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Differential Actions of Serotonin, Mediated by 5-HT_{1B} and 5-HT_{2C} Receptors, on GABA-Mediated Synaptic Input to Rat Substantia Nigra Pars Reticulata Neurons *In Vitro*

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The ability of serotonin to modulate GABA-mediated synaptic input to substantia nigra pars reticulata (SNr) neurons was investigated with the use of whole-cell patch-clamp recording from slices of rat midbrain. Fast evoked $GABA_A$ receptor-mediated synaptic currents (IPSCs) were attenuated reversibly ~60% by serotonin, which also caused an inward current with reversal potential of −25 mV. This inward current was blocked by the 5-HT_2 receptor antagonist ritanserin, whereas the IPSC depression was blocked by the 5-HT_{1B} receptor antagonist pindolol. The amplitude ratio of IPSC pairs (50 msec interpulse interval) was enhanced by serotonin (in ritanserin) and also by the $GABA_B$ receptor agonist baclofen (which also depressed the IPSC), consistent with a presynaptic site of action in both cases. In contrast, spontaneous tetrodotoxin-sensitive $GABA_A$ synaptic currents (sIPSCs) were increased in frequency by serotonin (an action that was sensitive to ritanserin, but not pindolol) but reduced in frequency by baclofen. SNr neurons therefore receive inhibitory synaptic input mediated by $GABA_A$ receptors from at least two distinct sources. One, probably originating from the striatum, may be depressed via presynaptic 5-HT_{1B} and $GABA_B$ receptors. The second is likely to arise from axon collaterals of SNr neurons themselves and is facilitated by an increase in firing via postsynaptic, somatodendritic 5-HT_{2C} receptor activation, but it is depressed by $GABA_B$ receptor activation. Thus, serotonin can both depolarize and disinhibit SNr neurons via 5-HT_{2C} and 5-HT_{1B} receptors, respectively, but excitation may be limited by GABA released from axon collaterals.

Key words: serotonin; substantia nigra pars reticulata; $GABA_A$ IPSPs; presynaptic receptors; baclofen; basal ganglia

The substantia nigra (SN) comprises two principal sets of projection neurons, both of which play important roles in the circuitry of the basal ganglia and thereby may influence critically the control of voluntary movement (Alexander and Crutcher, 1990). Although the dopamine-containing neurons of the substantia nigra pars compacta (SNc) have been the focus of much attention from cellular physiologists and pharmacologists in recent years (for review, see Kalivas, 1993; Lacey, 1993), the GABA-containing cells of the substantia nigra pars reticulata (SNr) have been comparatively neglected. However the SNr, together with the internal globus pallidus, constitutes a principal relay for basal ganglia output (Alexander and Crutcher, 1990; Chevalier and Deniau, 1990; Parent and Hazrati, 1995) and plays a clear role in movement initiation (Scheel-Kruger et al., 1977; Kilpatrick et al., 1982) by virtue of its influence on the thalamocortical pathway (Parent and Hazrati, 1995). Moreover, the SNr also gates propagation of generalized seizures (Gale, 1986; Depaulis et al., 1994) mediated by projections to the superior colliculus (Garant and Gale, 1987), which often arise from axonal branches of the same cells that innervate the thalamus (Deniau et al., 1978).

The substantia nigra receives innervation arising from the serotonin (5-hydroxytryptamine; 5-HT)-containing neurons of the dorsal raphe nucleus (Fibiger and Miller, 1977; Corvaja et al., 1993), and its functional role has been explored by several investigators, again with the main focus on dopamine neurons. However, the neurons of the SNr have been implicated in the contralateral turning (Oberlander et al., 1981; Kilpatrick et al., 1982) and stereotyped chewing behavior (Liminga et al., 1993) caused by intranigral serotonin application and also the anticonvulsant action of intranigral fluoxetine (the selective serotonin reuptake inhibitor; Pasini et al., 1992). Although *in vivo* experimentation has suggested an inhibitory action of serotonin on SNr neurons (Dray et al., 1976; Oberlander et al., 1981; Kilpatrick et al., 1982), we have demonstrated recently that serotonin can depolarize and excite rat SNr neurons in midbrain slices by a direct action on 5-HT_{2C}-like receptors (Rick et al., 1995). However, three serotonin receptor subtypes in the rat SN clearly have been identified by radioligand binding studies: 5-HT_{1B}, 5-HT_{2C} (Pazos and Palacios, 1985), and 5-HT_4 (Grossman et al., 1993). We have hypothesized that additional actions of serotonin in the SNr, possibly mediated by receptors other than 5-HT_{2C}, may contribute to its overall effect *in vivo* (Rick et al., 1995), and here we explore the modulation by serotonin of synaptic input to SNr neurons mediated by GABA. We show that serotonin acts on presynaptic 5-HT_{1B} receptors to depress evoked GABA-mediated synaptic input to SNr neurons. Furthermore, activation of the postsynaptic 5-HT_{2C} receptors promotes spontaneous synaptic activation of $GABA_A$ receptors, probably mediated by axon collaterals. These actions of serotonin are compared with those of the $GABA_B$ receptor agonist baclofen, which also acts both pre- and postsynaptically.

Some of these findings have been described previously in abstract form (Stanford and Lacey, 1995a).

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MATERIALS AND METHODS

Whole-cell patch-clamp recordings were made from parasagittal slices (300 μm thick) of rat (Wistar) midbrain obtained from animals 9–12 d old. Slices were prepared as described previously (Stanford and Lacey, 1995b). Slices were maintained in a recording chamber (0.75 ml) and perfused continuously at 2–3 ml/min with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, and glucose 10 buffered to pH 7.4 with Na_2HCO_3 (26 mM) and maintained at 33–35°C. Recordings were made with borosilicate glass pipettes (3–5 MΩ resistance) containing (in mM): K-gluconate 125, NaCl 10, $CaCl_2$ 1.0, $MgCl_2$ 2.0, BAPTA 10, HEPES 10, GTP 0.3, and Mg-ATP 2.0, adjusted to pH 7.25 with KOH (final K^+ concentration 165 mM). Cs-gluconate was substituted for K-gluconate in some experiments.

