Behavioral/Systems/Cognitive

Impact of Serotonin 2C Receptor Null Mutation on Physiology and Behavior Associated with Nigrostriatal Dopamine Pathway Function

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The impact of serotonergic neurotransmission on brain dopaminergic pathways has substantial relevance to many neuropsychiatric disorders. A particularly prominent role has been ascribed to the inhibitory effects of serotonin 2C receptor (5-HT2CR) activation on physiology and behavior mediated by the mesolimbic dopaminergic pathway, particularly in the terminal region of the nucleus accumbens. The influence of this receptor subtype on functions mediated by the nigrostriatal dopaminergic pathway is less clear. Here we report that a null mutation eliminating expression of 5-HT2CRs produces marked alterations in the activity and functional output of this pathway. 5-HT2CR mutant mice displayed increased activity of substantia nigra pars compacta (SNc) dopaminergic neurons, elevated baseline extracellular dopamine concentrations in the dorsal striatum (DSt), alterations in grooming behavior, and enhanced sensitivity to the stereotyped behavioral effects of d-amphetamine and GBR 12909. These psychostimulant responses occurred in the absence of baseline extracellular dopamine concentrations in the dorsal striatum (DSt), alterations in grooming behavior, and enhanced sensitivity to the stereotyped behavioral effects of d-amphetamine and GBR 12909. These psychostimulant responses occurred in the absence of phenotypic differences in drug-induced extracellular dopamine concentration, suggesting a phenotypic alteration in behavioral responses to released dopamine. This was further suggested by enhanced behavioral responses of mutant mice to the D1 receptor agonist SKF 81297. Differences in DSt D1 or D2 receptor expression were not found, nor were differences in medium spiny neuron firing patterns or intrinsic membrane properties following dopamine stimulation. We conclude that 5-HT2CRs regulate nigrostriatal dopaminergic activity and function both at SNc dopaminergic neurons and at a locus downstream of the DSt.

Introduction

The prominence of central serotonin (5-HT) and dopamine (DA) systems in the pathophysiology and treatment of neuropsychiatric disorders underscores the importance of understanding how these systems interact. It is established that the serotonin system regulates the mesoaccumbal and nigrostriatal dopaminergic pathways (Esposito, 2006; Alex and Pehek, 2007; Fink and Götthert, 2007). Serotonergic cell bodies in the raphe nuclei project to the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) and their terminal projections within the dorsal striatum (DSt) and nucleus accumbens (NAc), respectively (Bobillier et al., 1975; Fibiger and Miller, 1977; Hervé et al., 1987; Nedergaard et al., 1988). The serotonin system regulates the functional output of these pathways, but precise mechanisms remain to be clarified (Kelland et al., 1990, 1993; De Deurwaerdère et al., 1996; Saito et al., 1996).

Among the 14 serotonin receptor subtypes, the serotonin 2C receptor (5-HT2CR) is believed to play a particularly prominent role in the modulation of dopamine function (Di Matteo et al., 2002b; Esposito, 2006). 5-HT2CR mRNA is abundantly expressed on GABAergic neurons within the SNc and VTA and in the DSt and NAc (Mengod et al., 1990; Ward and Dorsa, 1996; Eberle-Wang et al., 1997). 5-HT2CRs are implicated in tonic and phasic modulation of the mesoaccumbal dopamine pathway. Pharmacological activation of 5-HT2CRs inhibits firing rates of VTA neurons and DA release within the NAc (Prisco et al., 1994; Di Matteo et al., 1998; Di Giovanni et al., 1999).

How 5-HT2CRs participate in the regulation of nigrostriatal DA system function is less clear. It has been suggested that 5-HT2CRs have less influence on the nigrostriatal than on the...
5-HT2CRs are also expressed in a number of basal ganglia structures regulated by the nigrostriatal DA system may occur through the actions of these receptors within the SNc. However, other studies indicate that 5-HT2CR agonist and antagonist treatments impact DA release in the DST and NAc in a similar manner (De Deurwaerdere and Spampinato, 1999; Gobert et al., 2000; Porras et al., 2002; De Deurwaerdere et al., 2004; Navailles et al., 2004). These conflicting results may be attributable to the use of different 5-HT2CR ligands with varying specificity and modes of action.

