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Regulation of Central Synaptic Transmission by 5-HT$_{1B}$ Auto- and Heteroreceptors

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ABSTRACT

Although 5-HT$_{1B}$ receptors are believed to be expressed on nerve terminals, their precise mode of action is not fully understood because of the lack of selective antagonists. The 5-HT$_{1B}$ receptor knockout mouse was used in the present investigation to assess the function of 5-HT$_{1B}$ receptors in the modulation of synaptic transmission in three areas of the central nervous system: the dorsal raphe, the ventral midbrain, and the nucleus accumbens. *N*-(3-Trifluoromethylphenyl)piperazine, a 5-HT$_{1B}$ receptor agonist, potently inhibited 5-HT$_{1A}$ receptor-mediated slow inhibitory postsynaptic potentials (IPSPs) in the dorsal raphe of wild-type but not knockout mice. Both synaptically released 5-HT and exogenous 5-HT caused a presynaptic inhibition that outlasted the postsynaptic hyperpolarization only in wild-type mice. In the ventral midbrain, 5-HT$_{1B}$ receptor-dependent inhibition of γ-aminobutyric acid$_B$ IPSPs in dopamine neurons was present in wild-type animals and absent in knockout animals. Similar results were obtained in the nucleus accumbens measuring glutamate-mediated excitatory postsynaptic currents in medium spiny neurons. Finally, cocaine, which blocks 5-HT uptake, inhibited IPSPs in the dorsal raphe and the ventral midbrain of wild-type but not knockout mice, whereas cocaine produced comparable inhibition of excitatory postsynaptic currents in the nucleus accumbens of both types of animals. These results indicate that 5-HT$_{1B}$ receptors function as autoreceptors and heteroreceptors to exert presynaptic inhibition of transmitter release in the central nervous system. Furthermore, this study underscores the role played by presynaptic 5-HT$_{1B}$ receptors in mediating the effects of cocaine on synaptic transmission.

Serotonin (5-HT) is a neuromodulator widely distributed in the central nervous system and has been implicated in a number of neurophysiological functions. The multiple actions of serotonin are mediated by at least 14 subtypes of membrane surface receptors, most of which are members of the G protein-coupled receptor superfamily (for review, see Barnes and Sharp, 1999).

The 5-HT$_{1B}$ receptor was initially identified as a [^{3}H]5-HT binding site with low affinity for spiperone in the rodent brain (Pedigo et al., 1981). Another [^{3}H]5-HT binding site, designated the 5-HT$_{1D}$ site, was found in other species, including human, dog, and guinea pig (Hoyer and Middlemiss, 1989). It had a distribution similar to that of the 5-HT$_{1B}$ site in rodents, although the pharmacological profile was distinct. Subsequent molecular biological studies have demonstrated that the 5-HT$_{1D\beta}$ receptor found in higher species is a close homolog of the rodent 5-HT$_{1B}$ receptor (Adham et al., 1992; Jin et al., 1992), leading to the recent reclassification of these receptors as the 5-HT$_{1B}$ subtype (Hartig et al., 1996). On the other hand, the 5-HT$_{1D\alpha}$ receptor, found in all mammalian species (Hamblin and Metcalf, 1991; Hamblin et al., 1992), is now termed the 5-HT$_{1D}$ receptor (Hartig et al., 1996).

5-HT$_{1B}$ receptors are expressed in many brain areas, including the basal ganglia and the midbrain raphe nuclei. 5-HT$_{1D}$ receptors seem to be colocalized with 5-HT$_{1B}$ receptors, although at much lower densities (Bruinvels et al., 1993). A unique feature of the expression pattern of 5-HT$_{1B}$ receptors is the mismatch in the distribution of 5-HT$_{1B}$ receptor mRNAs and 5-HT$_{1B}$ binding sites, which suggests that 5-HT$_{1B}$ receptors are expressed predominantly on nerve terminals of both 5-HT and non-5-HT neurons (Boschert et al., 1994). This anatomical distribution is consistent with the idea that 5-HT$_{1B}$ receptors function both as 5-HT auto- and heteroreceptors to control the release of neurotransmitters.

The most selective ligands at 5-HT$_{1B}$ and/or 5-HT$_{1D}$ recep-

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ABBREVIATIONS: IPSP, inhibitory postsynaptic potential; GABA, γ-aminobutyric acid; EPSC, excitatory postsynaptic current; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]-quinoxaline; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; TFMPP, *N*-(3-trifluoromethylphenyl)piperazine.

tors are agonists. Thus, previous pharmacological studies were based on a combination of selective agonists and relatively nonselective antagonists. The recently developed selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 has turned out to have a partial agonistic activity both in vitro and in vivo (Pauwels, 1997), compromising its usefulness as a probe for the functions of $HT_{1B/1D}$ receptors.

In the present study, the 5-HT_{1B} receptor knockout mouse was used to re-evaluate the involvement of 5-HT_{1B} receptors in modulating the release of neurotransmitters in the central nervous system. To this end, synaptic potentials or currents were examined in three specific brain areas: the dorsal raphe, the ventral midbrain, and the nucleus accumbens. Each of these areas is known to have presynaptic 5-HT_{1B} receptors, which have been suggested to inhibit synaptic transmission. These include 5-HT-mediated slow IPSPs in the dorsal raphe (Pan and Williams, 1989a), GABAergic slow IPSPs in the ventral midbrain (Johnson et al., 1992; Cameron and Williams, 1994), and glutaminergic EPSCs in the nucleus accumbens (Muramatsu et al., 1998). Furthermore, the effects of cocaine, which elevates extracellular 5-HT concentration by uptake blockade, were investigated. The results indicate that activation of presynaptic 5-HT_{1B} receptors produces potent inhibition of the release of 5-HT, GABA, and glutamate in the central nervous system and unequivocally demonstrate the participation of 5-HT_{1B} receptors in the actions of cocaine.

