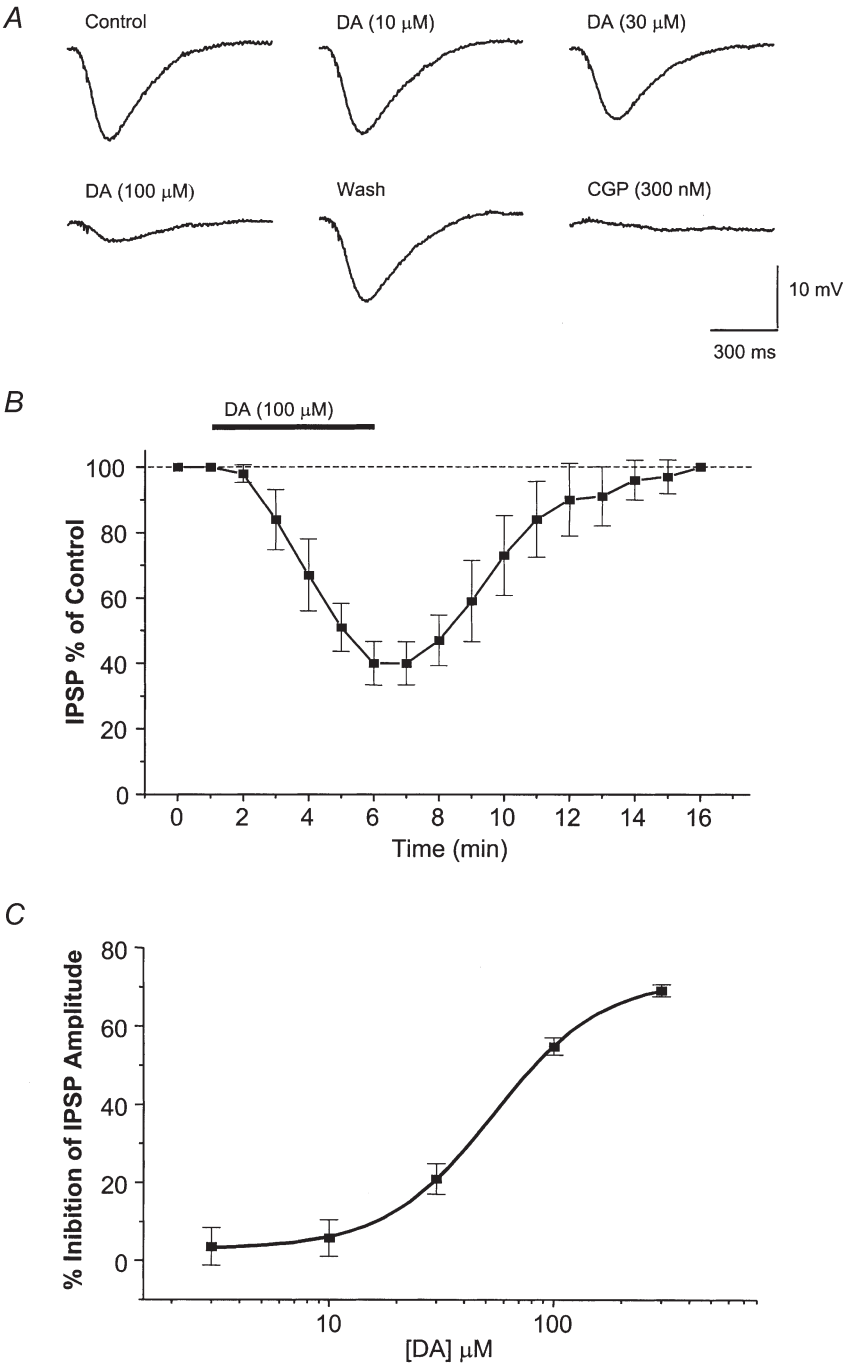

Figure 1. Dopamine inhibits the $GABA_B$ IPSP

A, example of the concentration-dependent and reversible inhibition caused by DA in the slow IPSP which was subsequently reduced by the $GABA_B$ receptor antagonist CGP (300 nM). *B*, time course of DA effects. Here and in subsequent graphs, the amplitudes of the IPSPs were normalized to the control amplitude determined for at least 5 min before application of DA. Each data point in this graph represents the mean ± S.E.M. of 7 cells. *C*, concentration-dependent curve of the reducing effects of DA on the $GABA_B$-mediated IPSP. Each point represents the mean ± S.E.M. of 25 cells.

