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Alterations in Dopamine Release But Not Dopamine Autorecepto Function in Dopamine D_3 Receptor Mutant Mice

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Dopamine (DA) autoreceptors expressed along the somatodendritic extent of midbrain DA neurons modulate impulse activity, whereas those expressed at DA nerve terminals regulate both DA synthesis and release. Considerable evidence has indicated that these DA autoreceptors are of the D_2 subtype of DA receptors. However, many pharmacological studies have suggested an autoreceptor role for the DA D_3 receptor. This possibility was tested with mice lacking the D_3 receptor as a result of gene targeting. The basal firing rates of DA neurons within both the substantia nigra and ventral tegmental area were not different in D_3 receptor mutant and wild-type mice. The putative D_3 receptor-selective agonist *R*(+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-(1)benzopyrano(4,3-*b*)−1,4-oxazin-9-ol (PD 128907) was equipotent at inhibiting the activity of both populations of midbrain DA neurons in the two groups of mice. In the γ-butyrolactone (GBL) model of DA autorec tor function, mutant and wild-type mice were identical with re ct to striatal DA synthesis and its suppression by PD 1289(*In vivo* microdialysis studies of DA release in ventral stri m revealed higher basal levels of extracellular DA in mutant ce but similar inhibitory effects of PD 128907 in mutant and c- type mice. These results suggest that the effects of PD 12 7 on dopamine cell function reflect stimulation of D_2 as opp d to D_3 receptors. Although D_3 receptors do not seem t e significantly involved in DA autoreceptor function, they y participate in postsynaptically activated short-loop feed k modulation of DA release.

Key words: D_3 receptors; mutant mice; dopamine autore tors; dopamine receptors; dopamine neurons; dopamine lease; dopamine synthesis

Discovery of the dopamine (DA) D_3 receptor (Sokoloff et al., 1990) generated considerable excitement regarding possible physiological and behavioral functions mediated by this member of the DA D_2 receptor subfamily (D_2, D_3, and D_4). Among the speculated roles for the D_3 receptor was that of an autoreceptor, the receptors expressed by DA neurons to provide feedback regulatory control. Initial characterization of the cloned D_3 receptor identified several D_3 receptor-preferring ligands that had been regarded previously as DA autoreceptor-selective (Svensson et al., 1986). In addition, D_3 receptor gene transcripts were detected by PCR in the rat ventral tegmental area (VTA) and substantia nigra (SN) and were absent after selective destruction of DA neurons with 6-hydroxydopamine, suggesting localization to DA neurons (Sokoloff et al., 1990). However, more recent studies have either failed to detect D_3 receptor mRNA in the rat midbrain (Landwehrmeyer et al., 1993; Meador-Woodruff et al., 1994; Richtand et al., 1995; Healy and Meador-Woodruff, 1996) or have demonstrated low and very restricted expression to la al regions of the SN and VTA, with only a portion of these cells o expressing tyrosine hydroxylase (Bouthenet et al., 1991; Dia et al., 1995).

Functionally, three classes of DA autoreceptors can be def d (for review, see Wolf and Roth, 1987). The soma and dendrite of midbrain DA neurons express autoreceptors that modulate r s of impulse activity (Bunney et al., 1973; Aghajanian and Bun y, 1977). Nerve terminals of these neurons express autorecep s that modulate DA synthesis (Kehr et al., 1972) and DA rel e (Farnebo and Hamberger, 1971). Each of these three autore tors exhibits pharmacological characteristics of the D_2 rece r subfamily (for review, see Clark and White, 1987; Wolf and R , 1987), and conclusive anatomical evidence indicates that A neurons express D_2 receptor mRNA (Meador-Woodruff et 1989; Mengod et al., 1989; Mansour et al., 1995). Despite limited anatomical evidence of the expression of D_3 recep mRNA in DA neurons (above), many pharmacological stud have suggested that D_3 autoreceptors modulate DA impulse f (Devoto et al., 1995; Kreiss et al., 1995; Lejeune and Millan, 19 Gobert et al., 1996; Tepper et al., 1997), DA synthesis (Melle al., 1993; Ahlenius and Salmi, 1994; Aretha et al., 1995; Gober al., 1995; Pugsley et al., 1995), and DA release (Damsma et 1993; Gainetdinov et al., 1994, 1996; Rivet et al., 1994; Gilbert al., 1995; Pugsley et al., 1995; Gobert et al., 1996; Routledge et 1996; Tepper et al., 1997). With notable exceptions (Tang et 1994; Nissbrandt et al., 1995; O'Hara et al., 1996; Tepper et 1997), most of these studies inferred a role for D_3 receptors bas on (1) correlations between DA agonist potency and relati

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binding affinities at cloned D_2 and D_3 receptors expressed in various cell lines, (2) comparisons between regional differences in DA agonist potency and relative expression of D_2 and D_3 receptors, or (3) antagonism of agonist effects by putative D_3 receptor-selective antagonists. These approaches are not conclusive because serious questions exist with respect to the selectivity of D_3 receptor ligands (Chio et al., 1994; Large and Stubbs, 1994; Potenza et al., 1994; Burris et al., 1995; Gonzalez and Sibley, 1995; Sautel et al., 1995).