Individual neurons of the substantia nigra pars reticulata were visualized with a differential interference contrast (Nomarski) optical system (Zeiss Axioscop FS microscope, Oberkochen, Germany) with a 40× water immersion objective. Recording pipettes were advanced while under positive pressure toward individual cells in the slice, and, on contact, tight seals of 10–20 GΩ were made by applying negative pressure. Then the membrane patch was ruptured by suction, and membrane current and potential were monitored with an Axopatch 1B patch-clamp amplifier (Axon Instruments, Foster City, CA). Whole-cell access resistances were in the range of 10–30 MΩ before electronic compensation by 65–80%. After initial determination in current-clamp mode, the compensated access resistance was monitored continuously in voltage clamp by measuring the size of the capacitance transient in response to a 5 mV hyperpolarizing step (Stuart et al., 1993) and checked intermittently in current clamp. Experiments were abandoned if the access resistance changed by >20%. Membrane potentials were corrected with respect to the null potential measured at the end of recording.

Synaptic events were evoked by focal bipolar stimulation of the slice 300–600 μm rostral to the recording site. Single shock stimulations (0.5 msec, 0.5–3 mA) were made at 30 sec intervals by a constant current stimulation unit (AMPI Isoflex, Israel). Voltage steps were generated by pCLAMP software (Axon Instruments); the resulting membrane currents, as well as those resulting from synaptic activation, were stored to computer disk and a DAT recorder for subsequent analysis and display on a chart recorder (Gould Easygraph, Hainault, UK). Numerical data derived from experimental manipulations on synaptic currents were quantified from the mean of five consecutive single events. All numerical data are expressed as mean ± SD unless otherwise stated.

Drugs were applied to the superfusate by exchanging the aCSF for one differing only by the addition of a known concentration of drug, with the exchange beginning after a dead time of 20–30 sec. Drugs used were 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (−)D-2-amino-5-phosphonopentanoic acid (D-AP5), bicuculline methiodide, and picrotoxin (all from Tocris Cookson, Bristol, UK); and baclofen, tetrodotoxin (TTX), and 5-hydroxytryptamine maleate (serotonin, 5-HT; all from Sigma, St. Louis, MO). Ritanserin (10 mM stock in methanol) and pindolol (10 mM stock in 3 mM HCl) were obtained from Research Biochemicals International (Natick, MA).

RESULTS

Substantia nigra pars reticulata neurons

The cells included in this study (40 in total) exhibited spontaneous action potential firing at rates of 11.7 ± 4.6 Hz (range 5–26 Hz; $n = 18$) at rest in current-clamp recording mode. This property distinguishes these SNr neurons from the dopamine-containing neurons of the substantia nigra pars compacta (SNc), which generally have slower firing rates (<6 Hz; Fig. 1*A*) (for review, see Lacey, 1993; Stanford and Lacey, 1995b). In voltage clamp, the resting potential (at which holding current was zero) was −51.8 ± 3.4 mV (range from −45 to −58 mV; $n = 23$). Hyperpolarizing voltage steps of 200 msec duration from −60 mV to −140 mV did not reveal any notable membrane rectification, time-dependent or otherwise, which is a distinct and well established property of the dopamine-containing neurons of the substantia nigra (Fig. 1*B*). Membrane conductance over this voltage range was 2.65 ± 1.56 nS ($n = 35$). These properties correspond to those of previous descriptions of SNr neurons derived from intracellular recording (Nakanishi et al., 1987; Hajós and Greenfield, 1994; Stanford and Lacey, 1996); although several cells with the characteristics of dopamine neurons were encountered within the SNr during the course of these experiments, they specifically were excluded from the present study.

Serotonin caused an inward current

Application of serotonin (3–10 μM) caused an inward current in 25 of the 27 cells tested at holding potentials of −50 to −80 mV, which was reversible on washout, as described previously (Rick et al., 1995). At −80 mV this current was 55.6 ± 23.3 pA, and it was 45.8 ± 16.7 pA at −50 mV (both with serotonin 10 μM). Current/voltage (*I/V*) plots were generated by depolarizing voltage ramps (20 mV/sec) from a holding potential of −100 mV to 0 mV in five cells, two of which were recorded with pipettes containing Cs^+ instead of K^+, and in the presence of TTX (1 μM). Comparison of *I/V* plots obtained before and during the application of serotonin showed the serotonin inward current to reverse polarity at −27 ± 6.1 mV ($n = 3$; K^+ in pipettes) or at −33 and −21 mV, respectively ($n = 2$; Cs^+ in pipettes, with TTX in perfusate), with mean reversal potential of −24.6 ± 5.2 mV for all five experiments, accompanied by a linear conductance increase over the whole voltage range examined (Fig. 2). The similar reversal potential obtained by using intracellular dialysis with Cs^+, which would be expected to block potassium channels, suggests that the ionic basis of the serotonin inward current did not involve a change in potassium conductance. The serotonin inward current, which is considered to be mediated by 5-HT_{2C} receptors on SNr neurons (Rick et al., 1995), was blocked by ritanserin (1 or 3 μM), the antagonist of 5-HT_2-type receptors (Baxter et al., 1995), in all nine cells tested (see Fig. 3*A*).

Depression of evoked $GABA_A$ IPSCs by serotonin

Focal stimulation 300–600 μm rostral to the recorded cell evoked fast synaptic currents that, in the presence of glutamate receptor antagonists CNQX (10 μM) and D-AP5 (50 μM), were blocked by TTX (1 μM; $n = 2$) and also the $GABA_A$ receptor antagonists picrotoxin (50 μM; $n = 3$) and bicuculline (10 μM; $n = 4$). These IPSCs were outward at −50 mV, becoming inward at potentials negative to −65 mV, close to the predicted Cl^- equilibrium potential, all of which are consistent with mediation by $GABA_A$ receptors.