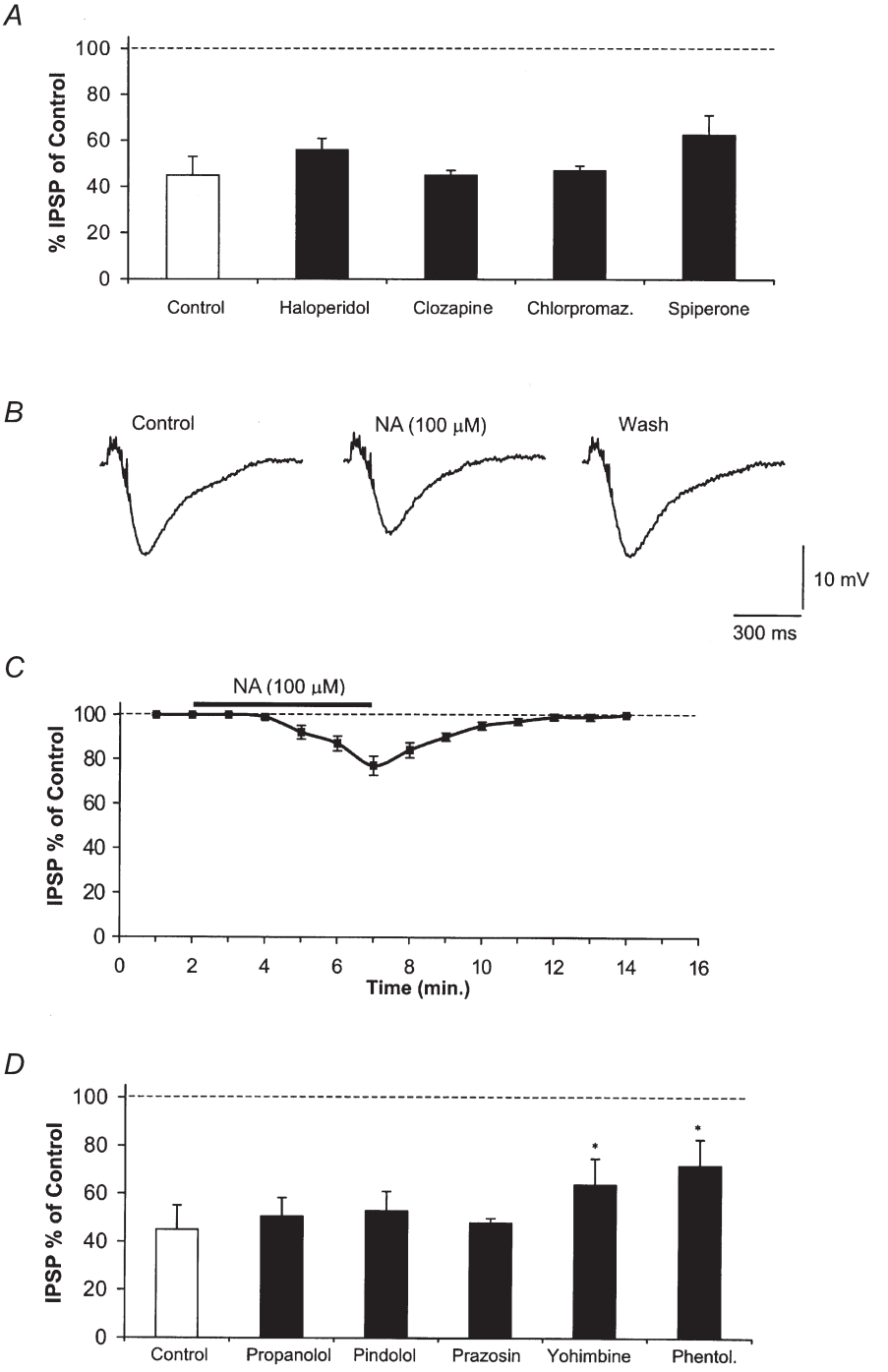

Figure 2. Dopaminergic antagonists do not change the DA-mediated reduction of the $GABA_B$ IPSP, NA mimics DA action while several noradrenergic antagonists are ineffective

A, the bars show that haloperidol (10 μM), clozapine (10 μM), chlorpromazine (Chlorpromaz, 10 μM) and spiperone (10 μM) had no significant antagonizing effect ($P > 0.05$ for each compound) on the DA-induced (100 μM) depression of the $GABA_B$ potentials. Each column shows the average of three to four experiments. *B*, reversible inhibition of an IPSP by noradrenaline (100 μM). *C*, time course of NA-induced depression of the IPSP. Each data point represents the mean ± S.E.M. of four cells. *D*, the graph shows the lack of antagonizing effect ($P > 0.05$) of several NA antagonists, propanolol (20 μM), pindolol (300 nM), prazosin (300 nM), on the DA-induced depression (100 μM) of the IPSP. Note that yohimbine (10 μM) and phentolamine (Phentol, 30 μM) caused an incomplete but significant ($P < 0.05$) reduction in the DA effect. Each column shows an average of four to five cells. Asterisks indicate significant reduction of the DA-induced effect.