It is possible that the impact of 5-HT2CR function on behaviors regulated by the nigrostriatal DA system may occur through the actions of these receptors within the SNc. However, 5-HT2CRs are also expressed in a number of basal ganglia structures outside the SNc. Moreover, 5-HT2CRs are expressed in basal ganglia structures outside the SNc have previously been implicated in movement disorders such as oral dyskinesias (Eberle-Wang et al., 1997). 5-HT2CRs expressed in basal ganglia structures outside the SNc have previously been implicated in movement disorders such as oral dyskinesias (Eberle-Wang et al., 1996).

Here, we use a genetic approach to investigate the role of 5-HT2CRs in physiology and behaviors associated with nigrostriatal dopamine pathway function. This was pursued in studies of mice bearing a null mutation of the htr2c gene by a combination of electrophysiological, pharmacological, neurochemical, and behavioral approaches.

Materials and Methods

Animals. Mice were produced in our rodent colony (5-HT2CR mutant and littermate wild-type controls) or were purchased from The Jackson Laboratory (wild-type C57BL/6J mice). The generation of 5-HT2CR null mutant mice has been described in detail previously (Tecott et al., 1995). The htr2c null mutation is congenic on a C57BL/6J background and maintained by mating 5-HT2CR heterozygous females and C57BL/6J males. Animals were weaned at 3 weeks and group housed in standard polycarbonate cages (16 × 27 × 12 cm) with 3–5 littermates per cage. Mice had ad libitum access to water and chow (PicoLab Mouse Diet 20, Purina Mills). The housing facility was maintained at 22°C with a 12-h-on/12-h-off lighting schedule, lights on at 7:00 A.M. Unless otherwise indicated, these studies used male mice 2.5–4 months old.

Drugs. GBR 12909 (a selective inhibitor of the dopamine reuptake transporter, Sigma-Aldrich; Tocris Bioscience) was dissolved by gentle heating and sonication. Animals received GBR 12909 doses of 0, 3, 10, and 30 mg/kg. SB 206553 (a 5-HT2CR inverse agonist, Sigma-Aldrich) was used at doses of 0, 2.5, 5, and 10 mg/kg. Quinpirole (Sigma-Aldrich) was used at doses of 0, 2.5, 5, and 10 mg/kg. Anesthesia was induced by 22°C with a 12-h-on/12-h-off lighting schedule, lights on at 7:00 A.M. Unless otherwise indicated, these studies used male mice 2.5–4 months old.

Microdialysis probe construction and implantation. Probe implantation occurred 12–48 h after dialysis testing. Concentric microdialysis probes consisted of 23 gauge stainless steel and silica capillary tubing. The dialysis membrane (inner diameter 240 μm, outer diameter 290 μm, 2 mm exposed for striatum, 1 mm exposed for NAc, AN 69 HF, Hospal) consisted of polyacrylonitrile/sodium methylsulfonate copolymer with an average pore size of 29 Å. Mice were anesthetized using inhalational (isoflurane or halothane, 2%–8% O2) or injectable (ketamine 100 mg/kg-xylazine 10 mg/kg, i.p.) agents. Lidocaine was used for local anesthesia. The animals were placed in a stereotaxic frame (Kopf Instruments), and probes were inserted into the striatum (+0.7 AP, −2.0 ML, −3.0 DV for standard microdialysis; +0.5 AP, +2.0 ML, −5.0 DV for no net flux microdialysis) or NAc (+1.5 AP, +0.8 ML, −5.3 DV, only net no flux microdialysis) per established atlas coordinates (Paxinos and Franklin, 2001). Probes were secured to the skull using dental cement. Mice were then returned to a standard polycarbonate cage with sawdust bedding and allowed to regain consciousness.

Single-cell recording data analysis. The mean number of cells per track was calculated by dividing the total number of DA neurons encountered by the total number of electrode tracks performed for either the SNc or the VTA. Burst analysis of DA neuronal firing was performed using RISI (Symbolic Logic). A total of 500–1200 consecutive spikes were recorded for each neuron in both wild-type and mutant mice. Burst firing, when present, was detected using an algorithm similar to that previously described (Grace and Bunney, 1984). The mean percentage of spikes occurring in bursts was used as a measure of bursting activity. Differences in the number of cells per track, the basal firing rate, and the bursting activity between wild-type and mutant mice were analyzed by two-tailed Student’s t test. All statistical analyses were performed with StatView version 5.0.1 (SAS Institute).

