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Abnormal presynaptic catecholamine regulation in a hyperactive SNAP-25-deficient mouse mutant

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Abstract

The consequences of a reduction in the presynaptic protein, SNAP-25, were investigated to determine the neurochemical basis of the marked hyperlocomotor activity in coloboma (Cm/+) mice. SNAP-25 is part of the minimal presynaptic machinery necessary for exocytotic neurotransmitter release. Reserpine treatment was used to deplete vesicular stores of catecholamines. Coloboma mice were more sensitive to the effects of reserpine than control mice. However, presynaptic regulation of dopamine (DA) release, as assessed by low-dose apomorphine challenge, was intact. There were region-specific reductions in in vivo tyrosine hydroxylation and the DA metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum and nucleus accumbens of Cm/+ mice. While hyperactivity is often associated with changes in DA concentration, norepinephrine (NE) concentration was significantly increased in the striatum and nucleus accumbens of the hyperactive mutant. The increase in NE may regulate the hyperactivity in these mice, as suggested by current hypotheses of the mechanisms underlying attention-deficit hyperactivity disorder (ADHD) and Tourette's syndrome.

Keywords: Presynapse; SNAP-25; Hyperactivity; Norepinephrine; Dopamine; ADHD; Tourette's syndrome; Locomotor activity; Mouse mutant; Coloboma

1. Introduction

Animal models of hyperactivity are of considerable interest, as they provide experimental paradigms for elucidating the biologic mechanisms underlying hyperactivity syndromes such as attention-deficit hyperactivity disorder (ADHD) or Tourette's syndrome. The mouse mutant coloboma carries a well-defined genetic abnormality that results in profound hyperactivity. The spontaneous hyperactivity exhibited by coloboma mice averages three times the activity of the control littermates; the most severely affected mice display activity 10 times greater than control mice (Hess et al., 1992). These mice carry a semidominant mutation (*Cm*), wherein the heterozygous state (*Cm*/+) results in a mutant phenotype while homozygous (*Cm*/*Cm*) mice die early in embryogenesis (Theiler and Varnum, 1981).

The genetic defect in coloboma mice is an $\sim$2 cM deletion mutation that encompasses the *Snap*, *PCLB*, and *Jag*1 genes including several as yet unidentified genes (Hess et al., 1994; Xue et al., 1999). The *Snap* gene encodes *syn*aptosomal *a*ssociated *p*rotein-25 kD (SNAP-25), a neuron-specific protein involved in exocytotic neurotransmitter release. SNAP-25 complexes with synaptobrevin and syntaxin to dock synaptic vesicles at the presynaptic membrane in readiness for Ca^{2+}-triggered neurotransmitter exocytosis (Horikawa et al., 1993; O'Connor et al., 1993; Sollner et al., 1993a,b). The addition of Ca^{2+} to the complex induces vesicle fusion, resulting in exocytotic transmitter release. Although SNAP-25 appears to be necessary for neurotransmitter release in all CNS neurons, it is differentially expressed with highest levels in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra, and cerebellar granule cells (Oyler et al., 1989). This pattern of expression is preserved in coloboma mice, but SNAP-25 mRNA and protein expression is reduced by 50% throughout the CNS (Hess et al., 1992). The reduction in SNAP-25 expression is critical for the manifestation of the hyperactive phenotype, as restoration of SNAP-25 expression to near normal by a SNAP-25 transgene resulted in normoac-

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tive coloboma mice, essentially rescuing the hyperactivity (Hess et al., 1996).

Catecholamine dysregulation has been implicated in the expression of hyperactivity. In another animal model of hyperactivity, administration of the neurotoxin 6-hydroxydopamine (6-OHDA) is administered to selectively lesioned catecholaminergic neurons and produces alterations in gross levels of activity. When administered to adult animals, 6-OHDA causes profound hypoactivity; however, when administered to young animals, it produces hyperactivity (Shaywitz et al., 1976). Further, experiments directly injecting dopamine (DA) agonists into the 6-OHDA-lesioned nucleus accumbens or specific lesions of the nucleus accumbens reproduce the hyperactivity and have implicated the nucleus accumbens as the site mediating this hyperactivity (Costall and Naylor, 1975; Jackson et al., 1975; Joyce and Koob, 1981; Koob et al., 1981). Another model implicating catecholamine regulation in hyperactivity are mice lacking the DA transporter. These mice are five to six times more active than their control littermates, similar to the levels of activity observed in coloboma mice (Giros et al., 1996). Their hyperactivity presumably results from the failure to clear DA from the synapse, resulting in persistent, unregulated DA stimulation.