affect the action of DA (100 μM) (Fig. 2*A*) ($P > 0.05$ for each compound). Furthermore, we tested the possibility that, in spite of the presence of their respective antagonists, the D_1 agonist SKF 38393 (3–10 μM) ($n = 4$) and/or the D_2 agonist quinpirole (10 μM) ($n = 3$) might have a depressant action on the IPSP. However, these compounds were ineffective ($P > 0.05$). The D_1/D_2 receptor agonist apomorphine (3 μM) was also without effect on the IPSP ($n = 3$) ($P > 0.05$). Next, we searched for possible effects of noradrenaline (NA) and the invertebrate biogenic amine octopamine on the $GABA_B$ IPSP. NA (10–100 μM) ($n = 4$) but not octopamine (30–100 μM) ($n = 3$) reversibly decreased the amplitude of the $GABA_B$ IPSP (Fig. 2*B* and *C*). Interestingly, the maximal inhibition caused by NA (100 μM) was only 45 % of that caused by an equimolar concentration of DA. The depressant effect induced by DA (100 μM) was not altered by specific adrenergic antagonists propanolol (20 μM, β1) ($n = 3$), pindolol (300 nM, β1/2 and also 5-HT_{1A}) ($n = 2$) and prazosin (300 nM, α1)($n = 3$), $P > 0.05$ for each compound). Interestingly, it was partially reduced by yohimbine (10 μM, α2) (42.5 ± 13.6 % of control) ($n = 5$, $P < 0.05$) and by relative high concentrations of the α1–α2 antagonist phentolamine (30 μM) (53.1 ± 26.2 % of control ($n = 4$); $P < 0.05$) (Fig. 2*D*). Conversely, the specific α2 agonist UK 14304 (10 μM) had no detectable effects on the amplitude of $GABA_B$ potentials ($n = 3$) ($P > 0.05$) (not shown).