To test possible autoreceptor roles for D_3 receptors more directly, we have used homologous recombination in embryonic stem cells to generate mice lacking the intact D_3 receptor gene (Xu et al., 1997). We used well characterized *in vivo* models of DA autoreceptor function to compare the D_3 receptor mutant mice with their wild-type littermates with respect to DA cell firing rates, DA synthesis, and DA release and inhibition of these functions by the putative D_3 receptor-selective agonist *R*(+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-(1)benzopyrano(4,3-*b*)−1,4-oxazin-9-ol (PD 128907) (Sokoloff and Schwartz, 1995). We reasoned that if D_3 receptors serve autoreceptor functions, then the basal firing rates of DA neurons, as well as levels of DA synthesis and release, should be altered in D_3 receptor mutant mice and that autoreceptor-mediated effects of PD 128907 should be absent or reduced in D_3 mutant mice. Our results fail to support an autoreceptor role for D_3 receptors but suggest that postsynaptic D_3 receptors may regulate DA release via short-loop feedback mechanisms.

MATERIALS AND METHODS

Mice. Mice were generated as detailed in Xu et al. (1997). They were shipped to the Chicago Medical School by commercial carrier. Mice were housed in like groups of three to four animals and were allowed 7–8 d to acclimate to the vivarium before use. All experimental procedures were conducted at the Chicago Medical School. All procedures were performed in strict accordance with the *National Research Council Guide for the Care and Use of Laboratory Animals* (1996) and were approved by our Institutional Animal Care and Use Committee.

Extracellular single-unit recordings. All methods for extracellular single-cell recordings were similar to those previously reported (White et al., 1995), although modified somewhat for the mouse (Xu et al., 1994). Briefly, mice were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a standard stereotaxic apparatus with a specialized adapter for the mouse. Body temperature was maintained at 36–37.5°C with a thermostatically controlled heating pad. A 28 gauge (3/8 inch) hypodermic needle was placed in a lateral tail vein through which additional anesthetic (as required) and drugs of study were administered. A burr hole was drilled in the skull, and the dura was retracted from the area overlying the VTA and SN, 0.4–1.3 mm anterior to lambda and 0.2–1.0 mm lateral to the midline. Recording electrodes were made by pulling glass tubing [outer diameter (o.d.), 2.0 mm], which was prefilled with fiberglass, and by breaking the tip back to a diameter of 1–2 μm. Electrodes were filled with 2 M NaCl saturated with 1% (w/v) fast green dye and typically exhibited *in vitro* impedances of 1–3 MΩ (at 135 Hz). Electrode potentials were passed through a high impedance amplifier/filter and displayed on an oscilloscope. Individual action potentials were discriminated electronically and monitored with an audio amplifier. Integrated rate histograms, generated by the output of the window discriminator, were plotted by a polygraph recorder, whereas digitized counts of cellular activity were obtained for off-line analysis. Electrodes were lowered to a point 0.5 mm above the VTA and SN and then slowly advanced with a hydraulic microdrive through the DA cell regions (3.2–5.0 mm ventral to the cortical surface). DA cells were identified by standard physiological criteria (Bunney et al., 1973; Wang, 1981; Sanghera et al., 1984) and were recorded for 3–6 min to establish a baseline firing rate. To determine the sensitivity of impulse-modulating somatodendritic autoreceptors, we administered PD 128907 to each mouse through the tail vein, using a cumulative dose regimen in which each dose doubled the previous dose, at 60–90 sec intervals. After agonist-induced inhibition, the D_2-class receptor antagonist eticlopride was administered (0.1–0.2 mg/kg) to reverse the effect and confirm receptor mediatic At the end of each experiment, the cell location was marked by ejectir st green dye, and the spot was verified by routine histological asses t (described below).

γ-Butyrolactone experiments. The L-aromatic amino acid decarbo se inhibitor NSD 1015 was administered 30 min before death (100 m g, i.p.). γ-Butyrolactone (GBL) was administered (750 mg/kg, i.p.) in before NSD 1015 to eliminate impulse flow in DA neurons (Walte nd Roth, 1976). PD 128907 was injected 5 min before GBL. Mice were ed by decapitation, and the brains were quickly removed. Dorsal and v al striatum were dissected on a chilled glass plate with the aid of a r se brain matrix designed to allow coronal sections to be cut rapidl nd reproducibly (Activational Systems, Warren, MI). Two slices (m each) were taken, beginning at the rostral boundary of the olfa cy tubercle. The most anterior slice was the source of ventral stri m, whereas both slices were used to obtain dorsal striatum. The v al striatum was dissected with angular cuts originating at the lateral ic- tory tracts and ending at the midline (average weight, 18.5 mg) he remainder of the striatal region from that slice as well as the s ar region from the more caudal slice was considered the dorsal str um (average weight, 35.8 mg). Tissues were kept at −80°C. To measure ue catechols, we weighed frozen tissues and then sonically disrupted th in a homogenization solution consisting of 100 mM $HClO_4$, 5 mM Na_2 , and α-methyl dopa, an internal standard. After centrifugation (25, × *g* for 10 min), aliquots of the supernatant were processed by alu na extraction as described previously (Galloway et al., 1986). Th 4- dihydroxyphenylalanine (DOPA) content of samples was deter ed using an HPLC system consisting of a Bioanalytical Systems (S) Phase II ODS 3 μm column (100 × 3.2 mm), a BAS LC4C electro n- ical detector, and a Scientific Systems model 222C HPLC pump he mobile phase consisted of 0.1 M NaH_2PO_4, 1 mM EDTA, 0. mM 1-octane-sulfonic acid, and 3% methanol, adjusted to pH 2.7 with s- phoric acid. DOPA content was quantified based on both interna nd external standards.