Abnormalities in monoamine transmission likely play a role in the regulation of locomotor activity in coloboma mice. Coloboma mice exhibit paradoxical behavioral and physiological responses to the indirect-acting monoamine agonist, amphetamine. Amphetamine, a psychostimulant that acts at the presynaptic terminal to promote catecholamine release, is effective in ameliorating the hyperactivity expressed in ADHD-affected children (Barkley, 1977; Shaywitz and Shaywitz, 1984). Low doses of amphetamine, which increase locomotor activity in normal mice, reduce locomotor activity in coloboma mice to near normal without the induction of stereotypy (Hess et al., 1996). The physiological response to low doses of amphetamine is similarly disrupted. Amphetamine causes an increased inhibition of dentate paired-pulse responses in coloboma mice, whereas amphetamine disinhibits these responses in normal mice (Steffensen et al., 1999). Further, monoaminergic neurotransmitter release is disrupted in coloboma mice. A reduction in both DA and serotonin release occurs specifically in the dorsal striatum, while release in the cortex and ventral striatum is apparently unaffected by the mutation (Raber et al., 1997). Despite the ubiquitous requirement for SNAP-25 in exocytotic neurotransmitter release, the effect of the coloboma mutation appears surprisingly specific. The presynaptic localization of SNAP-25 coupled with the obvious deficits in catecholaminergic responses suggests that abnormalities in presynaptic monoamine regulation, including monoamine vesicular release, may contribute to the hyperactivity in coloboma mice. Here, the role of presynaptic catecholamine regulation in the expression of coloboma mouse hyperactivity was examined.

2. Method

2.1. Animals

Coloboma (Cm/+) mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and thereafter bred at The Pennsylvania State University College of Medicine. In all experiments, coloboma mutant mice and wild-type control littermates (8–10 weeks of age) were produced by crossing Cm/+ mice with wild-type C3H/HeSnJ mice. Mutant (Cm/+) mice were identified at weaning by several characteristic phenotypes including head bobbing, ocular dysmorphology, and smaller body size. The coloboma mutants were also distinguished at this time by their profound hyperactivity. All procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

2.2. High-performance liquid chromatography (HPLC) analysis

Regional concentrations of monoamines [DA, norepinephrine (NE), and serotonin (5-HT)] and metabolites including 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection ($n = 15$/genotype). Brains were rapidly removed and dissected within 5 min over ice and frozen on dry ice. All mice were sacrificed between 10:30 and 12:00 h. The brain structures dissected included striatum, nucleus accumbens, hippocampus, cerebellum, thalamus, olfactory bulb, and brain stem. Tissues were stored at -70°C until HPLC analysis. The brain regions were homogenized by sonication in 0.1 M sodium acetate (pH = 5). The samples were then centrifuged for 10 min at $14{,}000 \times g$ at 4°C. The supernatants were filtered through microspin centrifuge filters (0.45 μm) for 2 min at $3000 \times g$. A 20 μl aliquot of the filtered solution was measured by HPLC with four coulometric electrochemical detectors. Electrochemical sensor potentials were set at 150, 250, 350, and 500 mV. Simultaneous separation of monoamines and metabolites occurred through a C18, MD-150 column (150 mm length × 3 mm id; ESA, Chelmsford, MA) with a mobile phase consisting of 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid sodium salt, 25 μM EDTA, and 8% acetonitrile (pH = 2.9) at a flow rate of 0.6 ml/min. The ratios of peak response for the dominant sensor to the other sensors were measured for each compound. Compounds were identified based upon the matching criteria of retention time, sensor ratio measures, and peak height to known standards. Compounds were quantified by comparing peak heights to those of standards on the dominant sensor. The in vivo activity of tyrosine hydroxylase (TH) in the coloboma mouse was assessed by measuring L-DOPA accumulation after subcutaneous (sc) administration of the decarboxylase inhibitor 3-

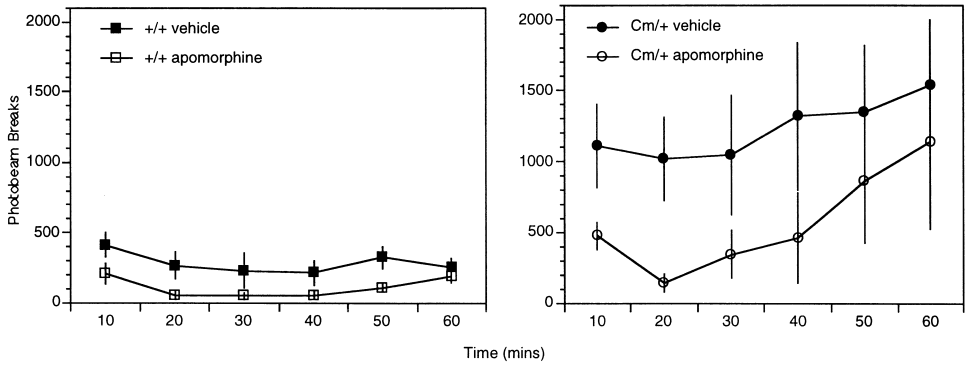

Fig. 1. Locomotor activity of control ($n=8$) and coloboma ($n=8$) mice after administration of vehicle or apomorphine (0.05 mg/kg). Mice were habituated to the test cages for 5 h before subcutaneous injection and photocell beam interruptions were recorded every 10 min for 2-h postinjection. Because drug effects of apomorphine were confined to the first 30 min, the graph represents the first hour of locomotor activity. The coloboma mouse drug-naive baseline activity is significantly greater than wild type; ANOVA for repeated measures indicated a significant genotype effect for vehicle treatment [$F(1,14)=5.74$, $P<.05$]. Data represent mean ± S.E.M.