Serotonin reduced reversibly the amplitude of these evoked IPSCs by 54.2 ± 23.8% (3 μM serotonin; $n = 11$) and 60.0 ± 22.5% (10 μM serotonin; $n = 11$; Fig. 3). Unlike the serotonin inward current, this effect was resistant to ritanserin (3 μM; $n = 7$; Fig. 3) but was blocked by pindolol (3 μM), the antagonist of 5-HT_{1B} receptors (Hoyer, 1989), in six of eight cells tested (Fig. 3*B*). In contrast, pindolol (3 μM) was without effect on the serotonin inward current in both cells tested.

Baclofen caused an outward current and a depression of $GABA_A$ IPSCs

The $GABA_B$ receptor agonist baclofen has been shown previously to inhibit extracellularly recorded single-unit SNr cell firing *in vitro* (Rick and Lacey, 1994). In the present study 30 μM baclofen caused a small outward current (33 ± 7.5 p at −50 mV; $n = 3$), whereas 3 μM baclofen was without effect. However, at the lower concentration of 3 μM, baclofen reduced reversibly the amplitude of the evoked $GABA_A$ IPSC by 77.7 ± 9.9% ($n = 6$; Fig. 4).

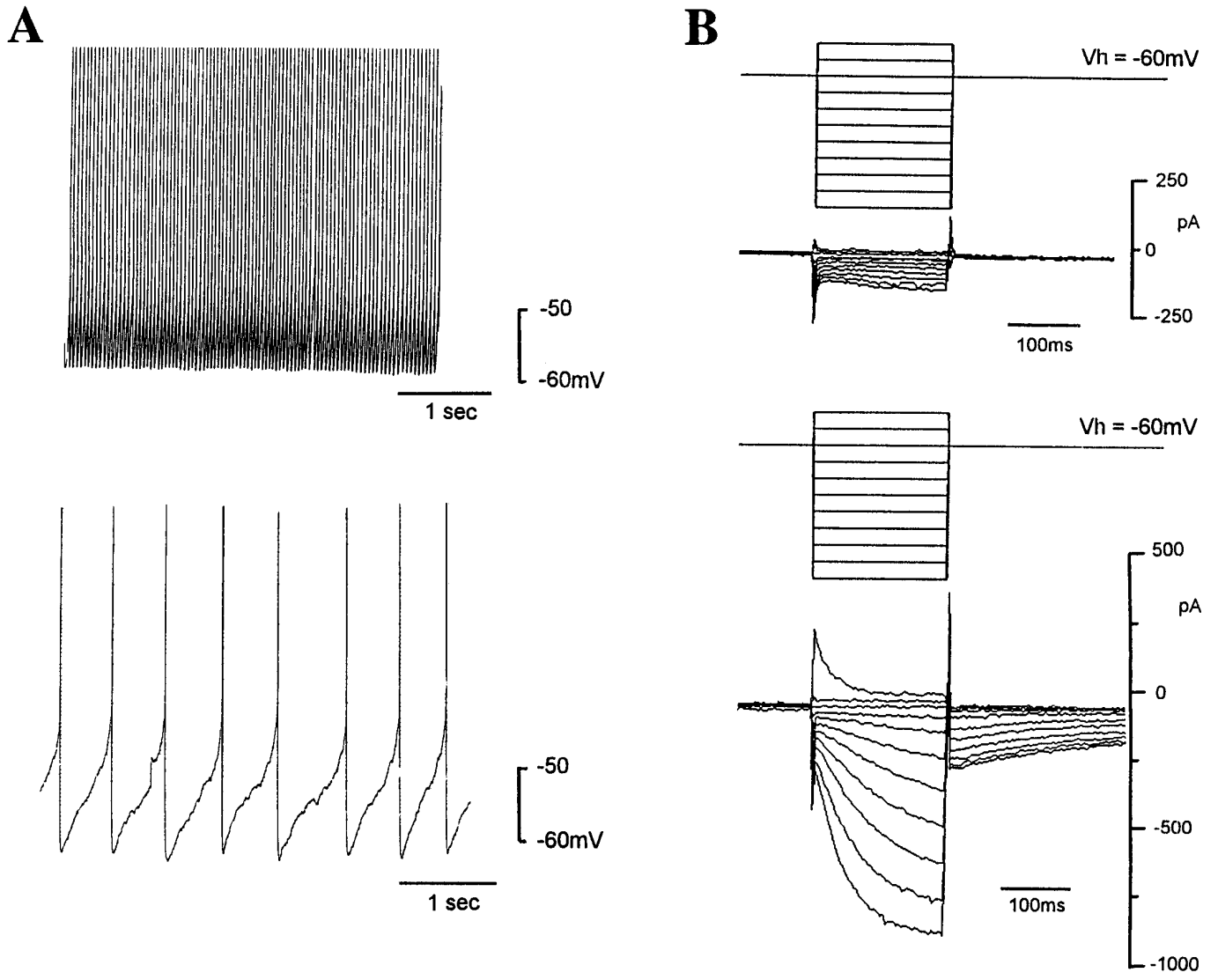

Figure 1. Substantia nigra pars reticulata neurons may be discriminated from dopaminergic neurons by their basic electrophysiological properties. *A*, Records of membrane potential from an SNr neuron (*top*) and a putative dopamine neuron (*bottom*), recorded at resting potential. Both cells fire action potentials spontaneously (full amplitude not reproduced) at regular rates but at markedly different frequencies (SNr neuron, 25 Hz; dopamine neuron, 1.75 Hz). *B*, Voltage steps (200 msec) to potentials in the range −40 to −140 mV performed under voltage clamp (holding potential −60 mV) demonstrate pronounced time-dependent inward rectification on hyperpolarization (I_h) in the dopaminergic neuron (*bottom records*), which is virtually absent in the SNr neuron (*top records*). The *top pair* of records shows the series of voltage steps applied to the same SNr neuron as in *A* and the resultant membrane currents, whereas the *bottom pair* shows the voltage steps and currents produced in the dopamine neuron in *A*.