In vivo *microdialysis experiments.* Mice used for dialysis experi ts weighed 28–35 gm. Concentric microdialysis probes were construc as described previously, with fused-silica inlet and outlet lines (Wolf l., 1994). Dialysis membrane (molecular weight cutoff, 6000; o.d., 25 m) was obtained from Spectrum (Los Angeles, CA). Data were no r- rected for *in vitro* probe recovery because probes may suffer differ ial alterations during insertion. Consistent with this assumption, dat ts corrected for recovery often exhibit greater variability than do thos at are uncorrected (Xue et al., 1996). Probes were stereotaxically impl ed under sodium Brevital (8 mg/kg, i.p.). Stereotaxic coordinates re, relative to bregma, anterior, 1.5 mm; lateral, 1.5 mm; and ventral, 2 .7 mm. Ventral coordinates indicate exposed regions of dialysis mem ne (2 mm). After surgery, mice were placed in Plexiglas cages (23 × 4 m) and allowed to recover overnight. Food and water were availab d *libitum*. Dialysis cages were equipped with balance arms (Instech ly- mouth Meeting, PA), and homemade liquid swivels and tethers re constructed from plastic syringes and tubing. These were used be se commercially available swivels were too heavy for use with mice. F es were perfused overnight at 0.3 μl/min with artificial CSF (aCSF n- sisting of (in mM): 2.7 KCl, 140 NaCl, 1.2 $CaCl_2$, 1 MgCl .3 NaH_2PO_4, and 1.7 Na_2HPO_4, pH 7.4. The next morning, the perf on rate was increased to 2 μl/min for 2–3 hr before the experimer as begun. Experiments consisted of 1 hr of perfusion with control aC to determine basal DA efflux, 1 hr of perfusion with aCSF containi PD 128907, and a 1 hr recovery period during which control aCS as perfused. Thus, administration of PD 128907 occurred ~20 hr er probe implantation. Fractions were collected every 20 min. After ch experiment, mice were anesthetized and perfused intracardially th normal saline followed by 10% formalin. Probe placement was exa ed in sections stained with cresyl violet. Only data from mice with v ed probe placements were included in the analysis. Dialysates were a- lyzed for DA content using the HPLC system described for GBL r- iments. Chromatographic conditions were optimized for early elut of DA to obtain maximum sensitivity. The mobile phase consisted of M NaH_2PO_4, 0.5 mM EDTA, 2 mM 1-octane-sulfonic acid, and 16% h- anol, adjusted to pH 4.9. Peaks were recorded using a dual-channel rt recorder and were quantified by comparison with the peak heig of external standards run with every experiment.

Drugs. PD 128907, 7-hydroxy-2-(di-*n*-propylamino)tetralin (7 H- DPAT), quinpirole, and eticlopride were obtained from Researcl lo- chemicals (Natick, MA). GBL was obtained from VWR Scientific hi-

cago, IL). NSD 1015 and alumina for column chromatography were obtained from Sigma (St. Louis, MO).

Statistics. Two-way repeated measures ANOVA (groups and dose) was used to analyze the electrophysiological and DOPA synthesis results when PD 128907 was administered. In both cases, dose was the repeated measure. Planned comparisons were made with Dunnett's test. Results from basal DOPA synthesis measures were analyzed with a two-way ANOVA (groups and treatment) with the treatments being NSD 1015 and NSD 1015 plus GBL. Basal levels of extracellular DA obtained with microdialysis were compared with a one-way ANOVA. Because of the significant difference between the two groups of mice on this measure, we used two-way (groups × fraction) repeated measures (fraction) analysis of covariance (ANCOVA) with basal DA as the covariate to compare the effects of PD 128907 on DA release.

RESULTS

Lack of evidence of impulse-modulating DA D_3 autoreceptors

PD 128907 produced a potent, dose-dependent suppression of firing of DA neurons in both the VTA and SN and did so equally well in mutant and wild-type mice (Fig. 1). Similar experiments conducted with other putative D_3 receptor-selective agonists, 7-OH-DPAT and quinpirole, produced identical results (data not shown). We also observed no difference in the basal firing rates of DA neurons recorded in the two groups of mice. Such a difference might be expected if D_3 receptors exert a tonic inhibitory influence on neuronal activity. These findings indicate that inhibition of DA cell activity by putative D_3 receptor-selective agonists is not mediated by stimulation of D_3 receptors and thus must be mediated by D_2 receptors. Because *in situ* hybridization histochemistry indicates restricted expression of D_3 mRNA in lateral portions of the rat SN and VTA (Diaz et al., 1995), we determined the sites of our recorded DA neurons using routine histological procedures and confirmed that DA neurons from these regions of the midbrain were included in our samples (Fig. 2).

Regulation of DA synthesis is unaltered in D_3 receptor mutant mice

We used the GBL model to determine whether the D_3 receptor null mutation altered control of DA synthesis by nerve terminal autoreceptors. In this model, GBL is used to inhibit impulse flow in DA neurons. Under these conditions, DA agonists can inhibit DA synthesis only via nerve terminal autoreceptors and not via long-loop feedback pathways or stimulation of somatodendritic impulse-modulating DA autoreceptors (Walters and Roth, 1976). Accumulation of the DA precursor DOPA was measured after administration of the L-aromatic amino acid decarboxylase inhibitor NSD 1015 to prevent conversion of DOPA to DA. DOPA accumulation was used as an index of the rate of tyrosine hydroxylation, the rate-limiting step in DA biosynthesis. Studies were performed in both dorsal and ventral striatum (including nucleus accumbens, olfactory tubercles, and islands of Calleja) because of reported differences in D_3 receptor expression in these terminal fields (for review, see Sokoloff and Schwartz, 1995).