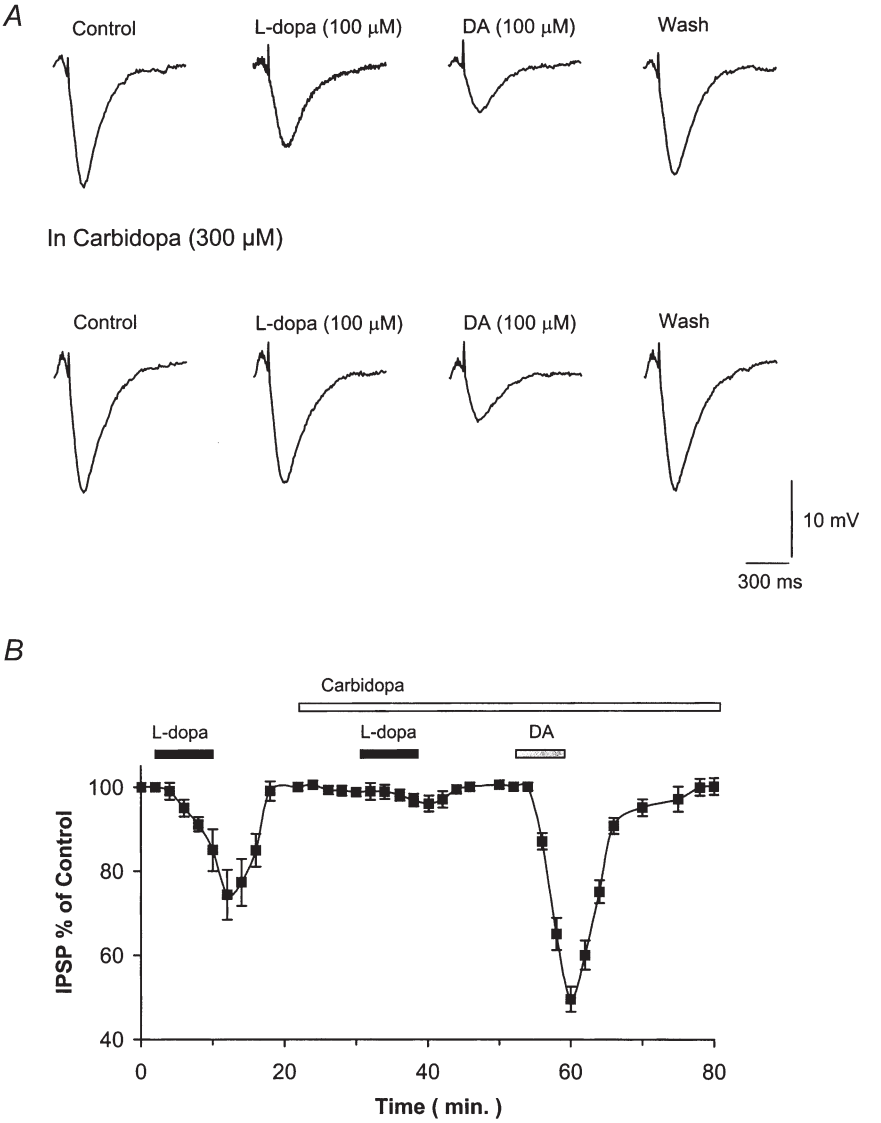

Figure 3. L-DOPA suppresses the $GABA_B$ IPSP through its metabolic transformation

A, reversible inhibition of the IPSP caused by L-DOPA and DA. Only the attenuation caused by L-DOPA but not that caused by DA is blocked by pretreatment of the slices with carbidopa (300 μM). *B*, time course of L-DOPA (100 μM) and DA (100 μM) effects and modification of L-DOPA action by carbidopa (300 μM). Each data point in this figure represents the mean ± S.E.M. of four cells.

L-DOPA acts throughout its metabolic transformation

Next we tested whether the DA precursor L-DOPA also reduced the amplitude of the $GABA_B$ IPSP. L-DOPA (100 μM) depressed this inhibitory potential to 70.2 ± 7.7 % ($n = 4$) of control (Fig. 3). It took longer than dopamine to act, 7–12 *versus* 2–4 min. The reduction of IPSP amplitude was due to transformation of L-DOPA into DA because it was selectively diminished to 97 ± 5 % ($n = 4$, $P < 0.05$) of control by a pretreatment of the slices with the DOPA-decarboxylase inhibitor carbidopa (300 μM, 15–25 min) (Mercuri *et al.* 1990).

The effects of dopamine on the $GABA_B$ inputs are presynaptic and specific

Dopamine did not affect the postsynaptic hyperpolarizations caused by the local application of baclofen (300 μM) (10–20 p.s.i, 8–10 ms) while it reduced the $GABA_B$ IPSP evoked on the same cells ($n = 5$) (Fig. 4*A*). This supports a presynaptic site of action. Next, we examined the possible effects of DA on the $GABA_A$-mediated IPSP. DA (30–100 μM) had no significant effect on the amplitude of the $GABA_A$ IPSP (Fig. 4*B*) ($n = 3$ $P > 0.05$).

Lack of effect of antagonists for presynaptic neuromodulators

We also tested whether a DA-induced release of adenosine underlies the depressant action of DA on the $GABA_B$ IPSP (Bonci & Williams, 1996). However the selective A_1 receptor antagonist DPCPX (3 μM) (Wu *et al.* 1995) did not affect DA action ($n = 3$, $P > 0.05$) on the $GABA_B$ IPSP (not shown). In addition a combination of atropine (3 μM) and MAP4 (500 μM) ($n = 2$) (to block muscarinic and group III presynaptic glutamate receptors, respectively) did not prevent the DA-induced depression of the $GABA_B$ IPSP (not shown).

Dopamine and serotonin have occluding presynaptic effects

In agreement with Johnson *et al.* (1992) the application of serotonin (5-HT; 100 μM) also inhibited the $GABA_B$ IPSP by 41.5 % ± 9.2 % ($n = 7$) throughout the activation of 5-HT_{1B} receptors (Fig. 5*A*). Thus, the 5-HT_{1B} receptor antagonist cyanopindolol (300 nM) reduced the depression of the $GABA_B$ IPSP caused by serotonin to 10 ± 0.9 % ($n = 5$, $P < 0.05$) (Johnson *et al.* 1992) while it did not affect DA action (Fig. 5). In addition, the depression of the IPSP caused by DA was not affected by pindolol (300 nM, 5-HT_{1A} and β1/2; 3 cells) and methysergide (10 μM; a serotoninergic antagonist that was reported to reduce the depressant action of DA on the excitatory synaptic events; Jones *et al.* 2000; 3 cells) ($P > 0.05$, for each compound). If the signalling pathway operated by 5-HT is also that engaged by DA, the IPSP inhibition caused by these neurotransmitters when bath applied in combination should be approximately the same as when each is applied