Materials and Methods

Animals. All experiments were performed on tissues obtained from wild-type and 5-HT_{1B} knockout inbred mice (20–53 days old). Both wild-type and knockout mice were bred in the Portland VA Veterinary Medical Unit. Mice were maintained at 22 ± 1°C with a 12-h/12-h light/dark cycle. The background strain comprised a mixture of the 129/SvPas, 129/Sv-ter, and 129/SvEvTac substrains of 129 inbred mice (Phillips et al., 1999).

Slice Preparation. The preparation of slices containing the dorsal raphe, the ventral midbrain, and the nucleus accumbens was as described previously (Pan et al., 1989; Cameron and Williams, 1994; Manzoni et al., 1998). Briefly, mice were anesthetized with halothane and killed. Brain slices (180–220 μm) were cut with a vibratome (Leica, Nussloch, Germany) in cold (4°C) physiological saline. For recordings from dorsal raphe neurons, coronal slices were taken at the level of the decussation of the cerebellar peduncle or where the aqueduct begins to open to the fourth ventricle. For recordings from midbrain dopamine neurons, horizontal slices were taken near the floor of the interpeduncular fossa. For recordings from medium spiny neurons, parasagittal slices containing the nucleus accumbens were cut. A single slice was placed in a recording chamber and superfused with warmed (35°C) physiological saline containing 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 11 mM glucose, 21.4 mM $NaHCO_3$, saturated with 95% O_2 and 5% CO_2.

Recordings in the Dorsal Raphe and the Ventral Midbrain. Cells were visualized with an upright microscope with infrared illumination, and whole-cell recordings of membrane potential were made with patch pipettes (1.8–2.5 MΩ) containing 115 mM K gluconate, 20 mM KCl, 1.5 mM $MgCl_2$, 1 mM BAPTA, 2 mM MgATP, 0.2 mM GTP, 10 mM Na_2 phosphocreatine, buffered with 10 mM HEPES, pH 7.3, 285 mOsm/kg. An Axopatch 1D amplifier (Axon Instruments, Foster City, CA) was used to record the data, which were filtered at 1 kHz, digitized at 5 kHz, and collected on a personal computer using AxoGraph 4 or pCLAMP 6 (Axon Instruments).

A single stimulus (500 μs) or a train of 10 stimuli (500 μs at 70 Hz) was delivered every 60 s to evoke synaptic potentials in dorsal raphe neurons or midbrain dopamine neurons, respectively, using a bipolar tungsten stimulating electrode placed near (30–100 μm) the soma. The stimulus intensity was adjusted to obtain a maximal IPSP in each cell. To evoke a pair of IPSPs in dorsal raphe neurons, two successive stimuli were applied at a 4-s interval, and the paired-pulse ratio was calculated by dividing the amplitude of the first IPSP by that of the second IPSP. When recordings were made in dopamine neurons, a small hyperpolarizing current (10–50 pA) was injected to maintain the membrane potential at −55 to −65 mV and stop the spontaneous firing. Traces of IPSPs are shown after subtracting the baseline membrane potential obtained during the 100-ms window before the stimuli, unless otherwise stated.

To isolate slow synaptic potentials, the superfusion medium contained a cocktail of antagonists which blocks fast synaptic potentials [i.e., NBQX (5 μM), picrotoxin (100 μM), and strychnine (1 μM) to block AMPA-, $GABA_A$-, and glycine-mediated synaptic potentials, respectively]. Slices were always pretreated with MK-801 (50 μM) to block the NMDA-mediated synaptic potential. Prazosin (100 nM) was further added to block the α_1-adrenergic receptor-mediated slow EPSP in dorsal raphe neurons, and eticlopride (100 nM) was included to block the dopamine D_2-mediated slow IPSP in midbrain dopamine neurons. No metabotropic glutamate receptor IPSP was observed in dopamine neurons with the whole-cell recording using K gluconate-based intracellular solution.

Recordings in the Nucleus Accumbens. Whole-cell recordings of membrane current were made with patch pipettes (2–4 MΩ) containing 128 mM KCl, 20 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.3 mM $CaCl_2$, 2 mM MgATP, 0.3 mM GTP, 0.2 mM cAMP, buffered with 10 HEPES, pH 7.3, 290 mOsm/kg. The holding potential was −70 mV. An Axopatch 200A amplifier (Axon Instruments) was used to record the data, which were filtered at 1 kHz, digitized at 5 kHz, and collected on a personal computer using ACQUIS-1 (Bio-Logic, Claix, France) or pCLAMP 6. To evoke EPSCs, extracellular stimuli (100 μs) were delivered every 30 s via bipolar tungsten stimulating electrodes at the prefrontal-accumbens border. The superfusion medium contained picrotoxin (100 μM) and strychnine (1 μM) to block $GABA_A$- and glycine-mediated synaptic currents, respectively. Two stimuli were applied at an interval of 50 ms to obtain a paired-pulse ratio, which was calculated by dividing the amplitude of the first EPSC by that of the second EPSC.

Drug Application. All drugs were applied by superfusion. Drugs used are as follows: 5-HT, cocaine, DAMGO, fenfluramine, picrotoxin, strychnine, and prazosin were from Sigma (St. Louis, MO); pindobind −5HT_{1A}, TFMPP, MK-801, and eticlopride were from RBI (Natick, MA); NBQX was from Tocris Cookson (Ballwin, MO); CGP35348 was a gift from Novartis (Basel, Germany); and GR 127935 was a gift from Glaxo Wellcome (Stevenage, UK).

Data Analysis. IPSP and EPSC amplitudes were measured, respectively, by averaging 20-ms and 2-ms windows around the peak and subtracting the average value obtained during 100-ms and 5-ms windows immediately before the stimuli. The time constants for the rising phase and decaying phase of the IPSP were obtained by fitting the IPSP trajectory with a double exponential function using AxoGraph 4. Data are expressed as mean ± S.E.M. Statistical significance was determined with unpaired Student's t test or Mann-Whitney U test. The difference was considered significant at $P < .05$.