Administration of GBL (with NSD 1015) increases DOPA formation in DA terminal fields because of a reduction in impulse-dependent DA release from nerve terminals and a resultant reduction in DA autoreceptor stimulation. The magnitude of the GBL-induced increase in DOPA formation therefore provides a measure of the magnitude of ongoing (tonic) suppression of DA synthesis by synthesis-modulating DA autoreceptors. We found that the magnitude of the GBL-induced increase in DOPA formation did not differ between D_3 receptor mutant and wild-type mice in either the dorsal or ventral striatum (Fig. 3). This argues strongly against a contribution of D_3 receptors to tonic

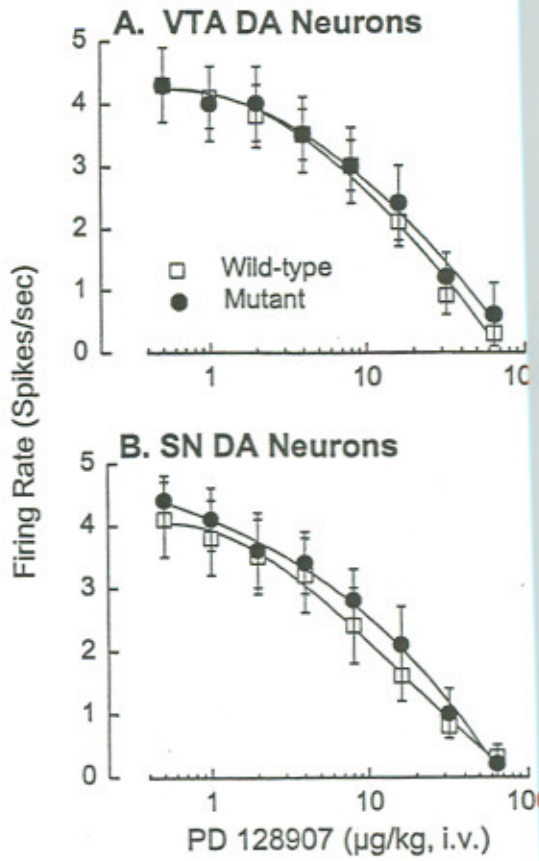

Figure 1. Similar inhibition of midbrain DA neurons by PD 128907 in D_3 receptor mutant and wild-type mice. *A*, Cumulative dose–response curves showing the significant dose-dependent suppression of VTA DA neuronal activity by PD 128907 ($F_{6,120} = 62.5$; $p < 0.001$) and the lack of difference between the wild-type and mutant mice with respect to this effect. There was also no significant difference between the groups with respect to basal firing rates, 4.3 ± 0.6 spikes/sec (mean ± SEM) in wild-type mice ($n = 12$) and 4.3 ± 0.5 spikes/sec in the mutant mice ($n = 10$). Eticlopride reversed the inhibition to 85–110% of the basal firing rate in every cell tested. *B*, Similar dose–response curves indicating dose-dependent inhibition of SN DA neurons by PD 128907 ($F_{6,138} = 51.5$; $p < 0.001$) and the lack of difference between wild-type and mutant mice with respect to this effect. There was also no significant difference in the basal firing rates for SN DA neurons in wild-type (4.1 ± 0.6 spikes/sec; $n = 12$) and mutant (4.3 ± 0.4 spikes/sec; $n = 13$) mice. Eticlopride reversed the agonist-induced inhibition to 82–112% of the basal firing rate in every cell tested. Each point represents the mean ± SEM.

autoreceptor-mediated modulation of DA synthesis. Furthermore, PD 128907 was equally potent at reversing GBL-induced DOPA formation in the mutant and wild-type mice (F 4), indicating that PD 128907 does not inhibit DA synthesis by stimulating D_3 autoreceptors but must do so via D_2 autoreceptors. In fact, at the highest dose tested, PD 128907 appeared somewhat more potent in the mutant compared with the wild-type mice (Fig. 4).

Regulation of DA release, but not PD 128907-induced suppression of DA release, is altered in D_3 receptor mutant mice

Extracellular DA levels were measured using *in vivo* microdialysis in the ventral striatum of freely moving mice. A representative probe placement is shown in Figure 5. To determine basal levels of DA efflux, we collected baseline samples of DA for 1 hr before administration of PD 128907. Basal DA efflux was consistently

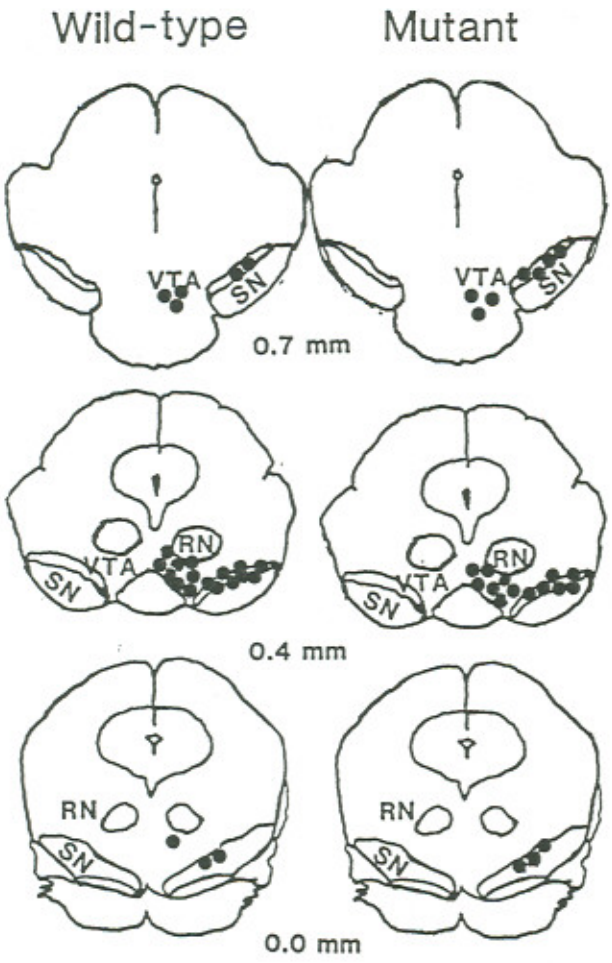

Figure 2. Recording sites for DA neurons within the mouse midbrain. Approximate recording sites (*filled circles*) for DA neurons within the ventral tegmental area (*VTA*) and substantia nigra (*SN*) of wild-type (*left*) and D_3 receptor mutant (*right*) mice. Coordinates are expressed as anterior to lambda. *RN*, Red nucleus.