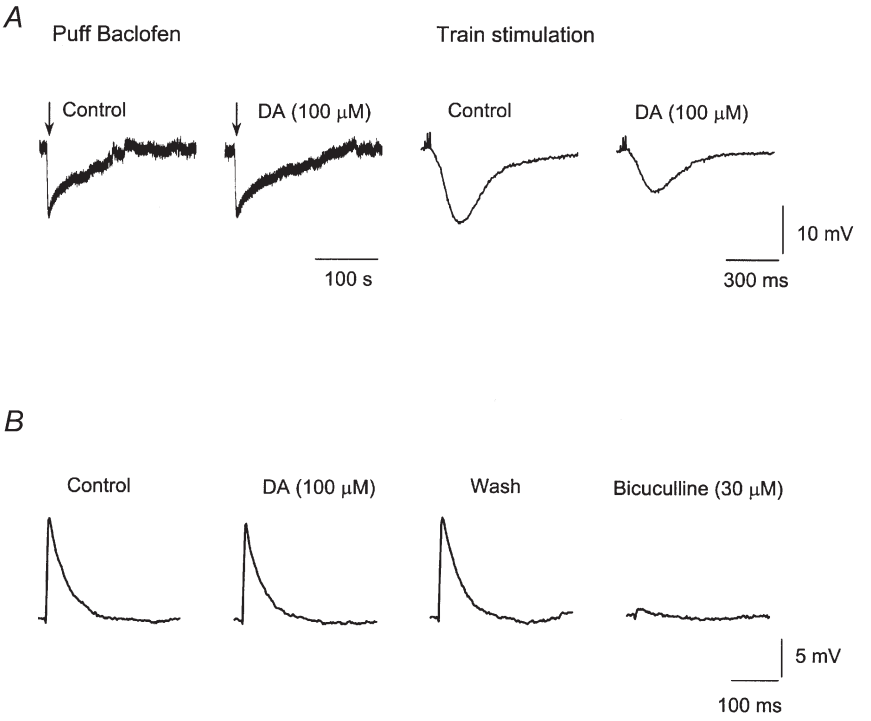

Figure 4. Dopamine selectively decreases the $GABA_B$-mediated IPSP by a presynaptic mechanism leaving the $GABA_A$ IPSP unaffected

A, dopamine (100 μM) reduces the $GABA_B$ IPSP without changing the membrane hyperpolarization caused by puffer applications of the $GABA_B$ agonist baclofen (300 μM, 10–20 p.s.i., 8–10 ms). *B*, lack of DA effects on the $GABA_A$ IPSP.

alone. Thus, we observed that the inhibition caused by DA (100 μM) was greatly reduced by the previous application of a maximal concentration of 5-HT (100 μM) (Fig. 5*C*). The lack of an additive effect supports the hypothesis that DA and 5-HT operate throughout the same signalling pathway activated by 5-HT_{1B} receptors and unconventional DA-recognising sites located on the GABA terminals impinging on the dopaminergic neurones.

The presynaptic actions of DA and 5-HT are independent of the modulation of high voltage-activated calcium, 4-AP and tolbutamide-sensitive potassium channels

The DA- and 5-HT-induced depression of GABA release on $GABA_B$ synapses could result either from the depression of calcium currents or the opening of potassium channels in presynaptic terminals. In the