Results

Dorsal Raphe Neurons. Whole-cell recordings of synaptic potentials were made at the resting membrane potential (−55 to −65 mV) from dorsal raphe neurons in wild-type and 5-HT_{1B} knockout mice. All experiments were carried out in slices treated with NBQX (5 μM), MK-801 (50 μM), picrotoxin (100 μM), strychnine (1 μM), and prazosin (100 nM) (see *Materials and Methods*). A single electrical stimulus

evoked a slow IPSP that was blocked by the selective 5-HT_{1A} receptor antagonist pindobind 5-HT_{1A} (1 μM) by 95 ± 3% and 100 ± 0% in wild-type and knockout mice, respectively (n = 3 in each group). This IPSP was reproducible throughout the duration of recordings (~3 h) with the application of stimuli at 60-s intervals. The presence of a 5-HT-mediated IPSP in the mouse dorsal raphe was expected from the experiments done in rats (Pan et al., 1989).

No Compensatory Alterations in 5-HT_{1B} Knockout Mice. The time course of the IPSP in wild-type and knockout animals was analyzed by examining the first IPSP evoked by a pair of stimuli separated by 4 s. This IPSP (P_1) was fitted to a double exponential function to obtain time constants of the rising and decaying phases of IPSP (Fig. 1A). The rise time constant reflects the activation kinetics of the underlying potassium conductance, whereas the rate of 5-HT uptake by the serotonin transporter is the major determinant of the decay time constant (Pan et al., 1989). It was found that both of these time constants were not significantly different between wild-type and knockout mice (Fig. 1B). Furthermore, the peak amplitude of P_1, which should be determined by the rise and decay time constants, the amount of 5-HT released, and the sensitivity of postsynaptic 5-HT_{1A} receptor, was also similar in wild-type (13.6 ± 0.9 mV, 3.7–23.5 mV, n = 34) and knockout animals (13.4 ± 0.8 mV, 6.2–23.3 mV, n = 37, $P >$ .8) (Fig. 1C). Thus, deletion of the presynaptic 5-HT_{1B} receptor caused no significant changes in the functioning of the serotonin transporter and the postsynaptic 5-HT_{1A} receptor, or in the presynaptic 5-HT release mechanism in the dorsal raphe.

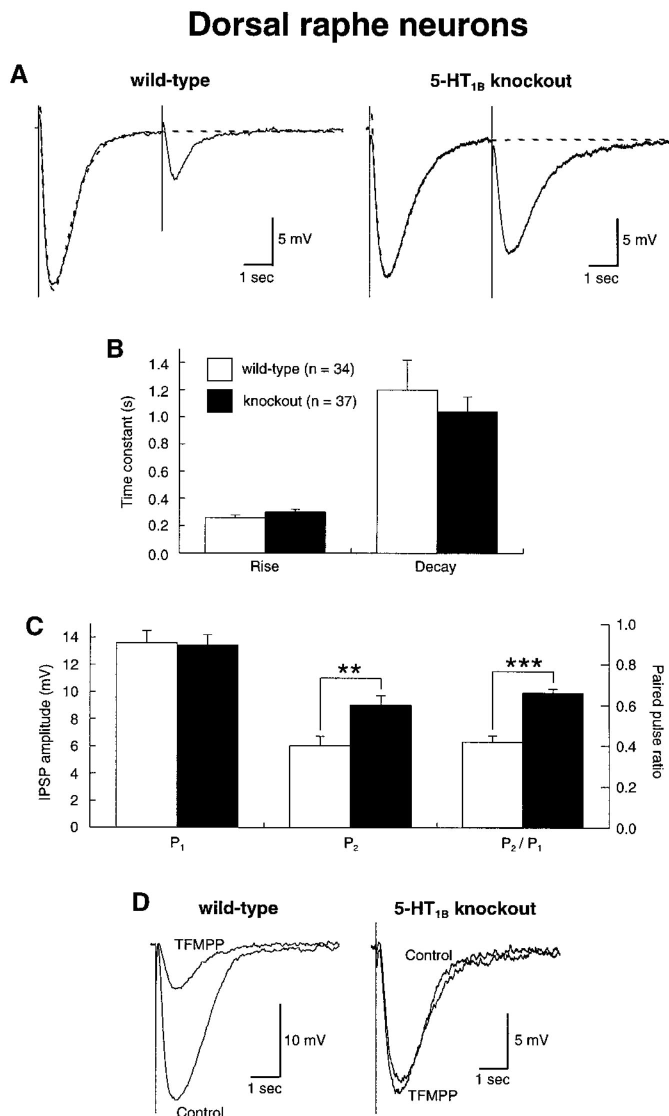

Fig. 1. Properties of 5-HT_{1A} IPSPs in the dorsal raphe of wild-type and 5-HT_{1B} knockout mice. A, representative traces of 5-HT_{1A} IPSPs in wild-type and knockout mice. IPSPs were evoked by a pair of stimuli separated by 4 s to obtain the first (P_1) and sond (P_2) IPSPs. Dashed lines are the double exponential fit to P_1. B, the time constants of the rising and decaying phases of IPSPs determined by the double exponential fit as in A. C, P_1 and P_2 amplitudes and the paired-pulse ratio (P_1/P_2). D, representative traces showing the effect of TFMPP (1 μM) in wild-type and knockout mice. Data are shown as means ± S.E.M. **$P < .01$; ***$P <$ .001.

Presynaptic 5-HT_{1B} Receptor-Mediated Inhibition of 5-HT_{1A} IPSPs Is Absent in Knockout Mice. Application of TFMPP (1 μM), a moderately selective 5-HT_{1B} receptor agonist, reduced the P_1 amplitude to 20 ± 10% of control in wild-type mice (n = 3), whereas it slightly augmented the P_1 amplitude in knockout mice (107 ± 9% of control, n = 3, $P <$ .005 versus wild-type) (Fig. 1D). TFMPP had no measurable effect on the baseline membrane potential nor did it block the hyperpolarization caused by application of the 5-HT_1 agonist 5-CT (100 nM; data not shown). Thus, it seemed that TFMPP selectively activated 5-HT_{1B} receptors without acting on 5-HT_{1A} receptors in this preparation. These data indicate that activation of presynaptic 5-HT_{1B} receptors potently inhibits evoked 5-HT release in the dorsal raphe.