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Single-cell recording procedures. Mice (n = 8 wild type, n = 8 5-HT2CR mutant) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted on a stereotaxic apparatus (SR-6, Narishige). Body temperature was maintained at 36–37°C. A 28 gauge needle was placed in a lateral tail vein through which additional anesthetic was administered and allowed to regain consciousness. The skull overlying both the SNc and the VTA was removed. The skull overlying both the SNc and the VTA was removed. The skull overlying both the SNc and the VTA was removed.
periment. Following equilibration, six baseline samples were collected at 10 min intervals. The perfusate was switched to aCSF containing 5 mM DA and allowed to equilibrate for 30 min followed by dialysis collection for 30 min. This same procedure was subsequently used for perchusates containing 10 and 20 mM dopamine. Following collection, dialysate samples were immediately frozen and stored at −70°C.

DA measurements. Dialysate aliquots were injected onto a reversed phase column (3 μm C18 stationary phase, multiple vendors). Samples were eluted with a mobile phase (acetonitrile, phosphate buffer, and an ion-pairing agent, ESA) for α2-aminophetamine studies; NaAc buffer (4.1 g/L) with MeOH (2.5% v/v), Tiritrepx (150 mg/L), OSA (150 mg/L), and TMACl (150 mg/L, pH 4.1) for GBR 12909 studies, and 17% acetonitrile (v/v) in a 25 mM NaH2PO4 buffer containing 0.1 mM Na–EDTA, 1.6 mM 1-decanesulfonic acid, and 14.4 mM triethyamine, pH 3.9, for no net flux studies. Pump flow rates were 0.25 ml/min, 0.35 ml/min, and 35 μl/min for α2-aminophetamine, GBR 12909, and no net flux studies, respectively. For α2-aminophetamine studies, DA was detected using an amperometric electrode (Model 5041, ESA) at a potential of +175 mV coupled to a coulometric detector (Coulonchem II, ESA). For GBR 12909 studies, DA was detected using a glassy carbon electrode at a potential of +500 mV (vs Ag/AgCl reference electrode) coupled to an Intracorder detector (Aptec, Leyden). For no net flux studies, DA was detected using a glassy carbon electrode at a potential of +700 mV (vs Ag/AgCl reference electrode) as controlled by an amperometric detector (EG&G 400, Princeton Applied Research).

Video and stereo behavior. Differences in motor and stereotypy responses between GBR 12909 and α2-aminophetamine have previously been noted (Hooks et al., 1994); we thus used stereotypy rating scales appropriate for each treatment. Motor stereotypy in response to GBR 12909 treatment was scored using a modified version of a previously published protocol (Creese and Iversen, 1973). Six distinct behaviors were noted: asleep (score 1), wake and inactive (score 2), awake locomotion (score 3), wake “route tracing” locomotion (score 4), intermittent oral/grooming stereotypies (score 5), and continuous oral/grooming stereotypies (score 6). Motor stereotypy in response to α2-aminophetamine treatment was scored using a modified version of a previously published protocol (Chartoff et al., 2001). Eight distinct behaviors were noted: no movement, ambulation, oral stereotypy, sniffing, rearing, vigorous grooming, taffy pulling, and climbing. For both GBR 12909 and α2-aminophetamine treatment, behaviors were scored at the start and every 10 s into the trial. For SKF 81297 and quinpirole treatments, videos were scored for the entire 10 min recording period. Investigators were blinded to genotype and drug treatment status. For each treatment dose, repeated-measures ANOVA was conducted with time as a within-subject factor and genotype and treatment as between-subject factors.

Slice preparation and electrophysiology. Mice P22-P28 mice were anesthetized with halothane and decapitated, the brain was rapidly removed, and coronal slices (300 μm) were cut in 4°C aCSF using a VT1000S (Leica). Slices were recovered at 32°C in carben-bubbled aCSF (126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 2.4 mM CaCl2, 18 mM NaHCO3, and 11 mM glucose, with pH 7.2–7.4, 301–305 mM) for 30 min to 5 h. During experiments, slices were continuously perfused (using a peristaltic pump, ~2 ml/min) with carben-bubbled aCSF warmed to 31–32°C and supplemented with CNQX (10 μM, to block AMPA-type glutamate miniature EPSPs) and picrotoxin (50 μM, to block GABAa receptors). All reagents were batch applied.