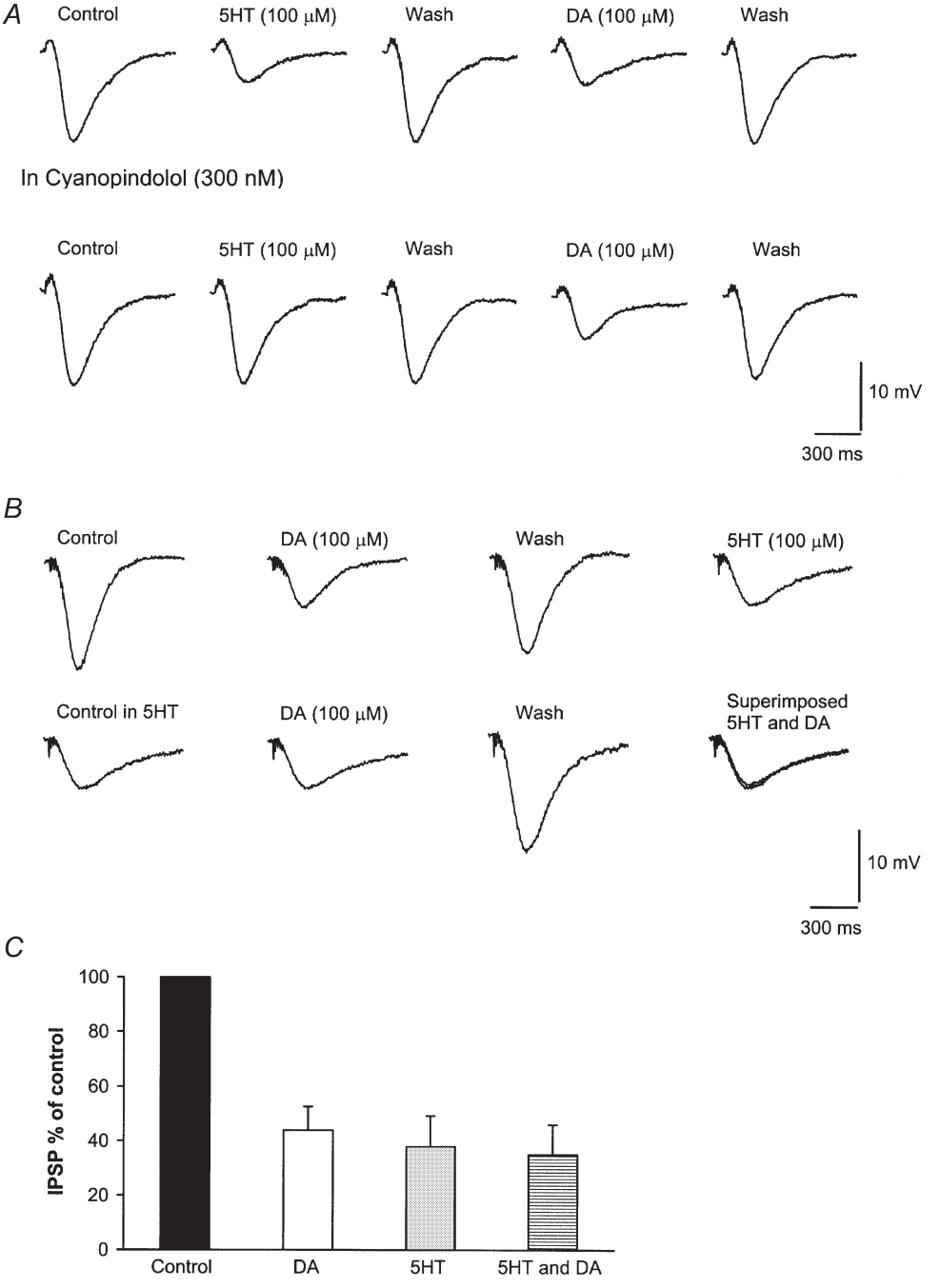

Figure 5. Activation of 5-HT_{1B} presynaptic receptors occludes the effects of DA on the $GABA_B$ IPSP

Examples of IPSP inhibition caused by 5-HT and DA. Only the 5-HT but not the DA effects are antagonized by the 5-HT_{1B} receptor antagonist cyanopindolol (*A*). The inhibition of the IPSP caused by 5-HT is very similar to that induced by DA (*B*). The graph shows that the inhibition of the $GABA_B$ IPSP caused by DA and 5HT is not additive. Each column shows the average of four to eight cells (*C*).

presence of the non-selective antagonist of the N/Q- and P-type calcium channels ω-CgTx MVIIC (2 μM) (Wheeler *et al.* 1994), a steady-state reduction of the amplitude of the $GABA_B$ IPSP to 30 ± 12 % ($n = 6$ of control) was obtained in 10–15 min.