The peak amplitude of the second IPSP of the pair (P_2), evoked 4 s after P_1, was depressed compared with P_1 in both wild-type and knockout mice (paired-pulse depression; Fig. 1A). However, P_2 amplitude was significantly smaller in wild-type mice than in knockout mice. Hence, the magnitude of paired-pulse depression was significantly larger in wild-type mice (Fig. 1C). These results suggest that synaptically released 5-HT can activate presynaptic 5-HT_{1B} autoreceptors to inhibit subsequent release of 5-HT, and that this mechanism accounts for part of the paired-pulse depression of the 5-HT_{1A} IPSP.

Persistent Effect of 5-HT on the Presynaptic 5-HT_{1B} Receptor. The effect of 5-HT released by the first stimulus on presynaptic 5-HT_{1B} receptors outlasted the duration of the postsynaptic 5-HT_{1A} receptor-mediated IPSP. After 4 s, the 5-HT_{1A} IPSP produced by the first stimulus had recovered but the second stimulus caused a much smaller IPSP (Fig. 1A). We next examined whether this differential kinetics of presynaptic versus postsynaptic effects of synaptically released 5-HT could also be observed for exogenously applied 5-HT. Superfusion of 5-HT (1 μM) caused a membrane hyperpolarization of 16.1 ± 3.2 mV (n = 4) and 17.6 ± 2.4 mV (n = 3) in wild-type and knockout mice, respectively ($P >$ .7) (Fig. 2B), further confirming the similar sensitivity of the postsynaptic 5-HT_{1A} receptor in both animals. In wild-type mice, 5-HT (1 μM) almost completely abolished the IPSP (7 ± 3% of control, n = 4) (Fig. 2B). After washout of 5-HT, the membrane hyperpolarization recovered rapidly (~3 min). However, inhibition of the IPSP recovered much more slowly over the time course of ~10 min. Thus, a significant reduction in the IPSP amplitude was observed even when the membrane potential had completely recovered (Fig. 2A), in-

dicating that 5-HT-induced inhibition of the IPSP was not simply caused by occlusion of the IPSP by the membrane hyperpolarization. In contrast, in the same experiments with knockout mice, the IPSP was largely occluded but not abolished. In the presence of 5-HT (1 μM), the IPSP was still 31 ± 6% of control ($n = 3$, $P < .05$ versus wild-type) (Fig. 2D). In addition, after washout of 5-HT, the membrane hyperpolarization and IPSP inhibition recovered over a similar time course (Fig. 2, C and D), suggesting that postsynaptic occlusion was mainly responsible for the IPSP inhibition. We observed a small inhibition of IPSP transiently remaining after hyperpolarization had subsided in one cell from knockout mice, which may reflect a presynaptic effect of 5-HT not mediated by 5-HT_{1B} receptors. Taken together, these results show that the effect of 5-HT on presynaptic 5-HT_{1B} receptors is more persistent than that on postsynaptic 5-HT_{1A} receptors.

The Effects of 5-HT Uptake Inhibition by Cocaine. Application of cocaine increased the decay time constant of IPSP, resulting from the blockade of 5-HT uptake (Fig. 3A and B). This action was similar in wild-type and knockout mice, indicating that the sensitivity of the serotonin transporter was not different. However, cocaine affected the peak amplitude of the IPSP differently in two groups of animals (Fig. 3, A and C). Superfusion of cocaine (1 μM) increased the P_1 amplitude to 128 ± 8% and 152 ± 29% in wild-type and knockout animals, respectively ($n = 5$ each, $P > .4$). Increasing the concentration of cocaine from 1 μM to 10 μM caused a reduction in the P_1 amplitude to 88 ± 13% of control in wild-type mice ($n = 5$), whereas the P_1 amplitude remained at the same level in knockout mice (148 ± 19% of control, $n = 5$, $P < .05$ versus wild-type). Under the conditions of these experiments, cocaine had no effect on the membrane potential in either group of animals. These results indicate that an increase in the ambient concentration of 5-HT produced by cocaine acts selectively on presynaptic 5-HT_{1B} receptors to inhibit 5-HT release.

Dopamine Neurons in the Ventral Midbrain. A train of 10 extracellular stimuli was applied to evoke synaptic potentials mediated by $GABA_B$ receptors in midbrain dopamine neurons. The membrane potential was maintained at −55 to −65 mV to stop the spontaneous firing and $GABA_B$ IPSPs were isolated pharmacologically (see *Materials and Methods*). IPSPs thus recorded were completely blocked by the $GABA_B$ receptor antagonist CGP35348 (100 μM) in both wild-type and knockout mice ($n = 3$ each). The average amplitude of the IPSP was similar in wild-type (10.2 ± 1.1 mV, 4.6–22.1 mV, $n = 18$) and knockout mice (10.5 ± 0.8 mV, 5.2–17.8 mV, $n = 20$, $P > .8$).

Application of 5-HT (10–30 μM) caused a marked reduction of the IPSP amplitude in wild-type mice, although it failed to affect IPSPs in 5-HT_{1B} knockout mice (Fig. 4, A and B). On the other hand, the μ-opioid receptor agonist DAMGO (300 nM), which is known to presynaptically inhibit the $GABA_B$ IPSP (Shoji et al., 1999), reduced the IPSP amplitude to a similar degree in wild-type and knockout mice, indicating that the machinery required for the presynaptic inhibition is intact in knockout mice. 5-HT produced no measurable change in the membrane potential in both animals. Thus, 5-HT acts on presynaptic 5-HT_{1B} receptors on GABAergic terminals to inhibit the release of GABA on $GABA_B$ receptors.