Depression of the $GABA_A$ IPSC by serotonin and baclofen was attributable to presynaptic actions

To determine the site of action of serotonin and of baclofen in depressing the amplitude of the evoked IPSC, we adopted a paired-pulse protocol. $GABA_A$ IPSCs were evoked by single shocks of equal strength and duration paired at 50 msec intervals, with the stimulus strength adjusted in each experiment so that the second IPSC was always greater in amplitude (by 46 ± 38%; n = 8) than the first. Both serotonin (3 μM; in the presence of 3 μM ritanserin) and baclofen (3 μM) reduced the absolute amplitude of the IPSPs, but they also increased the degree of this paired-pulse facilitation (Fig. 4). Thus, the ratio of the amplitudes of the second IPSC to the first was increased significantly by serotonin (by 29.2 ± 14.7%; $p < 0.05$ with paired t test; n = 4) and by baclofen (60.5 ± 17.5%; $p < 0.01$; n = 4). Such a change in the paired-pulse ratio of synaptic current amplitudes is attributable to an action of both serotonin and baclofen on presynaptic 5-HT_{1B} and $GABA_B$ receptors, respectively, on the GABA-releasing nerve terminals (Davies et al., 1990; Travagli and Williams, 1996), causing reduction of GABA release. A postsynaptic site of action would be expected to reduce both the paired IPSCs to a similar extent, leaving the paired-pulse ratio unchanged.

Spontaneous $GABA_A$ IPSPs were facilitated by serotonin but depressed by baclofen

In the presence of glutamate receptor antagonists CNQX (10 μM) and D-AP5 (50 μM), 30% of cells (12/40) exhibited discernible spontaneous fast outward currents (sIPSCs; recorded at −50 mV). These were blocked by TTX (1 μM; n = 2; Fig. 6*A*), suggesting their dependence on spontaneous activity of neurons within the preparation, and also by picrotoxin (50 μM; 1 cell; Fig. 5*B*) and were therefore considered to be $GABA_A$ receptor-

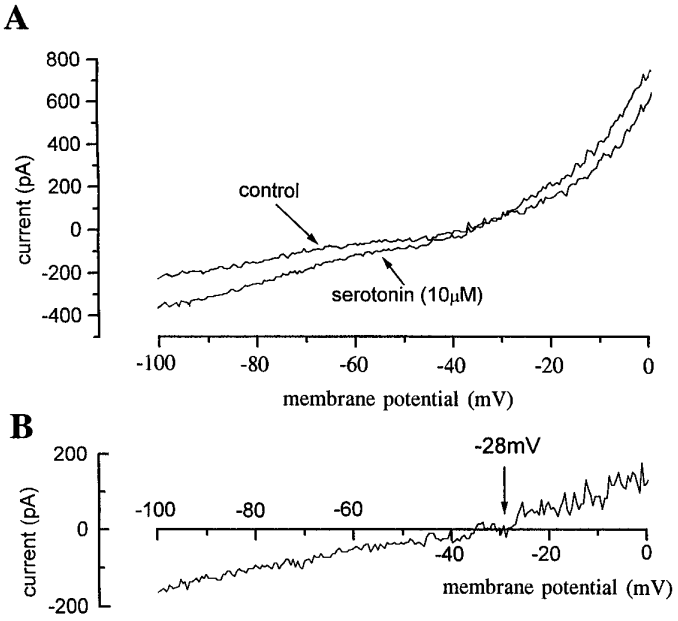

Figure 2. The serotonin inward current is voltage-independent and accompanied by a conductance increase, with a reversal potential at approximately −25 mV. *A*, Steady-state current/voltage plots obtained with a ramp depolarization (20 mV/sec) under voltage clamp from −100 to 0 mV before (*control*) and during application of serotonin (10 μM). Serotonin caused an inward current at potentials negative to −28 mV, reversing to an outward current between −28 and 0 mV. *B*, The serotonin current derived from *A* by digital subtraction is plotted against membrane potential. The current reverses polarity at −28 mV, and the conductance is essentially linear over the voltage range examined.

mediated synaptic currents. Serotonin (3–10 μM) caused a clear and reversible increase in the frequency of these sIPSCs in all five cells tested (Fig. 5*C*), an action that additionally was observed in the presence of pindolol (3 μM; $n = 2$), but not ritanserin (3 μM; $n = 2$). In contrast, baclofen (3 μM) reduced the frequency of spontaneous IPSCs in both cells tested (Fig. 5*D*).

DISCUSSION

Depression of the evoked IPSC by serotonin: a presynaptic action mediated by 5-HT_{1B} receptors

The depression of the evoked IPSC by serotonin was resistant to ritanserin but blocked by pindolol (3 μM; Fig. 3). Pindolol is a selective 5-HT_{1B} receptor antagonist (Hoyer, 1989) and, unlike ritanserin, does not block the serotonin inward current (present study) or the excitation of SNr neurons by serotonin (Rick et al., 1995), suggesting that this pindolol-sensitive site of the IPSC depression by serotonin is presynaptic. Although 5-HT_{1B} binding in rat SN has been demonstrated (Bruinvels et al., 1993b), the location of 5-HT_{1B} receptors on exclusively presynaptic sites is suggested by (1) the loss of radioligand binding to 5-HT_{1D} receptor binding in guinea pig SN (homologous to 5-HT_{1B} receptors in rat) after lesion of the striatonigral pathway (Waeber et al., 1990) and (2) the lack of messenger RNA encoding the 5-HT_{1B} receptor in SN (but considerable amounts in striatum; Bruinvels et al., 1993a). The enhancement of the paired-pulse ratio accompanying the IPSC inhibition seen with serotonin (Fig. 4) also points to a presynaptic location for the 5-HT_{1B} receptors. This resembles the report of presynaptic inhibition (via 5-HT_{1D} receptors) by serotonin of evoked glutamate release onto guinea pig substantia gelatinosa neurons (Travagli and Williams, 1996). Presynaptic 5-$HT_{1B/1D}$ heteroreceptors acting to reduce transmitter release also have been reported in locus coeruleus (Bobker and Williams, 1989a).