Subsequently, increasing the intensity of the stimulating current restored the initial amplitude of the $GABA_B$ IPSP. In the presence of ω-CgTx MVIIC, either DA (100 μM) or 5-HT (100 μM) still depressed the $GABA_B$ IPSP by 68 ± 5 % ($n = 3$) and 61 ± 8 % ($n = 3$), respectively (Fig. 6). Remarkably, a similar degree of depression was observed in control conditions. In fact, DA (100 μM) and 5-HT (100 μM) reduced the $GABA_B$ IPSP by 65 ± 8 % ($n = 3$) ($P > 0.05$) and 60 ± 5 % ($n = 3$), ($P > 0.05$) of control, respectively. We also examined the possibility that DA and 5-HT could modulate L-type calcium channels to decrease GABA transmission by perfusing the L-type antagonist nifedipine (10 μM) (Bonci *et al.* 1998). In the presence of nifedipine, the amplitude of the $GABA_B$ IPSP did not significantly change and DA (100 μM) ($n = 3$) and 5-HT (100 μM) ($n = 3$) still suppressed the $GABA_B$ potential in a manner not different with respect to control conditions ($P > 0.05$ for both neurotransmitters) (Fig. 6). Alternatively, DA and 5-HT could control GABA release by modulating potassium channels in the presynaptic terminals. The depression of voltage-dependent potassium currents by the application of 4-AP (300 μM) rapidly (3–6 min) increased the amplitude of the $GABA_B$ potential by 300 ± 30 % ($n = 3$) of control. However, by decreasing the intensity of the stimulating current we restored the initial amplitude of the inhibitory potential. In the presence of 4-AP, DA (100 μM) ($n = 3$) and 5-HT (100 μM) ($n = 3$) both reduced the $GABA_B$ potential to 48 ± 5 % ($n = 3$) and 60 ± 10 % ($n = 3$) as in control conditions ($P > 0.05$ for each compound) (Fig. 6). In the presence of the K-ATP channel blocker, tolbutamide (1 mM), DA (100 μM) ($n = 3$) and 5-HT (100 μM) ($n = 3$) also caused an inhibition of the $GABA_B$ IPSP as in control conditions ($P > 0.05$ for each compound) (Fig. 6). Tolbutamide alone had no effect on the amplitude of the $GABA_B$ potential.

DISCUSSION

This paper describes an unconventional and novel depressant effect of dopamine on the $GABA_B$ IPSP, which is not mediated via the activation of D_1- and D_2-like receptors. In addition, the observation that we could antagonize the depressant effects of L-DOPA by blocking the DOPA-decarboxylase enzymes supports the specificity of the DA-induced depression of the $GABA_B$ IPSPs. In an area, reach of dopaminergic neurones L-DOPA mainly produces DA. However, the scant noradrenergic innervation of the substantia nigra pars compacta also suggests that DA and not NA mediates the depression of the $GABA_B$ IPSP.

While it is generally accepted that DA facilitates GABA release on $GABA_B$ synapses in the ventral midbrain by stimulating presynaptic D_1 receptors (Cameron & Williams,

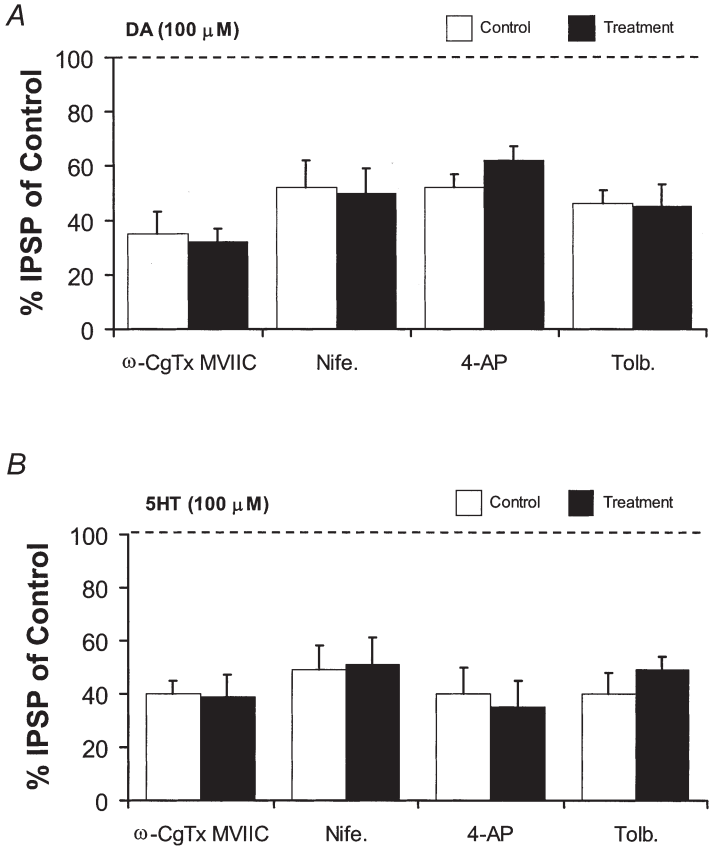

Figure 6. The superfusion of calcium and potassium blockers did not prevent DA and 5HT presynaptic actions

Dopamine (*A*) and serotonin (*B*) presynaptic actions in control and drug-treated slices (Nife, nifedipine; Tolb, tolbutamide). Columns show means of three experiments Note that either the DA- or the 5-HT-mediated inhibition of neurotransmitter release are not significantly different from control during the different experimental conditions ($P > 0.05$).