Whole-cell recordings were made from D1 medium spiny neurons (MSNs), identified as previously described (Hopf et al., 2003), using a potassium methanesulphonate-based internal solution (KOH 0.98% (v/v), methanesulfonic acid 0.76% (v/v), hydrochloric acid 0.18% (v/v), 20 mM HEPES, 0.3 mM EGTA, 2.8 mM NaCl, 2.5 mM MgATP, and 0.25 mg/ml GTP, pH 7.2–7.4, 275–285 mM). Current pulses were applied using Clampex 9.2 and 700A patch amplifier in current-clamp mode (Molecular Devices). After each current pulse was used for time points before and after drug addition. Spike firing rates during the 3 min before addition of the reagent were averaged and this value normalized to 100%. Statistical significance was determined for the average spike firing change during the last 2 min of exposure to reagents. All statistics were performed using a two-tailed, unpaired Student’s t test.

Receptor autoradiography. Mice underwent rapid decapitation, brain dissection, and tissue freezing on powdered dry ice. Coronal sections (20 μm thickness) were cut on a cryostat with appropriate histological reference slides and stored desiccated at −20°C until ready for tissue processing. All radioligand binding reactions occurred within 1 week of cutting. Sections were preincubated in buffer (50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, and either 40 mM ketanserin (to block nonspecific 5-HT2A and 5-HT2C receptor binding in the D2 reaction) or 0.5 mM DTG, 0.1 μM pindolol (to block nonspecific 5-HT1AR receptor binding in the D1 reaction) for 1 h before indirect...
percentage bursting activity (Fig. 1). Increases in dopaminergic neuron tonic firing rate (Fig. 1) represented as mean SNc neurons from mutant mice have overall higher-frequency activity spike trains than those seen in wild-type mice. Data are

Duncan's post hoc test revealed that SNc or the VTA (Fig. 1) differences in the number of spontaneously active DA neurons or latency to first syntactic grooming episode were observed.

Results

SNc dopaminergic neurons display increased basal firing rates and bursting activity in 5-HT<sub>2C</sub>R mutant mice

Extracellular microelectrode recordings were obtained to assess how the htr<sup>2C</sup>- mutation influenced baseline electrophysiological properties of midbrain dopaminergic neurons. No significant differences in the number of spontaneously active DA neurons were found between wild-type and mutant mice, either in the SNc or the VTA (Fig. 1A). At the SNc, statistically significant increases in dopaminergic neuron tonic firing rate (Fig. 1B) and percentage bursting activity (Fig. 1C) were noted in 5-HT<sub>2C</sub>R mutant mice compared with wild types. Basal activity of SNc DA neurons in mutant mice increased by 20%, whereas bursting activity of these neurons increased by almost 50%. Interspike interval histograms revealed a skew toward lower-frequency activity in 5-HT<sub>2C</sub>R mutant mice compared with wild types (Fig. 1D, E). This probably occurs as a result of the increased bursting behavior, leading to an increase in long interspike intervals between the bursts, although the mean frequency is increased. No statistically significant differences in either basal or percentage bursting activity were observed between 5-HT<sub>2C</sub>R mutant and wild-type mice at the VTA (Fig. 1B, C).

Extracellular dopamine is increased in the DSt and NAc of 5-HT<sub>2C</sub>R mutant mice

To determine whether alterations in tonic and phasic SNc dopaminergic neuron activity were reflected at the dopaminergic terminal fields, we performed no net flux microdialysis to measure DSt and NAc baseline extracellular dopamine concentrations in awake, behaving mice. Estimates of extracellular DA concentrations in each brain region were calculated from the mean of three samples at each perfusate concentration (0, 5, 10, and 20 nM DA). First-order regressions were used to obtain slope and intercept values, which were solved for the point of no net flux (zero intercept on the y-axis) (Parsons and Justice, 1994).

Figure 2, A and C, depicts data where the microdialysis probe was placed within the DSt. DSt extracellular dopamine concentrations were nearly twice as large in mutant mice compared with wild types. Extracellular dopamine concentrations in the NAc were modestly increased in mutant mice compared with wild types (Fig. 2B, C).