5-$HT_{1B/1D}$ receptor activation also depresses the slow $GABA_B$ receptor-mediated IPSP in dopamine neurons of the ventral tegmental area (VTA; Johnson et al., 1992; Cameron and Williams, 1994). However, the fast $GABA_A$ IPSP was unaffected by serotonin (Johnson et al., 1992), leading these authors to conclude that this serotonin-sensitive synaptic input to dopamine neurons in the VTA, which was most likely of striatal origin (Waeber et al., 1990), was mediated solely by $GABA_B$ receptors. If, by the same argument, the 5-HT_{1B} receptor-sensitive component of GABA-mediated synaptic input to SNr also is derived from the striatum, then it seems that, in contrast to the dopamine neurons in VTA, striatal input to SNr uses postsynaptic $GABA_A$ receptors. Indeed, using the same stimulus protocol as reported by Johnson et al. (1992), we have been unable to elicit a $GABA_B$ IPSC in SNr neurones, although a $GABA_B$ IPSC was readily demonstrable in SNc dopamine neurons (I. M. Stanford and M. G. Lacey, unpublished observations). Thus the serotonin-sensitive striatonigral input may use exclusively $GABA_A$ receptors on SNr neurons and $GABA_B$ receptors on dopamine neurons.

Pre- and postsynaptic actions of baclofen

The $GABA_B$ receptor agonist baclofen also considerably depressed the amplitude of the IPSC, accompanied by an increase in the paired-pulse ratio (Fig. 5), again strongly supporting a presynaptic site of action. Although inhibition of GABA release in SN by terminal "autoreceptors" of the $GABA_B$ type has been demonstrated [Giralt et al. (1990) but also see Waldmeier et al. (1989)]; it cannot be assumed necessarily that this was from exclusively striatonigral terminals. Pallidonigral fibers (Smith and Bolam, 1991), as well as intrinsic afferents (see below), also may have made a contribution to the IPSC. The postsynaptic action of baclofen was weak and, at a concentration of 3 μM (sufficient to depress the IPSC by 78%), it was without effect on membrane current. Although able to reduce cell firing rate (Rick and Lacey, 1994), this low postsynaptic sensitivity to baclofen contrasts with the robust responses to $GABA_B$ receptor activation in dopamine neurons (Lacey, 1993).

The serotonin inward current

The ritanserin-sensitive serotonin inward current, which we have demonstrated previously to be mediated by postsynaptic 5-HT_{2C} receptors (Rick and Lacey, 1994), was insensitive to pindolol. The firing rate increase induced by serotonin was also insensitive to pindolol and the 5-HT_4 antagonist GR113808, ruling out 5-HT_{1B} and 5-HT_4 receptors in this effect (Rick and Lacey, 1994). The serotonin inward current was accompanied by a voltage-independent conductance increase over the voltage range examined (−100 to 0 mV). Although excitations mediated by 5-HT_2 receptors in the nucleus accumbens (North and Uchimura, 1989), facial motoneurons (Larkman and Kelly, 1992), and cingulate cortex (Tanaka and North, 1993) have been shown to be attributable primarily to a decrease in resting potassium conductance, the increased conductance and apparent insensitivity of the reversal potential (−25 ± 5 mV) of the serotonin inward current to intracellular Cs^+ suggests this is not the case here. Moreover, the voltage independence of the serotonin current and the lack of a pronounced I_h in SNr neurons (Fig. 1*B*) renders it unlikely that augmentation of this current by serotonin plays a major role, as described elsewhere (McCormick and Pape, 1990; Takahashi and