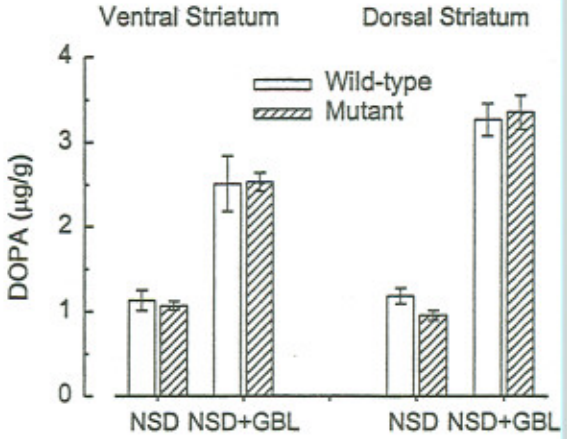

Figure 3. Similar GBL-induced increase in striatal DOPA forma in D_3 receptor mutant and wild-type mice. Basal levels of DOPA, me ed after inhibition of L-aromatic amino acid decarboxylase with NSD 5, did not significantly differ in wild-type and D_3 receptor mutant m in either the ventral or dorsal striatum. GBL significantly increased l PA formation in both ventral ($F_{1,20} = 58.58$; $p < 0.001$) and dorsal (F = 261.79; $p < 0.001$) striatum, as a result of the cessation of im e-dependent DA release and the relief of tonic autoreceptor-me ed inhibition of tyrosine hydroxylase activity. Again, there were no s ifi-cant differences in the GBL-induced increase in the two groups of e. Error bars indicate SEM. Sample size is six for all groups except f he NSD alone group (dorsal striatum in mutants) where $n = 5$.

and significantly higher in mutant mice (Fig. 6*A*). After collection of baseline samples, PD 128907 was applied by reverse dialysis for 1 hr, followed by a 1 hr recovery period. Initial experiments in wild-type mice indicated a small (15%), although statistically significant, decrease in DA efflux during perfusion with 100 nM PD 128907 (data not shown); however, to maximize our ability to detect potential differences between the wild-type and mutant mice, we used a higher concentration of PD 128907 (1 μM). At this concentration, PD 128907 significantly decreased DA efflux in both mutant and wild-type mice, as determined by comparisons of fractions during drug infusion to the weighted mean of three baseline fractions (Fig. 6*A*). When these data were expressed as the percent of basal DA release (Fig. 6*B*), an apparent group difference was observed. To determine whether this was attributable to the difference in basal DA efflux or to a blunting of the inhibitory effects of PD 128907 in mutant mice, we performed an analysis of covariance with basal DA as the covariate. The results confirmed the significant difference in basal DA levels but indicated that when this covariate was controlled, there was no significant difference between mutant and wild-type mice with respect to the suppression of DA release by PD 128907, i.e., the absolute decrease in DA release produced by the agonist was not different in the two groups of mice. Nevertheless, it is apparent that there was a lag in the onset of the drug-induced suppre on in the mutant mice; thus, marked decreases in DA efflux re observed during the first 20 min of PD 128907 application i he wild-type mice, whereas no effect was observed during this p od in the mutants (Fig. 6).

DISCUSSION

Autoreceptor regulation of impulse flow and DA synthesis is mediated primarily by DA D_2 receptors

Our results indicate that D_3 receptors may be involved i he regulation of DA release but not via autoreceptor mechan s. Moreover, D_3 receptors do not seem to contribute significan o functional pools of either somatodendritic impulse-modulati or nerve terminal synthesis- or release-modulating DA autor p-tors. The putative D_3 receptor-selective agonist PD 12890 as equally potent at inhibiting DA cell firing and decreasing A synthesis in D_3 receptor mutant mice and their wild-type l r-mates. Therefore, the ability of PD 128907 and other putativ D_3 receptor-selective ligands to regulate DA neuronal activity d DA synthesis, demonstrated by many laboratories (see intr o-tory remarks), could not have been by activation of D_3 rece rs but must have been mediated by the well-accepted D_2 autor p-tor population. With respect to impulse-regulating DA au e-ceptors, our findings are consistent with a recent report indic g a complete loss of autoreceptor-mediated hyperpolarizatio nd suppression of activity of midbrain DA neurons in mice la g the DA D_2 receptor gene (Mercuri et al., 1997). Taken toge r, these findings demonstrate the power of the gene knoc ut approach in the identification of functions mediated by indiv al members of a receptor family.