Increased syntactic grooming chain failures and altered grooming behaviors in 5-HT<sub>2C</sub>R mutant mice

Given the above electrophysiological and neurochemical evidence that 5-HT<sub>2C</sub>-loss increases nigrostriatal dopaminergic activity, we proceeded to determine whether these changes were accompanied by altered DSt-organized behaviors. One such behavior is syntactic grooming. More than 15 min of overall grooming activity (pooled over the 10 d of observation) were obtained for all mice studied. In general, the majority of observed grooming behavior was nonsyntactic. The percentage of grooming activity occurring within syntactic chains (5%, total observation) was similar to that reported by other groups (Aldridge and Berge, 1998). No statistically significant phenotypic differences in syntactic grooming initiation rate, syntactic grooming frequency, or latency to first syntactic grooming episode were observed. However, 5-HT<sub>2C</sub>R mutant mice spent significantly more time in syntactic phase 3 and significantly less time in syntactic phase 4 (Fig. 3A).

5-HT<sub>2C</sub>R mutant mice also demonstrated gross differences in nonsyntactic grooming behavior compared with wild-type mice (Fig. 3B). While there were no significant differences in overall grooming duration between wild-type and 5-HT<sub>2C</sub>R mutant mice.
A 2.5 mg/kg treatment in wild-type and 5-HT2CR mutant mice. LA in response to D-amphetamine, locomotion increased more rapidly, peaking 30 min after injection, declining at a slower rate, and finally attaining baseline values 180 min after injection (Fig. 4B). An early but smaller peak in locomotion (10 min after injection) was observed after 10 mg/kg dose of d-amphetamine, but locomotion was rapidly suppressed, remaining at minimal levels until 110–120 min after injection, followed by another rise in locomotion lasting until >210 min after injection (Fig. 4C). Overall, 5-HT2CR mutant mice showed less LA response to d-amphetamine.

The transient decrease in d-amphetamine-induced LA has been proposed to result from an increase in focused stereotypies (Yates et al., 2007). Our data indicate that the expression of these stereotypies may be enhanced in 5-HT2CR mutant mice. To test this hypothesis, we measured motor stereotypy in response to 2.5, 5, and 10 mg/kg d-amphetamine in wild-type and 5-HT2CR mutant mice. After 2.5 mg/kg d-amphetamine, there were no significant phenotypic differences in stereotypic activity (Fig. 4D). However, detailed analysis of stereotypic behaviors revealed that 5-HT2CR mutant mice spent significantly more time in rearing and jumping (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Following 5 mg/kg d-amphetamine, 5-HT2CR mutant mice spent significantly more time in total stereotypy than did wild type (Fig. 4E). At the highest dose of 10 mg/kg, mice were almost fully engaged in orofacial stereotypy, but there was no significant phenotypic difference (Fig. 4F).

5-HT2CR mutant mice demonstrate increased stereotypic behaviors following selective DAT blockade

We then examined stereotypic behavioral responses to the selective DAT antagonist GBR 12909 (Heikkila and Manzino, 1984) in 5-HT2CR mutant and wild-type mice. We used a modified Creese-Iversen scale to score stereotyped behaviors in wild-type and 5-HT2CR mutant mice treated with GBR 12909 (Fig. 5A). We also examined the impact of 5-HT2CR pharmacological blockade (using SB 206553) on stereotypic behavioral responses to GBR 12909 (Fig. 5B). 5-HT2CR mutant mice displayed increased stereotypic behaviors in response to intermediate doses of GBR 12909. Similarly, mice receiving both SB 206553 and intermediate doses of GBR 12909 displayed greater stereotypic behaviors compared with wild-type mice receiving GBR 12909.

No phenotypic difference in striatal DA release after d-amphetamine or selective DAT blockade

Given the phenotypic differences we observed in locomotor activity and stereotypy following psychostimulant administration, we hypothesized that we would find phenotypic differences in psychostimulant-evoked Dst dopamine extracellular concentrations. We examined dopamine release in the Dst of 5-HT2CR mutant and wild-type mice in response to 2.5 and 5 mg/kg d-amphetamine (Fig. 6A,B). A dose of 2.5 mg/kg d-amphetamine induced a sharp increase in DA release, which peaked 40 min after the injection (883.7 ± 114.13 and 731 ± 92.7% increase for wild-type and 5-HT2CR mutant mice respectively, NS). Similarly, a 5 mg/kg d-amphetamine dose induced a sharp increase in DA release, which peaked 40 min after injection (1072.35 ± 140.3 and 1242.6 ± 190.7% increases in wild-type and 5-HT2CR mutant mice respectively, NS). However, neither genotype nor any of its relevant interactions were found to be significant by repeated-measures ANOVA. Thus, we were unable to detect any phenotypic differences in d-amphetamine-evoked Dst extracellular dopamine concentrations.