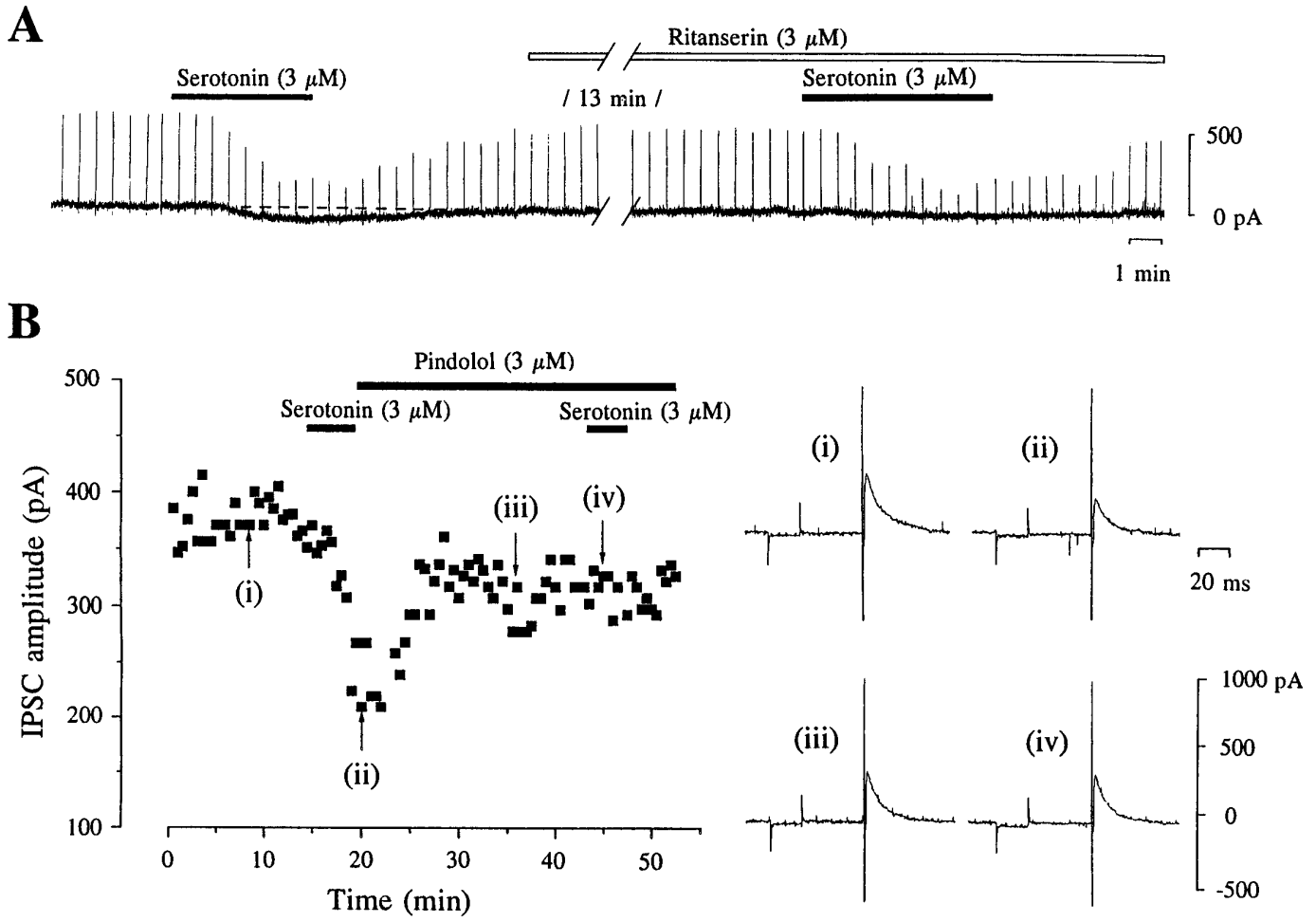

Figure 3. Serotonin depresses the evoked IPSC. Although the serotonin inward current is blocked by the 5-HT_2 antagonist ritanserin, the depression of the IPSC by serotonin is blocked by the 5-HT_{1B} antagonist pindolol. *A*, Serotonin (3 μM; *filled bars*) causes an inward current and also reversibly depresses the IPSC amplitude (transient outward currents evoked every 30 sec). In the presence of ritanserin (3 μM; *open bar*) serotonin still depresses the IPSC, but the inward current is blocked. Shown is a continuous record of membrane current, except for a 13 min break where indicated, from cell clamped at −50 mV. CNQX (10 μM) and D-AP5 (50 μM) are present throughout. *B*, *Left*, Plot of time course of experiment from another cell: serotonin (3 μM) reversibly depresses the evoked IPSC (*ii*) but was ineffective in the presence of pindolol (3 μM; *iv*). *Right*, Single records from this experiment, at times indicated on the plot, showing the IPSC preceded by current resulting from −5 mV test step. The cell was voltage-clamped at −50 mV in the continued presence of ritanserin (3 μM) as well as CNQX (10 μM) and D-AP5 (50 μM).

Berger, 1990; Bobker and Williams, 1989b). A more likely mechanism underlying this current is an increased nonselective cation conductance. 5-HT_{2C} receptors couple to phosphatidylinositol turnover (Conn et al., 1986) and intracellular calcium elevation (Watson et al., 1995) in choroid plexus epithelial cells; such a transduction mechanism well may be involved in the direct serotonin excitation of SNr neurons.

Spontaneous IPSCs represent recurrent axon collateral input

Spontaneous GABA-mediated IPSCs were detected in 12 of 40 cells. Because they were blocked by TTX in both cells tested, they probably depended on action potential firing and thereby on intact, spontaneously active GABA-containing cells within the preparation. Indeed, a tonic activation of $GABA_A$ receptors on SNr neurons in rat brain slices has been demonstrated previously (Rick and Lacey, 1994), and spontaneous action potential firing is abolished by TTX in serotonin-sensitive SNr neurons (Rick et al., 1995). The most likely source of the sIPSCs is the axon collateral network of the SNr projection neurons themselves, for which there is both anatomical (Karabelas and Purpura, 1980; Deniau et al., 1982) and physiological evidence (Deniau et al., 1982). However, although evidence for there being GABA-containing interneurons in SNr is scant (Wilson et al., 1977; Francois et al., 1979), they cannot be ruled out of consideration completely as a source of this input. The increased rate of sIPSCs seen with serotonin (Fig. 5*C*), which was blocked by ritanserin, but not pindolol, is consistent with the somatodendritic excitation of these neurons by serotonin, resulting in increased GABA release from their collaterals. A parallel may be drawn between these observations and those on the actions of norepinephrine in the hippocampus, in which α_1-adrenoceptor activation both depolarized interneurons directly and increased the frequency and amplitude of spontaneous IPSCs recorded in pyramidal cells, an effect that was not seen in TTX (Bergles et al., 1996). Additionally, 5-HT_2 and α_1 receptor activation both depolarize interneurons, causing spontaneous glycine and GABA-mediated synaptic potentials, respectively, in other neurons of the trigeminal nucleus (Grudt et al., 1995).