Our findings stand in contrast to recent reports in which i-sense oligodeoxynucleotides have been used to reduce D_3 re p-tor expression (Nissbrandt et al., 1995; Tepper et al., 1). Nissbrandt and colleagues (1995) found that intracerebrover o-ular administration of a D_3 receptor antisense oligodeoxyn e-

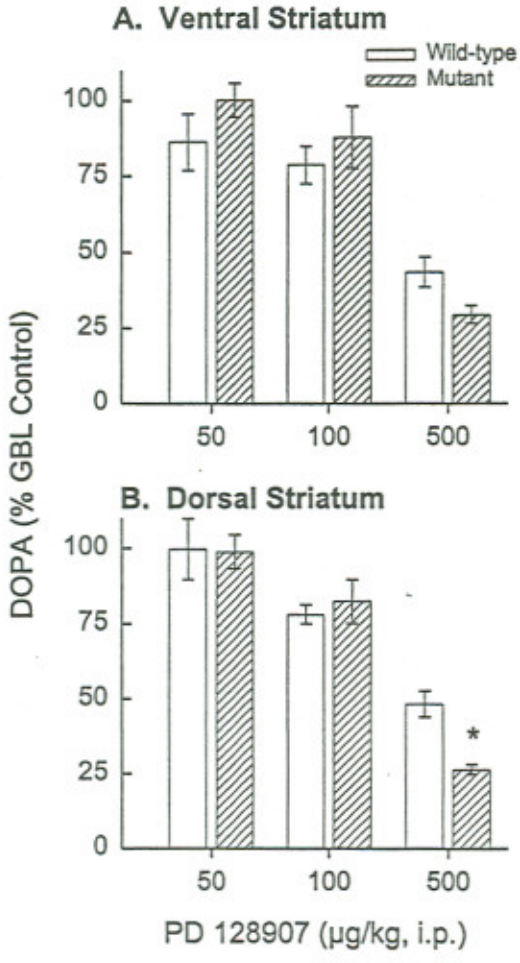

Figure 4. Similar inhibition of GBL-induced striatal DOPA formation by PD 128907 in D_3 receptor mutant and wild-type mice. *A*, Reversal of GBL-induced DOPA synthesis by PD 128907 ($F_{3,57} = 29.97$; $p < 0.001$) in the ventral striatum was not significantly different in D_3 receptor mutant and wild-type mice. Data are presented as the percent of DOPA levels measured in mice administered GBL plus NSD without PD 128907 (wild type, 2.51 µg/gm/30 min; mutant, 2.53 µg/gm/30 min). Sample sizes for the 0, 0.05, 0.1, and 0.5 mg/kg doses of PD 128907 are 16, 4, 6, and 6 for the mutant and 19, 3, 5, and 6 for the wild-type mice, respectively. *B*, Reversal of GBL-induced DOPA synthesis by PD 128907 ($F_{3,57} = 25.23$; $p < 0.001$) in the dorsal striatum was also not significantly different in the two groups of mice; basal levels were 3.26 ng/gm/30 min for the wild-type and 3.40 ng/gm/30 min for the mutant mice. Error bars indicate SEM. Sample sizes for the 0, 0.05, 0.1, and 0.5 mg/kg doses of PD 128907 are 21, 4, 6, and 6 for the mutant and 14, 3, 5, and 5 for the wild-type mice, respectively. Note that the effect of the highest dose of PD 128907 on DA synthesis within the dorsal striatum was actually significantly greater in the mutant than in the wild-type mice (*$p < 0.05$; Dunnett's test).

otide enhanced basal levels of DA synthesis in the nucleus accumbens but failed to affect the suppression of synthesis by the nonselective DA receptor agonist apomorphine. More recently, Tepper and coworkers (1997) reported a nearly equivalent (50%) reduction in the inhibitory effects of apomorphine on the activity of rat SN DA neurons after intra-SN infusion of antisense oligodeoxynucleotides complementary to the initial coding region of either the DA D_2 or D_3 receptor. Despite considerably greater expression of D_2 than of D_3 receptors in the SN, Tepper et al. (1997) argued that D_3 receptor coupling to transduction events may be more efficient such that D_2 and D_3 receptors play equivalent functional roles as DA autoreceptors.