We then examined dopamine release in the Dst of 5-HT2CR mutant and wild-type mice in response to 3 and 10 mg/kg GBR...
5-HT2CR mutant mice display enhanced D1 receptor agonist-induced stereotypy

The above findings suggest that 5-HT2CRs influence behavioral responses to dopamine release. This raises the possibility that 5-HT2CR mutant mice have enhanced behavioral responses to DA receptor activation. Figure 7 displays LA in response to 10 mg/kg injection of the D1 receptor agonist SKF 81297. SKF 81297 increased LA in both wild-type and 5-HT2CR mutant mice relative to saline-injected controls. However, the magnitude of the increase was diminished in 5-HT2CR mutant mice. Stereotypy observed during these trials was mainly orofacial in nature, with 5-HT2CR mutant mice displaying a robust enhancement of stereotypy relative to the wild-type mice (mutant mice spent 86.91 ± 6.92% time in focused stereotypy compared with 21.29 ± 6.18% for wild-type mice, F(1,6) = 49.98, p < 0.001). We further examined the effect of quinpirole (0.6, 2, and 6 mg/kg) on LA and stereotypy. Quinpirole treatment evoked no phenotypic effects on LA or stereotypy.

Absence of phenotypic difference in striatal D1 and D2 receptor binding

The enhanced responses of 5-HT2CR mutant mice to D1 receptor stimulation may reflect a phenotypic difference in D1 receptor expression. To test this hypothesis, 3H receptor-ligand-binding autoradiography was used to assess D1 and D2 receptor binding in DSt and NAc. No phenotypic differences in receptor binding in DSt and NAc were observed in either of these regions when comparing wild-type to 5-HT2CR mutant mice (supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

Absence of phenotypic difference in D1 receptor activation of DSt MSNs

Brain slices were used to examine the firing properties of DSt MSNs. Neurons were brought to approximately −80 mV by passage of DC current via the patch amplifier, and then a series of 300 ms current pulses was delivered every 30 s. Dopamine, acting via the D1 receptor, can enhance action potential firing in the DSt (Surmeier et al., 1995; Hernández-López et al., 1997). In agreement, the selective D1 receptor agonist SKF 81297 dose-dependently enhanced firing in wild-type and 5-HT2CR mutant mice, with significant enhancement of action potential generation at 10 μM (supplemental Fig. 3A, available at www.jneurosci.org as supplemental material) but not 3 μM (supplemental Fig. 3C, available at www.jneurosci.org as supplemental material). However, there were no phenotypic differences in firing enhancement. SKF 81297 did not alter the input resistance, measured using a 33.3 pA hyperpolarizing pulse, suggesting that there was no effect of D1 receptor activation on inwardly rectifying potassium channels (supplemental Fig. 3B, D, available at www.jneurosci.org as supplemental material). Finally, as shown in supplemental Table 1, there were no phenotypic differences in a number of basic firing parameters related to action potential waveform and input resistance (n = 14 cells from six wild-type mice, n = 14 cells from five 5-HT2CR mice), suggesting that 5-HT2CR deletion did not alter the basal function of several channels active during firing.

Discussion

These studies suggest that 5-HT2CRs have a significant role in controlling nigrostriatal physiology and behavior. We demonstrate that 5-HT2CR-R loss increases SNc dopaminergic neuron firing rates and DSt extracellular dopamine concentrations and enhances behaviors associated with DSt activation. Loss of 5-HT2CR function enhances psychostimulant-induced stereotypy. This enhancement occurs without phenotypic differences in the elevation of psychostimulant-induced striatal extracellular dopamine concentrations. Thus, loss of 5-HT2CR function may be accompanied by enhanced behavioral responses to released dopamine. Phenotypic differences in stereotypic behavior following selective D1 receptor agonist stimulation support this hypothesis. These differences were not attributable to increases in DSt D1 receptor expression or MSN response to dopaminergic stimulation. Thus, these findings suggest that 5-HT2CRs inhibit behaviors associated with nigrostriatal function at multiple loci.