However, the relative sensitivity of the sIPSCs to depression by

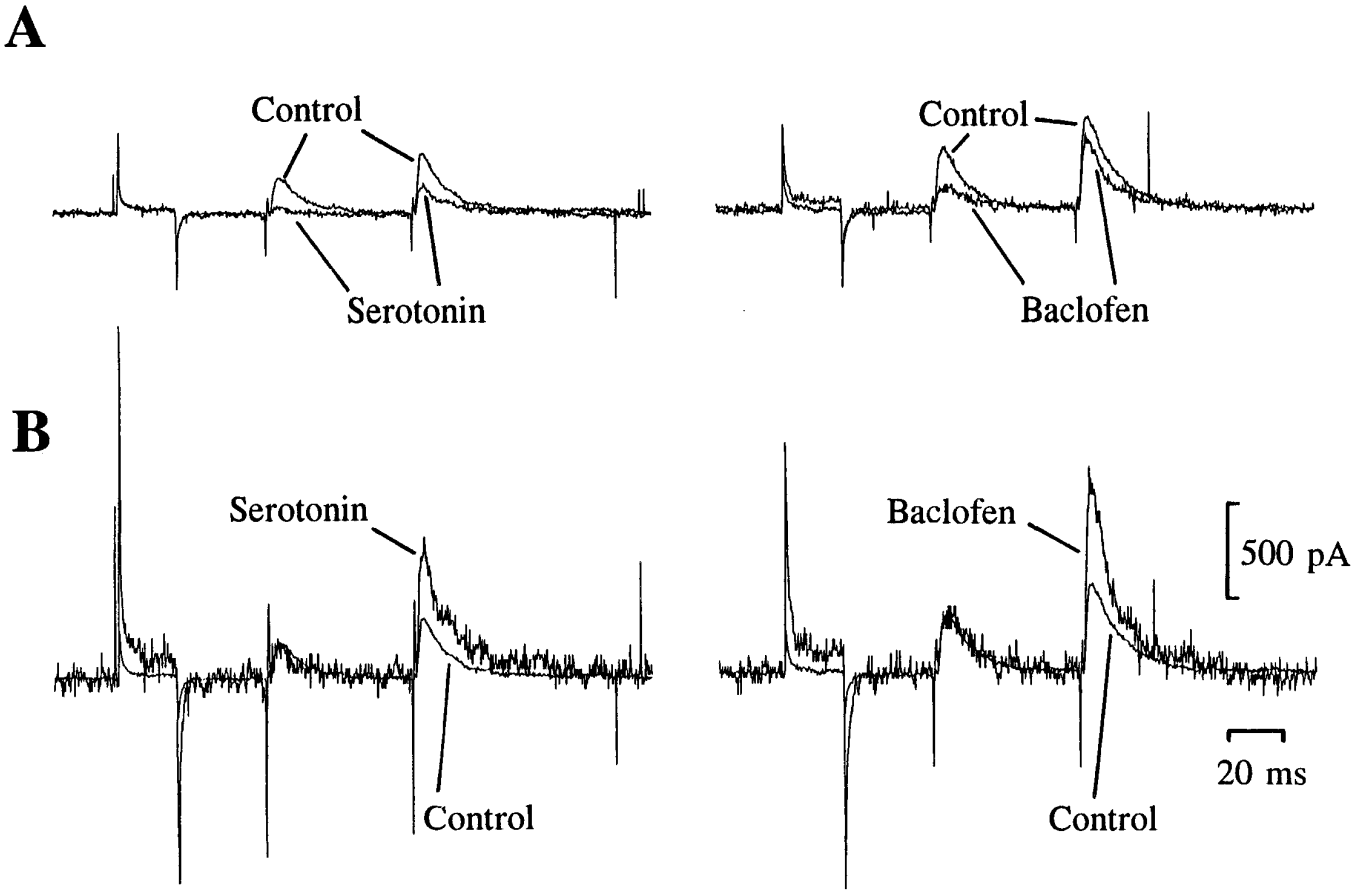

Figure 4. Both serotonin and baclofen enhance the paired-pulse IPSC ratio. *A*, Superimposed records from two different cells showing the effects of serotonin (3 μM; *left*) and baclofen (3 μM; *right*), relative to control in each case, on successive IPSCs evoked with the same stimulus at an interpulse interval of 50 msec. In control conditions, paired-pulse facilitation was observed in which the second IPSC was larger than the first. Both serotonin and baclofen reduced IPSC amplitude, but the second IPSC of the pair was reduced to a lesser extent than the first. Shown are IPSCs preceded by current resulting from +5 mV test step. Holding potential is −50 mV. Ritanserin (3 μM), CNQX (10 μM), and D-AP5 (50 μM) were present throughout. *B*, Same data as in *A*, but records in the presence of serotonin and baclofen are rescaled digitally so that the first IPSC of the pair is of similar amplitude to control. The second IPSC is clearly larger relative to control in the presence of both serotonin and baclofen, indicating that the depression of the IPSC (in *A*) by serotonin and baclofen occurs at a presynaptic locus.

baclofen, as compared with the somatic membrane current itself, implicates $GABA_B$ receptors on the terminals of axon collaterals themselves in this effect (Fig. 5*D*). Again, this may parallel the situation in the hippocampus, in which depression by baclofen of sIPSCs in hippocampal neurons arising from interneurons has been attributed to a reduction of calcium influx into presynaptic terminals (Doze et al., 1995).

Implications for the regulation of SNr output by serotonin

Studies of the actions of serotonin on SNr neurons *in vivo* indicate that 5-HT has a net inhibitory action (see the introductory remarks). Moreover, to comply with the original hypothesis of Gale (1985) that "inhibition of nigral efferents reduces susceptibility to generalized seizures," an inhibitory action of serotonin would be required to account for the anticonvulsant effect of intranigral fluoxetine (Pasini et al., 1992). Our results show that serotonin not only will excite SNr neurons directly (Rick et al., 1995; present study) but also will disinhibit them by reducing GABA release from (probably) striatonigral terminals. However, the ability of serotonin to promote increased lateral inhibition via GABA release from axon collaterals (Chevalier and Deniau, 1990) would serve to offset these excitatory influences. For this to translate into a net inhibitory action on SNr output and to disinhibit both the superior colliculus and the thalamocortical pathway, the extent and influence of the collateral network in the SNr would have to be considerably greater *in vivo* than in our brain slice preparation. It should be noted that, although no clear evidence was obtained for modulation of evoked GABA release by any serotonin receptor other than 5-HT_{1B}, it remains possible that, under different conditions, a significant role of 5-HT_4 receptors, considered to be located on striatonigral neuron terminals (Patel et al., 1995), could emerge.