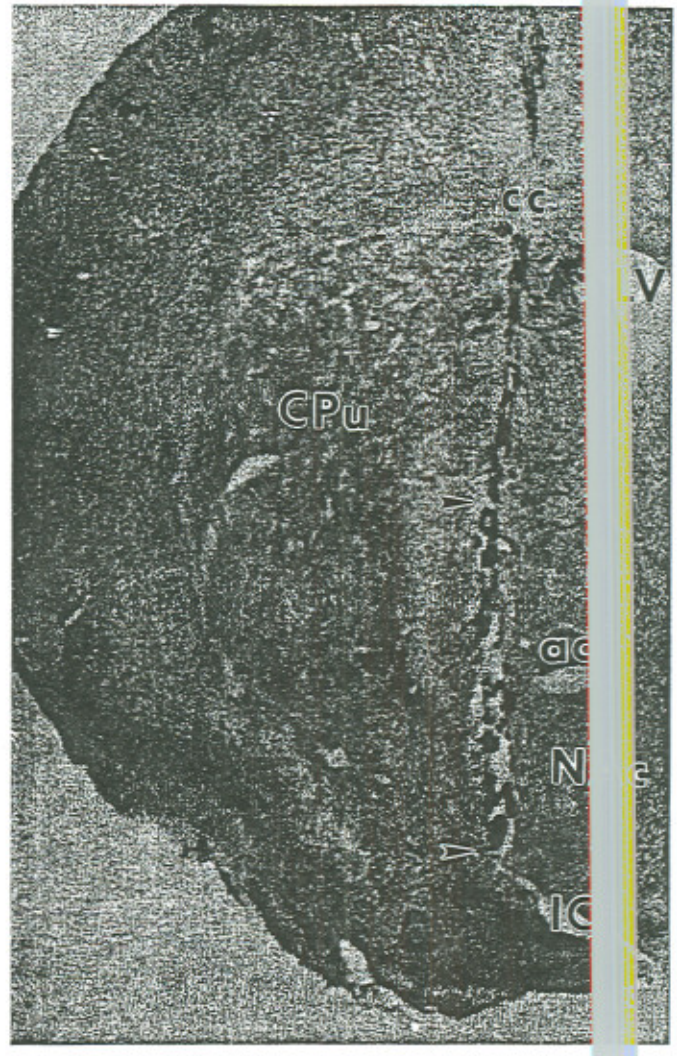

Figure 5. Localization of microdialysis probe in the ventral str m. In this coronal section of the mouse forebrain, the *tract* left by the rodialysis probe can be seen traversing the ventral striatum. Only th ttom 2 mm of the probe (located between the *arrowheads*) consisted pen dialysis membrane. *NAc*, Nucleus accumbens; *CPu*, caudate- men; *LV*, lateral ventricle; *ICj*, island of Calleja; *ac*, anterior comm ; *cc*, corpus callosum.

The results of these antisense oligodeoxynucleotide dies raise the possibility that in our D_3 receptor mutant m D_2 receptors had compensated for the loss of D_3 recept and thereby masked any functional loss in our assays. If this ue, then it is interesting that the reciprocal does not seem to ue, i.e., that D_3 receptors are not able to compensate for the s of D_2 receptors (Mercuri et al., 1997). This would suggest D_2 receptors are capable of autoregulation independently, eas D_3 receptor autoregulatory activity would be dependent on- current D_2 autoreceptor stimulation. Although there are de- finitive findings that eliminate the possibility of compens n of D_3 receptor function by D_2 autoreceptors, we think th his explanation is unlikely because of the following: (1) suc m- pensation would have to be precise given the almost i cal effects observed in the mutant and wild-type mice duri lec- trophysiological and neurochemical experiments, (2) suc m- pensation would have to be specific to certain receptor fu ons given that differences between mutant and wild-type mi ere

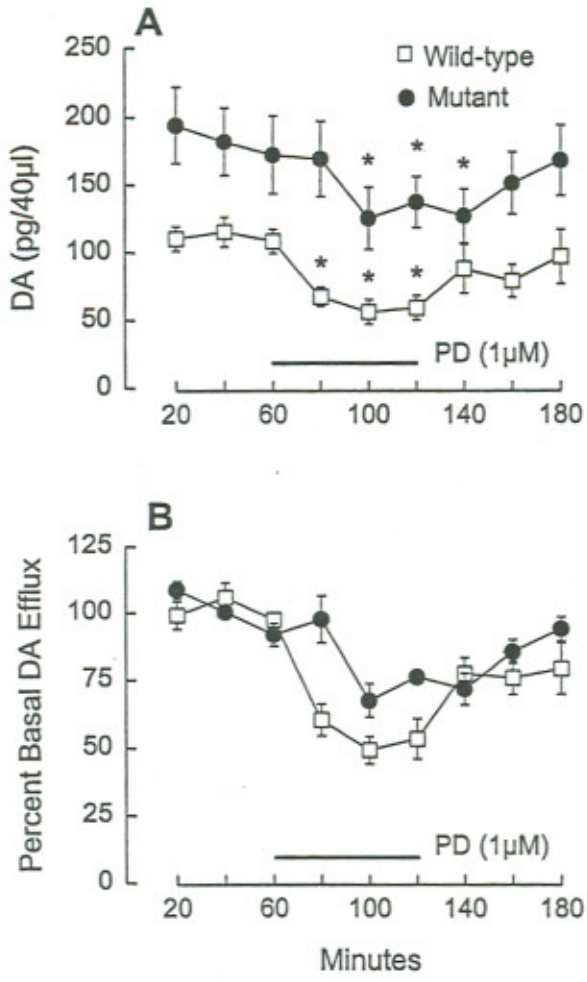

Figure 6. Regulation of basal DA efflux, but not PD 128907-induced suppression of DA release, is altered in D_3 receptor mutant mice. *A*, After 2–3 hr of perfusion, three baseline fractions (20 min each) were collected (0–60 min). Basal DA efflux was significantly higher in mutant mice ($F_{1,9} = 26.9$; $p = 0.0008$). Values for basal levels in wild-type mice were ~13 nM, which is comparable with values obtained in our previously published studies of rat NAc (e.g., see Wolf et al., 1994). PD 128907 was applied for 60 min (*horizontal bar*), beginning after collection of the third baseline fraction. PD 128907 significantly reduced DA efflux in both wild-type and mutant mice ($F_{1,10} = 7.798$; $p = 0.019$). *Asterisks* indicate points that differ significantly ($p < 0.01$; Dunnett's test) when compared with the weighted mean of the three predrug baseline values. *B*, When the results shown in *A* are expressed as percent of basal DA efflux, the inhibitory effect of PD 128907 appears blunted in the mutant mice. However, ANCOVA indicated that this apparent effect was solely attributable to the initial differences in basal DA efflux, i.e., there was no difference between the two groups with respect to the release-suppressing effects of PD 128097 when basal DA levels were controlled as a covariate. Results are expressed as mean ± SEM (n = 6/group).

observed in basal DA efflux and in certain behavioral measures (Accili et al., 1996; Xu et al., 1997), (3) there are no increases in D_2 receptor binding densities caused by the D_3 receptor mutation (Accili et al., 1996; Xu et al., 1997), (4) knock-out of the DA D_2 receptor leads to a complete loss of autoreceptor function in the SN (Mercuri et al., 1997), and (5) there have been no documented cases of compensatory upregulation of related receptor subtypes after deletion of G-protein coupled receptors (for review, see Tecott et al., 1996).