Superfusion with cocaine (1 μM) and the 5-HT releasing agent fenfluramine (10 μM) significantly inhibited the IPSP in wild-type mice (Fig. 4, C and D). Both of these drugs had no effect on the membrane potential or the time course of the IPSP. In contrast, IPSPs were marginally affected by cocaine (1 μM) and fenfluramine (10 μM) in knockout mice (Fig. 4C, D). These data indicate that increasing the ambient concentration of endogenous 5-HT with cocaine or fenfluramine inhibits GABA release through activation of presynaptic 5-HT_{1B} receptors.

Medium Spiny Neurons in the Nucleus Accumbens. Whole-cell recordings were made at a holding potential of

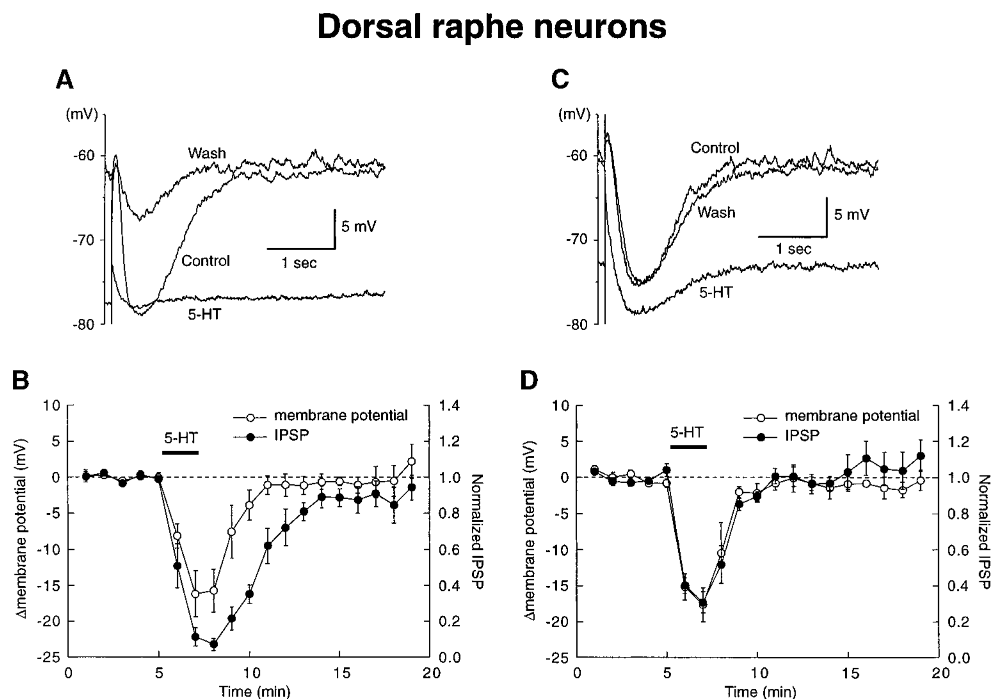

Fig. 2. The effects of exogenous 5-HT on the membrane potential and IPSPs in the dorsal raphe. A and C, representative traces of IPSPs illustrating the effects of 5-HT (1 μM) in wild-type (A) and 5-HT_{1B} knockout (C) mice. Traces of IPSPs immediately before application of 5-HT (Control), in the presence of 5-HT (5-HT), and at the time when the membrane potential had recovered to control level after washout of 5-HT (Wash) are shown. The baseline membrane potential before the stimulus is not subtracted in these traces to depict the hyperpolarization induced by 5-HT. B and D, summary graphs illustrating the time course of the effects of 5-HT (1 μM) in wild-type ($n = 4$; B) and knockout ($n = 3$; D) mice. The baseline membrane potential before the stimulus is plotted after subtraction of the mean membrane potential obtained during the 5-min period before application of 5-HT, and the IPSP amplitude is normalized to the mean amplitude obtained during the same 5-min period. Data are expressed as mean ± S.E.M.

−70 mV from medium spiny neurons in the nucleus accumbens. Glutamate EPSCs elicited by focal extracellular stimuli were isolated by blocking IPSCs with receptor antagonists (see *Materials and Methods*). The 5-HT_{1B} receptor agonist TFMPP (1 μM) inhibited EPSCs in wild-type mice (Fig. 5, A and B). This inhibition was associated with an increase in the paired-pulse ratio (151 ± 41% of control, n = 5), consistent with a presynaptic locus of action. Superfusion with fenfluramine (10 μM) also caused a reduction in the EPSC amplitude (Fig. 5B). Both of these drugs failed to affect EPSCs in knockout mice. These results show that activation of presynaptic 5-HT_{1B} receptors inhibits glutamate release and that this can be achieved by the endogenous 5-HT.

Cocaine at 1 μM had no significant effect on EPSCs, whereas 10 μM cocaine reduced the EPSC amplitude to a similar degree in both wild-type and knockout mice (Fig. 5C). Thus, cocaine inhibits glutamate EPSCs through a mechanism independent of 5-HT_{1B} receptors in the nucleus accumbens.