1993), our results demonstrate that, at least under particular conditions, DA strongly depresses (in a concentration-dependent manner), the $GABA_B$ potentials by activating sites with a new pharmacological profile. Therefore, these unconventional sites are activated by DA and NA and not by the invertebrate biogenic amine octopamine (Degen *et al.* 2000). In addition, they are not stimulated by D_1- and D_2-like agonists and not affected by a series of DA antagonists.

Although most NA antagonists were ineffective, the α2 adrenergic-blocking drugs yohimbine and phentolamine partially reduced DA action on the $GABA_B$ potentials. Conversely, the α2 adrenergic agonist UK 14304 was ineffective. This suggests the existence of functional DA-recognising sites, which are affected by α2-adrenergic antagonists but not by agonists.

The clear-cut depression exerted by DA on the slow $GABA_B$ IPSP without changing the fast inhibitory component ($GABA_A$) favours a specialised action of this catecholamine at the $GABA_B$ synapses in the ventral midbrain. The functional distinction is eventually sustained by anatomical differences between GABAergic terminals making separate connections on $GABA_A$ and $GABA_B$ receptors on the dopaminergic cells, being the $GABA_B$ responses probably caused by synapses arising from the forebrain and the $GABA_A$ from local interneurones (Lacey *et al.* 1989; Johnson *et al.* 1992; Sugita *et al.* 1992; Shoji *et al.* 1999). Interestingly, the novel sites of DA action are very likely located on the same GABAergic terminals bearing $5\text{-}HT_{1B}$ receptors. This is supported by the fact that the DA-induced depression of the $GABA_B$ IPSPs can be occluded by the application of 5-HT, suggesting that DA, which is released from dopaminergic cells dendrites (Cheramy *et al.* 1981), uses the same machinery operated by presynaptic $5\text{-}HT_{1B}$ receptors on the GABAergic afferents (Johnson *et al.* 1992).

Based on the experimental evidence that the DA- and the 5-HT-induced depression of the $GABA_B$ IPSPs are neither sensitive to the inhibition of N-, P/Q- and L type calcium channels nor to the depression of 4-AP- and K-ATP-sensitive potassium channels, we suggest that different subtypes of presynaptic channels could be involved in the inhibition of GABA release. Alternatively, DA and 5-HT could mediate inhibition of neurotransmitter release one step beyond an increased permeability to ions by directly regulating exocytotic fusion through the activation of G-proteins (Blackmer *et al.* 2001).

The presynaptic modulation of GABA release caused by DA might be an important phenomenon for the development of addiction, since it has also been demonstrated that the psychostimulant cocaine also causes a specific depression of the $GABA_B$ synaptic transmission mainly stimulating presynaptic 5-HT receptors (Cameron & Williams, 1994). The absence of any effect of 5-HT-, adenosine-, muscarine- and metabotropic glutamate receptor antagonists increases the possibility that DA activates unique sites to reduce $GABA_B$ IPSPs. There are conflicting results in the literature descibing the net effect of dopamine and dopaminergic agonists on GABA efflux in the ventral mesencephalon, namely that D1 receptors are mainly stimulatory and D2 receptors are mainly inhibitory (Arbilla *et al.* 1981; Kelly *et al.* 1985; Mattuszewich & Yamamoto, 1999). However, relatively high doses of DA preferentially cause a depression of GABA release in *in vivo* experiments (Korf *et al.* 1981; Chesselet, 1984). It is not difficult to imagine circumstances where DA could reduce, instead of increase, the release of GABA in the ventral midbrain. Thus, chronic treatment with high doses of neuroleptics, which block the classical D_2-/D_1-like receptors (Sedvall *et al.* 1995), could facilitate the depression of the $GABA_B$ IPSPs mediated by endogenously released DA. Additionally, the larger and sustained efflux of DA either caused by L-DOPA treatment of parkinsonian patients or by the compulsive intake of amphetamine/cocaine in addicts might inhibit the release of GABA at the $GABA_B$ synapses in the ventral midbrain. This might activate, under particular circumstances, the mesostriatal and mesocortical dopaminergic systems (Gonon, 1988) and could be involved in the production of extrapyramidal side effects and/or therapeutic actions of antipsychotics (Grace, 1991; Abi-Dargham *et al.* 2000; Kegeles *et al.* 2000). Alternatively, the presynaptic actions of DA described here could be involved in determining the beneficial/side effects of L-DOPA in parkinsonian patients (Chase *et al.* 1998) and might be involved in the addictive effects of psychostimulants (Wise & Bozarth, 1987; White, 1996).

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