It is also possible that the difference between findings from antisense knock-down and genetic knock-out studies reflects differences between mice and rats with respect to D_3 rec tor contributions to autoregulation. However, there does not se to be a difference in the levels of D_3 receptors expressed i e midbrain of these two species (Mercuri et al., 1997). More r, recent studies have demonstrated that putative D_3 autorec r effects in rats are in fact caused by D_2 receptors, incl g hyperpolarization and suppression of DA neuron activity (w- ery et al., 1996). Given the low abundance and highly restr d expression of D_3 mRNA by DA neurons (Diaz et al., 1995) s certainly possible that a small number of DA neurons ex s functional D_3 autoreceptors. However, any autoreceptor e occurring in such a small neuronal population is unlikely to t significant impact on the *in vivo* activity of the mesotelencep c DA systems as a whole, unless such a role is accomplished by w levels of D_3 receptors the mRNA of which is below the dete n limit of current *in situ* hybridization (Bouthenet et al., 1991; z et al., 1995), reverse transcriptase-PCR (Valerio et al., 1994), d ribonuclease protection (Richtand et al., 1995) assays. Altho h we cannot exclude this possibility and the findings of Tepper e l. (1997) certainly support it, we believe the bulk of the evid e argues that D_2 receptors are primarily responsible for autor - lation of DA neuronal function.

D_3 receptors may contribute to local regulation of DA release

Our microdialysis studies indicated a significant increase in b l DA efflux in the ventral striatum of D_3 receptor mutant m s compared with their wild-type littermates. Although "no- t flux" dialysis studies are often needed to quantify difference n basal transmitter efflux *in vivo* (for review, see Justice, 1993), p difference between D_3 receptor mutant and wild-type mice w quite robust, with all but one mutant mouse showing higher ba DA efflux than any of the wild-type mice. Increased basal l efflux might be expected if nerve terminal D_3 autorecept normally exert a tonic inhibitory influence on DA release. Ho ever, the results obtained with PD 128907 are incompatible w this simple interpretation because this putative D_3 recept selective agonist inhibited DA release by the same absolu amount in D_3 receptor mutant and wild-type mice. Thus, reduction of DA autoreceptor modulation occurred as a result the D_3 receptor mutation. Although the percent decrease caus by PD 128907 was smaller in the mutant mice, this effect w attributable to their higher basal DA levels, a factor known reduce the ability of DA agonists to suppress DA release (C beddu and Hoffman, 1982; Dwoskin and Zahniser, 1986; f review, see Wolf and Roth, 1987).

An alternative explanation for our findings is that DA relea is normally modulated both by D_2 release-modulating autorece tors and by negative feedback pathways engaged by postsynapt D_3 (and other D_2-class) receptors. Loss of D_3 receptor-mediate inhibitory feedback would explain increased basal DA efflu whereas activation of D_2 release-modulating autoreceptors by P 128907 would explain the normal inhibitory effects on DA releas This model is consistent with the D_3 mRNA findings indicatin that D_3 receptors are distributed primarily within target neuror of the ascending DA systems. In ventral striatal terminal field where the D_3 receptor is most highly expressed (nucleus accum bens shell, olfactory tubercle, and islands of Calleja), there i good agreement between levels of D_3 receptor mRNA and D receptor binding sites (Diaz et al., 1995), suggesting that most D receptors exist on dendrites, soma, or local terminals of intrinsi neurons. If postsynaptic D_3 receptors are coupled to feedbac

pathways that normally exert an inhibitory influence on DA release, the loss of such feedback might lead to increased basal DA efflux but leave D_2 autoreceptor-mediated effects intact.

Negative feedback pathways engaged by postsynaptic D_3 receptors might involve retrograde messengers, short-loop paths (either mono- or multisynaptic) terminating on DA terminals, or striatomesencephalic (long-loop) projections regulating impulse flow at the level of DA cell bodies and thereby influencing impulse-dependent DA release from nerve terminals. We do not favor the latter mechanism because (1) we observed no differences in basal firing rates of DA neurons in mutant and wild-type mice, and (2) previous work has shown that local application of DA agonists by reverse dialysis inhibits DA release via local mechanisms and not via long-loop feedback effects on DA impulse flow (Timmerman et al., 1990). As for the first two possibilities, it has long been known that intrinsic striatal neurotransmitters (e.g., acetylcholine, GABA, enkephalin, and substance P) as well as corticostriatal afferents (e.g., glutamate) may modulate DA release (for review, see Chesselet, 1984), and similar effects of the retrograde messenger nitric oxide have recently been demonstrated (Lonart et al., 1993). DA agonists, when applied locally by reverse dialysis, could conceivably engage such local mechanisms, in addition to exerting effects via nerve terminal DA autoreceptors. Indeed, the slower onset of the PD 128907-induced suppression of DA release in the mutants suggests that at least two processes, one of which is faster and involves D_3 receptors, are responsible for this effect in the wild-type mice.

Conclusions

Our findings are incongruent with recent claims that D_3 receptors function as DA autoreceptors. D_3 receptors may contribute to regulation of DA release, but the mechanism may involve postsynaptically activated feedback as opposed to nerve terminal autoreceptors. If so, then D_3 receptors would not only be subject to anterograde regulation by unknown factors released by DA neurons (Lévesque et al., 1995) but might also engage feedback mechanisms to control DA release. In conclusion, the results obtained with D_3 mutant mice support a large body of earlier work suggesting that D_2 receptors constitute the vast majority of DA autoreceptors and that the ability of D_3-preferring ligands to alter these functions *in vivo*, as demonstrated by numerous studies in normal rats (see introductory remarks), reflects their lack of selectivity for D_3 receptors and must be attributed to stimulation of D_2 receptors.

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