5-HT2CR-R loss evokes a tonic activation of nigrostriatal dopaminergic neurotransmission

In mice lacking 5-HT2CRs, SNc dopaminergic neurons displayed elevated tonic firing rates and an increased percentage of burst-firing cells. 5-HT2CR-Rs are expressed on GABAergic neuronal subpopulations (Di Giovanni et al., 2001) of the SNc [by in situ hybridization (Eberle-Wang et al., 1997)] and VTA [by immunocytochemistry (Bubar and Cunningham, 2007)]. Thus, loss of 5-HT2CR-R-evoked activity within these GABAergic cells could diminish inhibitory drive onto SNc neurons. This is consistent with
pharmacological studies suggesting that 5-HT2C-Rs suppress SNc neuronal firing [Di Giovanni et al. (1999) and Porras et al. (2002), using SB-206553] and burst firing [Blackburn et al. (2006), using SB-200646A].

Surprisingly, our data demonstrate that the htr2c null mutation has a greater impact on SNc cell firing rate and bursting properties than on those of VTA dopaminergic neurons. Previous studies clearly demonstrate that pharmacological 5-HT2C-R activation/inhibition evokes a corresponding inhibition/activation of VTA neuronal firing (Di Giovanni et al., 1999, 2000; Di Matteo et al., 2001, 2002a; Blackburn et al., 2006), for review, see Giorgetti and Tecott, 1999, 2000; Di Matteo et al., 1999, 2000); (for review, see Giorgetti and Tecott, 2004). Many of these studies suggest that systemic modulation of 5-HT2C-R activity alters VTA neuronal activity to a greater extent than SNc neuronal activity (Di Matteo et al., 2001, 2002a; Blackburn et al., 2002). However, a number of factors complicate this interpretation. Studies that use different 5-HT2C-R antagonists have yielded disparate results. For example, previous reports suggest that 5-HT2C-R antagonism increases (Di Giovanni et al., 1999) or has no effect on (Di Matteo et al., 1999) SNc dopaminergic neuronal firing rates. Some of these issues may relate to differences in the extent to which antagonist compounds block 5-HT2C-R constitutive activity (De Deurwaerde`re et al., 2004).

Increases in SNc neuron firing rate and bursting were accompanied by increases in extracellular DSt dopamine concentration as determined by no flux methods. This finding is also concordant with previous studies demonstrating increased striatal dopamine concentrations following treatment with 5-HT2C-R antagonists (Navailles et al., 2004). Conversely, extracellular striatal dopamine concentrations were decreased after systemic 5-HT2C-R agonism (Di Matteo et al., 2004). Moreover, constitutive 5-HT2C-R activity in absence of bound ligand has been proposed to decrease striatal dopamine concentrations (De Deurwaerde`re et al., 2004).

In accord with the observed changes in baseline SNc neuronal firing properties and DSt extracellular DA concentrations, we observed altered grooming behavior in 5-HT2C-R mutant mice. Increases in total grooming have previously been observed following treatment with dopamine D1 receptor agonists (Berridge and Aldridge, 2000) and in mice expressing a hypoactive dopamine reuptake transporter (Berridge et al., 2005). Like 5-HT2C-R mutant mice, the dopamine reuptake transporter hypomorphic mice displayed elevated nonsyntactic grooming event rates. 5-HT2C-R mutant mice also demonstrate a dysregulation of syntactic grooming, a behavior specifically linked to DSt function (Cromwell and Berridge, 1996; Aldridge and Berridge, 1998; Aldridge et al., 2004). Similar to what is observed following D1 receptor agonist treatment, 5-HT2C-R mutant mice spend less time in the final phase (phase 4) of syntactic grooming chains compared with wild-type mice (Matell et al., 2006).
though these findings are consistent with an enhancement of nigrostriatal dopamine system activity, it is also possible that the absence of 5-HT2CR from basal ganglia structures could contribute to the observed grooming phenotypes.