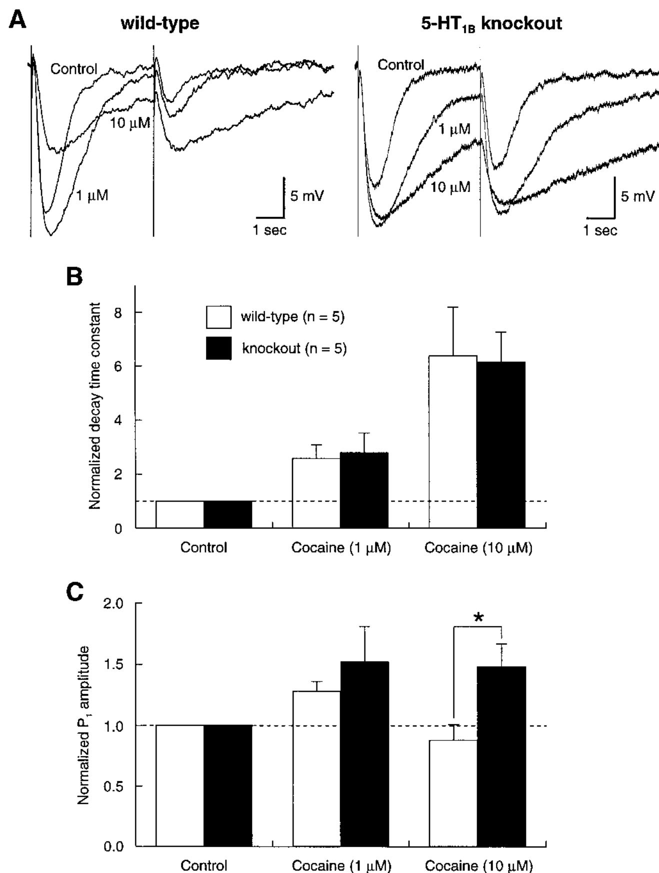

Fig. 3. The effects of cocaine on IPSPs in the dorsal raphe. A, representative traces of IPSPs illustrating the effects of cocaine in wild-type and 5-HT_{1B} knockout mice. B, a summary bar graph showing the effects of cocaine on the time constants of the rising and decaying phases of IPSP. Sequential application of increasing concentrations of cocaine (1 μM and 10 μM) was made in each cell. The time constants are normalized to those obtained in the absence of cocaine. C, a summary bar graph depicting the effects of cocaine on the P_1 amplitude. The P_1 amplitude is normalized to that obtained in the absence of cocaine. Data are expressed as mean ± S.E.M. *$P < .05$.

Discussion

Using the 5-HT_{1B} receptor knockout mouse, this study demonstrates that the presynaptic 5-HT_{1B} receptor functions both as an autoreceptor to inhibit 5-HT release in the dorsal raphe and as a heteroreceptor to inhibit the release of GABA and glutamate in the ventral midbrain and the nucleus accumbens, respectively. Endogenous 5-HT, released either electrically, spontaneously or pharmacologically, can activate presynaptic 5-HT_{1B} receptors on all of these three types of terminals (i.e., serotonergic, GABAergic, and glutaminergic). Furthermore, the cocaine-induced inhibition of the 5-HT-mediated IPSP in the dorsal raphe and the GABAergic IPSP

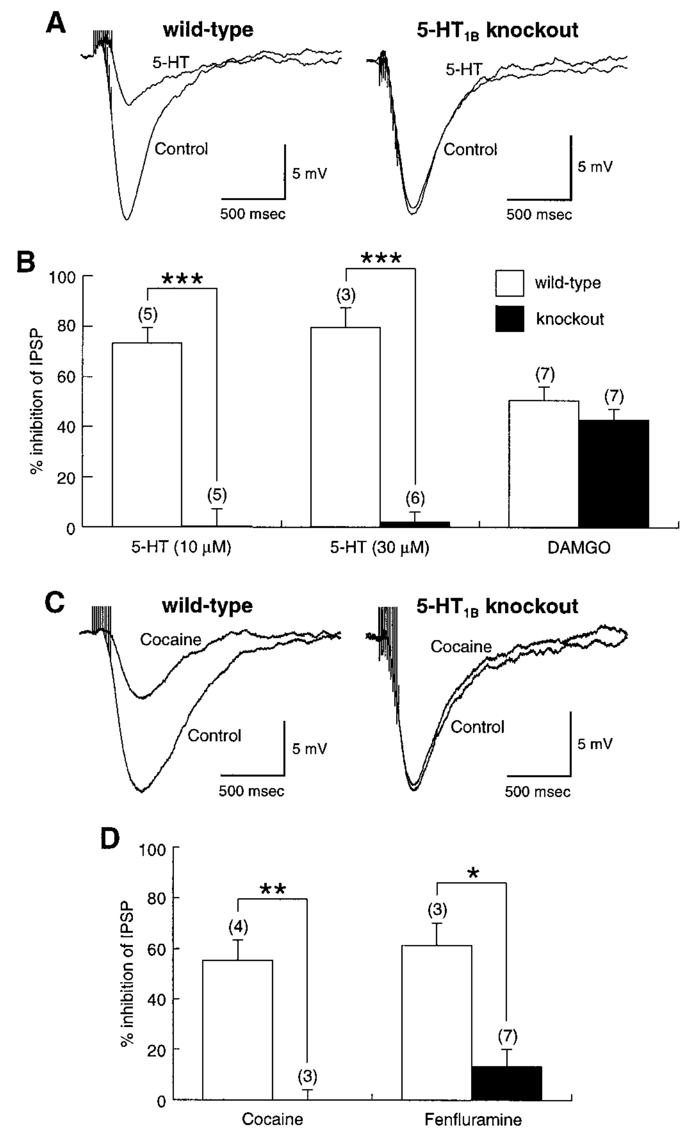

Fig. 4. 5-HT_{1B} receptor-mediated inhibition of $GABA_B$ IPSPs in midbrain dopamine neurons. A, representative traces of $GABA_B$ IPSPs showing the effect of 5-HT (10 μM) in wild-type and 5-HT_{1B} knockout mice. IPSPs were evoked by a train of 10 stimuli (70 Hz). B, A summary bar graph of the inhibition of $GABA_B$ IPSPs by 5-HT (10 μM, 30 μM) and DAMGO (300 nM). C, representative traces of IPSPs depicting the effect of cocaine (1 μM) in wild-type and knockout mice. D, a summary bar graph of the inhibition of IPSPs by cocaine (1 μM) and fenfluramine (10 μM). Data are shown as means ± SEM. *$P < .05$; **$P < .01$; ***$P < .001$.

in the ventral midbrain was mediated by the activation of presynaptic 5-HT_{1B} receptors. In contrast, presynaptic 5-HT_{1B} receptors were not involved in the effects of cocaine on the glutaminergic transmission in the nucleus accumbens.