Ritanserin has been shown to increase excitability of SNc and VTA dopamine neurons *in vivo* (Ugedo et al., 1989). However, it has been demonstrated that, in addition to innervating other SNr neurons, axon collaterals of SNr neurons also innervate dopamine neurons in SNc (Tepper et al., 1995). Thus, rather than representing a direct action, this excitation may be a consequence of ritanserin reducing serotonin-stimulated GABA release from SNr collaterals and thereby disinhibiting dopamine neurons. Indeed, the rather inconsistent results of functional pharmacological studies of serotonin actions on dopamine neurons both *in vivo* (Kelland et al., 1990) and *in vitro* (Pessia et al., 1994) may, at least in part, reflect an indirect contribution arising from serotonin actions on SNr neurons.

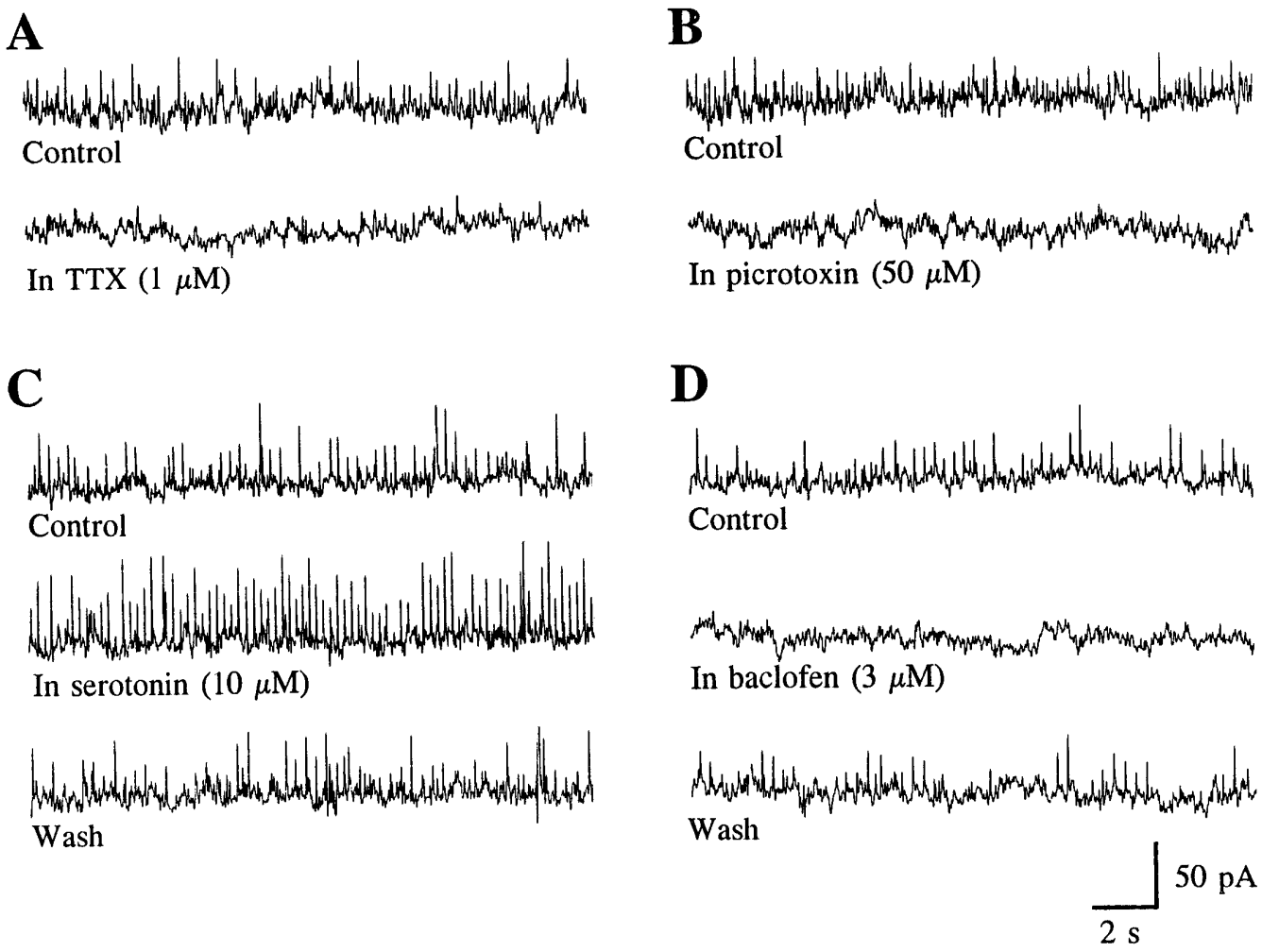

Figure 5. Spontaneous IPSCs mediated by GABA are reduced in frequency by baclofen but enhanced by serotonin. *A*, Spontaneous transient outward currents were abolished by TTX (1 μM) and also (*B*) by picrotoxin (50 μM). *C*, Spontaneous IPSCs were increased in frequency by serotonin (10 μM), which was reversible on washout. *D*, Spontaneous IPSCs were abolished reversibly by baclofen (3 μM). Records are from four different cells voltage-clamped at −50 mV, with CNQX (10 μM) and D-AP5 (50 μM) present throughout in all cases.

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