5-HT2CR loss enhances psychostimulant-induced motor activity

Both locomotor stereotypies and focused stereotypies (such as grooming and gnawing) are prominent behaviors associated with psychostimulants. Typically, locomotor stereotypic behaviors are observed at lower psychostimulant doses, while focused stereotypies predominate with higher psychostimulant doses. In 5-HT2CR mutant mice, focused stereotypies were the prevalent behavior following d-amphetamine treatment. In contrast, locomotor stereotypies were more prevalent in wild-type mice except at the highest tested d-amphetamine dosage. In mutant mice, behavioral responses were characterized by greater duration of focused stereotypy and greater sensitivity to focused stereotypy. Because d-amphetamine nonspecifically influences monoamine release and uptake, we examined the consequences of selective DA transporter blockade using GBR 12909. Similarly, focused stereotypies were observed more frequently in 5-HT2CR mutant mice than in wild-type littermates following GBR 12909 treatment.

Unexpectedly, we did not observe phenotypic differences in DSt extracellular dopamine concentrations following administration of either GBR 12909 or d-amphetamine. Nevertheless, we observed enhanced focused stereotypies in 5-HT2CR mutant mice. The finding of phenotypic differences in psychostimulant-evoked nigrostriatal behaviors in the absence of differences in nigrostriatal extracellular dopamine concentration raised the possibility that behavioral responses to released dopamine are enhanced by the htr2c mutation.

5-HT2CR loss enhances behavioral sensitivity to D1 receptor activation

Phenotypic differences in the response to dopamine release could occur through changes within signaling pathways activated by D1-like and/or D2-like receptors. 5-HT2CR mutant mice demonstrated enhanced sensitivity to the behavioral effects of the D1 receptor agonist SKF 81297, while no phenotypic differences were noted in behavioral responses to the D2 receptor agonist quinpirole. Thus, D1 receptor signaling pathways may contribute substantially to the enhanced behavioral responses of 5-HT2CR mutant mice to psychostimulants.

In light of these findings and the known expression of 5-HT2CRs within the DSt (Alex et al., 2005), we evaluated D1 receptor expression and function in wild-type and mutant mice. We found no phenotypic difference in DSt D1 receptor expression by autoradiography. We then examined DSt MSN responses to D1 receptor stimulation. Electrophysiological studies revealed enhancement of firing after D1 receptor activation, as has been described (Hernández-López et al., 1997). However, no significant phenotypic differences in the D1 receptor firing enhancement were noted. Additionally, we did not observe gross phenotypic differences in MSN action potential waveform or other basic parameters. Thus, we did not detect significant phenotypic differences in intrinsic striatal function.

Within the basal ganglia, information from the “direct” [consisting of striatal to substantia nigra reticulata/globus pallidus interna (SNr/GPi) projections] and “indirect” [consisting of striatal to globus pallidus exterum (GPe) to subthalamic nucleus (STN) projections] pathways is integrated to produce an inhibi-
tory output that is fed back to thalamic motor centers (Graybiel, 2004). In addition to its expression in the SNC and DSt components of these circuits, 5-HT2C-Rs are highly expressed in the STN and SNr/GPi (Pompeiano et al., 1994; Wright et al., 1995; Eberle-Wang et al., 1997). Serotonin application to STN neurons in slice preparations evokes a large inward current that can be blocked by 5-HT2C-R antagonists (Shen et al., 2007). This is accompanied by increases in cell firing (Fox et al., 1998; Stanford et al., 2005; Invernizzi et al., 2007; Shen et al., 2007). Furthermore, serotonin application to SNr neurons in slice preparations evokes an excitatory inward current that is blocked by 5-HT2C-R antagonists (Stanford and Lacey, 1996). Therefore, decreases in 5-HT2C-R function could decrease SNr activity. The extent to which functional perturbations within the STN and Gpi contribute to the phenotypic abnormalities observed here warrants further investigation.

We conclude that 5-HT2C-Rs have a substantial role controlling the nigrostriatal dopaminergic system and the behaviors it regulates. Previous studies have already shown that 5-HT2C-Rs have a role in mesoaccumbal dopaminergic system physiology. 5-HT2C-Rs are thus key regulators of the two major CNS ascending dopaminergic systems, and may play an important role integrating serotonergic and dopaminergic signaling. 5-HT2C-Rs may thus prove to be a clinically relevant target for the development of drugs to treat CNS disorders in which dopaminergic systems have been implicated (Di Matteo et al., 1999), including schizophrenia, substance abuse, attention-deficit/hyperactivity disorder, Parkinson’s disease, and drug-induced movement disorders.

References


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