Presynaptic 5-HT_{1B} Receptor as an Autoreceptor in the Dorsal Raphe. 5-HT_{1A} IPSPs exhibited paired-pulse depression when two IPSPs were elicited 4 s apart. The magnitude of paired-pulse depression was reduced significantly in knockout mice, indicating a significant contribution of the activation of presynaptic 5-HT_{1B} autoreceptors. Paired-pulse depression of slow synaptic potentials has been described for 5-HT_{1A} IPSPs in the nucleus prepositus hypoglossi (Bobker and Williams, 1990) and for $GABA_B$ IPSPs in the ventral midbrain and the CA1 region of the hippocampus (Davies et al., 1990; Cameron and Williams, 1994). In all these cases, neurotransmitters released by the first stimulus were suggested to activate presynaptic autoreceptors to suppress their own release. Our data with 5-HT_{1B} receptor knockout mice provides the most compelling evidence for the involvement of presynaptic autoreceptors in this form of frequency-dependent fatigue of the slow synaptic transmission.

Paired-pulse depression of ~35% was observed even in knockout mice. It was not caused by desensitization of the postsynaptic 5-HT_{1A} receptor, because hyperpolarization produced by prolonged application of 5-HT showed no desensitization (data not shown). 5- HT_{1D} receptors are colocalized with 5-HT_{1B} receptors on nerve terminals in several brain areas, including the dorsal raphe (Bruinvels et al., 1993). Thus, activation of presynaptic 5- HT_{1D} receptors could be responsible for the paired-pulse depression observed in 5-HT_{1B} knockout mice. In line with this, it has been reported that 5- HT_{1D} receptors mediate suppression of 5-HT release in the midbrain raphe nuclei of 5-HT_{1B} knockout mice (Piñeyro et al., 1995). Alternatively, this paired-pulse depression may well be caused by heterosynaptic depression by neurotransmitters other than 5-HT. Because GABA and norepinephrine are known to be released by electrical stimulation in the dorsal raphe (Yoshimura et al., 1985; Pan and Williams, 1989b), the $GABA_B$ receptor antagonist CGP35348 (100 μM) and the α_2-adrenergic receptor antagonist idazoxan (1 μM) were tested. Neither had an effect on the paired-pulse ratio (data not shown). However, the involvement of other neurotransmitters cannot be ruled out. Finally, it is possible that presynaptic depletion of synaptic vesicles containing 5-HT plays a role in this depression.

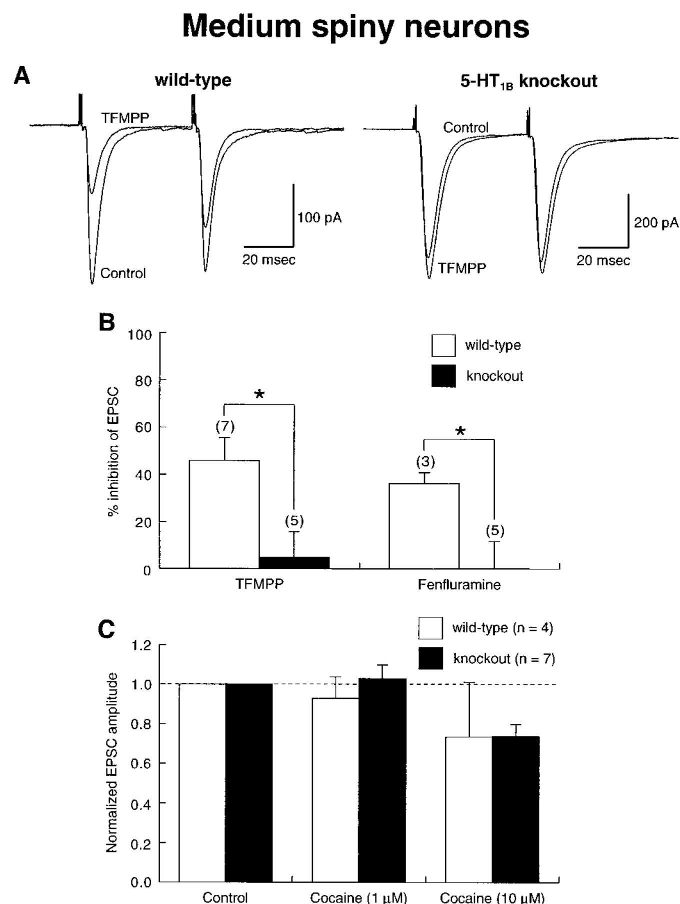

Fig. 5. 5-HT_{1B} receptor-mediated inhibition of glutamate EPSCs in the nucleus accumbens. A, Representative traces of EPSCs illustrating the effect of TFMPP (1 μM) in wild-type and 5-HT_{1B} knockout mice. EPSCs were evoked by a pair of stimuli separated by 45 ms. B, a summary bar graph showing the inhibition of EPSCs by TFMPP (1 μM) and fenfluramine (10 μM). C, a summary bar graph depicting the effect of cocaine on EPSCs. Sequential application of increasing concentrations of cocaine (1 μM and 10 μM) was made in each cell. The EPSC amplitude is normalized to that obtained in the absence of cocaine. Data are expressed as mean ± S.E.M. $^*P < .05$.

The action of 5-HT on presynaptic 5-HT_{1B} receptors seems to persist longer than that on postsynaptic 5-HT_{1A} receptors. Thus, 5-HT released by electrical stimulation could inhibit subsequent release of 5-HT through activation of 5-HT_{1B} receptors after 4 s (paired-pulse depression), when the hyperpolarization mediated by postsynaptic 5-HT_{1A} receptors had decayed. Likewise, the inhibition of IPSP caused by exogenous 5-HT (1 μM) lasted longer (~10 min) after washout than the hyperpolarization, which decayed rapidly (~3 min). This differential kinetics of the effects of exogenous 5-HT was not observed in knockout mice. It can be accounted for if 5-HT acts more potently on 5-HT_{1B} receptors than on 5-HT_{1A} receptors, so that lower concentrations of 5-HT can selectively activate presynaptic 5-HT_{1B} receptors without activating postsynaptic 5-HT_{1A} receptors. Indeed, it has been reported that $GABA_B$ agonists act more potently on presynaptic than postsynaptic $GABA_B$ receptors in the hippocampus, resulting in a similar long-lasting presynaptic effect compared with the postsynaptic effect (Davies et al., 1990; Isaacson et al., 1993). However, lower concentrations of 5-HT (100–300 nM) produced marginal hyperpolarization with no long-lasting inhibition of the IPSP even in wild-type mice. This is consistent with the similar potency of 5-HT on the cloned 5-HT_{1A} and 5-HT_{1B} receptors in expression systems (Fargin et al., 1989; Maroteaux et al., 1992). Therefore, the more persistent effect of 5-HT on presynaptic 5-HT_{1B} receptors than on postsynaptic 5-HT_{1A} receptors was not caused by a difference in the sensitivity of these receptors, but instead should be caused by the persistence of 5-HT at the presynaptic site compared with the postsynaptic site. The mechanism for this is unclear at present.

Cocaine (1 μM) augmented the amplitude and duration of the 5-HT-mediated IPSP in the dorsal raphe in both wild-type and knockout mice. This augmentation can be accounted for by the increase in the decay time constant of the IPSP resulting from 5-HT uptake blockade by cocaine (Pan and Williams, 1989a). Increasing the concentration of cocaine to 10 μM produced a decrease in the IPSP amplitude only in wild-type mice. Similar inhibition of the IPSP by relatively

high concentrations of cocaine was observed in rat dorsal raphe (Pan and Williams, 1989a) and guinea pig prepositus hypoglossi (Bobker and Williams, 1991). The absence of this inhibition in 5-HT_{1B} knockout mice indicates that it results from the activation of presynaptic 5-HT_{1B} receptors by increased ambient 5-HT.

The increase in ambient 5-HT caused by cocaine failed to activate postsynaptic 5-HT_{1A} receptors, as opposed to the hyperpolarization observed in a previous study with intracellular recording (Pan and Williams, 1989a). It is possible that, with whole-cell recording in the present study, uptake inhibition did not elevate the extracellular concentration of 5-HT enough at the soma of the recorded cell, which was located close to the surface of the slice.

Presynaptic 5-HT_{1B} Receptor as a Heteroreceptor in the Ventral Midbrain and the Nucleus Accumbens. 5-HT, the 5-HT_1 agonist TFMPP, or the 5-HT releasing agent fenfluramine suppressed GABAergic and glutaminergic transmission in the ventral midbrain and the nucleus accumbens, respectively, only in wild-type mice. These results confirm previous pharmacological studies reporting the involvement of presynaptic 5-HT_{1B} receptors in these effects (Johnson et al., 1992; Cameron and Williams, 1994; Muramatsu et al., 1998). The ventral midbrain and the nucleus accumbens are known to receive substantial 5-HT innervation from the median and dorsal raphe (Parent et al., 1981). Moreover, ultrastructural studies have found evidence for nonjunctional communications made by these 5-HT terminals in the ventral midbrain and the nucleus accumbens (Hervé et al., 1987; Van Bockstaele and Pickel, 1993). Thus, endogenous 5-HT may well diffuse extrasynaptically to act on presynaptic 5-HT_{1B} receptors expressed at non-5-HT terminals. Indeed, cocaine inhibited $GABA_B$ IPSPs in midbrain dopamine neurons from wild-type mice but not from knockout mice, showing that facilitating the diffusion of endogenous 5-HT by uptake blockade can induce activation of presynaptic 5-HT_{1B} receptors at GABAergic terminals in the ventral midbrain. A similar conclusion was reached on a pharmacological basis in a previous study done in slices from guinea pig (Cameron and Williams, 1994). In the nucleus accumbens, on the other hand, cocaine produced a comparable inhibition of glutaminergic EPSCs in both wild-type and knockout mice, excluding the involvement of 5-HT_{1B} receptors. This is in accord with the previous report showing that cocaine suppresses glutaminergic transmission in the nucleus accumbens through an increase in endogenous extracellular dopamine levels and subsequent activation of presynaptic dopamine receptors (Nicola et al., 1996). It should be noted that a relatively high concentration (10 μM) was required to obtain this inhibitory effect of cocaine in the nucleus accumbens compared with the concentration of cocaine (1 μM) that caused inhibition of GABAergic transmission in the midbrain. This is consistent with the known lower affinity of cocaine for the dopamine transporter than for the serotonin transporter (Ritz et al., 1987).

Significance. Studies on the functions of 5-HT_{1B} receptors have been confounded by the lack of a potent and selective antagonist. GR 127935, a benzanilide compound, was originally developed as a highly selective $HT_{1B/1D}$ receptor antagonist (Skingle et al., 1993). However, accumulating evidence has demonstrated that it has a considerable intrinsic activity at $HT_{1B/1D}$ receptors both in vitro and in vivo (Pauwels, 1997). In line with this, GR 127935 (1 μM) inhibited $GABA_B$ IPSPs and occluded the effect of 5-HT in midbrain dopamine neurons (data not shown). On the other hand, the 5-HT_{1B} receptor knockout mice have been used successfully to probe the functions of 5-HT_{1B} receptors in behavior and neuropsychiatric disorders (Scearce-Levie et al., 1999). Although some researchers have suggested that developmental compensations are responsible for the increased vulnerability to cocaine in 5-HT_{1B} receptor knockout mice (Rocha et al., 1998; Shippenberg et al., 2000), our results show that 5-HT_{1B} receptors are indeed responsible for the effects of cocaine on synaptic transmission in the ventral midbrain and the dorsal raphe, two brain areas believed to be important for cocaine sensitization (Parsons and Justice, 1993). Furthermore, deletion of 5-HT_{1B} receptors seemed to induce no compensatory changes in the properties of synaptic transmission recorded with brain slices. Thus, the impact of ablation of the 5-HT_{1B} receptor protein could be unambiguously detected when the assay was performed at the site of its expression (i.e., the presynaptic terminal).

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