hydroxybenzylhydrazine HCl (NSD-1015, 150 mg/kg; Sigma, St. Louis, MO) to wild-type ($n=10$) and coloboma ($n=10$) mice 40 min before sacrifice. Data were analyzed using ANOVA.

2.3. Behavioral testing

Prior to experiments, the mice were maintained in group cages with a reverse 12-h light and dark cycle. The mice were allowed free access to both food and water. Locomotor activity was quantified by placing coloboma and control mice into individually automated Plexiglass boxes (29.2 × 50.5 cm) with 12 2-cm high infrared beam detectors arranged in a 4 × 8 grid (San Diego Instruments; San Diego, CA). Computer-recorded beam breaks were accumulated every 10 min for the duration of the 2-h test period, with changes in beam status assessed 18 times/s. Equal numbers ($n=8$/genotype) of sex and age-matched (8–10 weeks) coloboma and control wild-type littermates were habituated to test cages for at least 5 h with access to food and water ad libitum. Locomotor activity was assessed 1 h after the start of the dark cycle. Photocell beam interruptions were analyzed by ANOVA.

Mice were injected subcutaneously with reserpine (0.1–2 mg/kg) to prevent storage of monoamines or the mixed D_1/D_2 DA receptor agonist apomorphine (0.05–8 mg/kg; RBI, Natick, MA). Apomorphine hydrochloride was dissolved in 0.9% NaCl and 0.2% ascorbic acid immediately prior to each session. Reserpine was first dissolved in 5% glacial acetic acid and then subsequently diluted in water to obtain the appropriate dose. Reserpine was administered 24 h before the locomotor testing session. Apomorphine was given immediately prior to the start of the behavioral test. For both reserpine and apomorphine, locomotor activity was

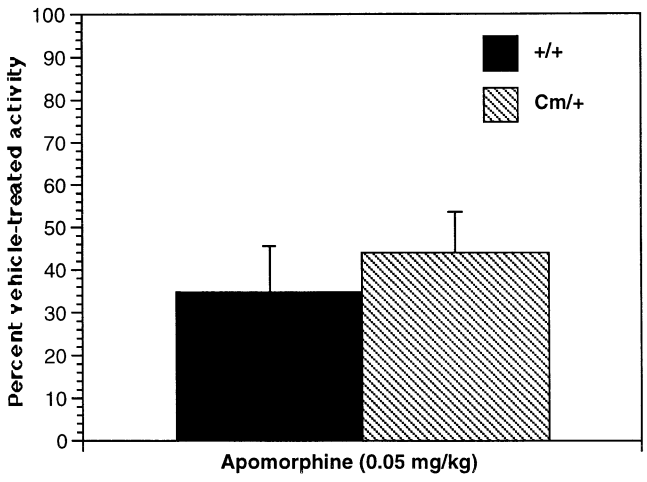

Fig. 2. Apomorphine-induced reduction in locomotor activity expressed as a percent of vehicle-treated activity in wild-type ($n=8$) and coloboma mice ($n=8$). Locomotor activity after apomorphine injection is expressed as a percent of vehicle-treated activity. Data are from the first 30 min of the testing session during the maximal effect of apomorphine (see Fig. 1). The percent reduction in locomotor activity caused by apomorphine did not differ between the genotypes (unpaired t test, $P=.55$).

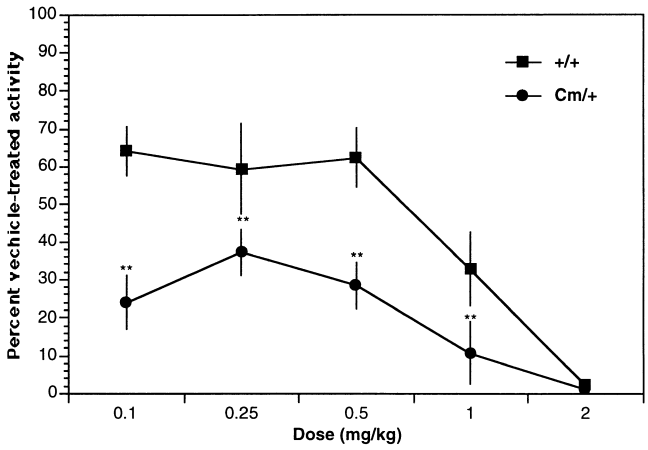

Fig. 3. Dose-dependent decrease in locomotor activity following reserpine treatment. The reserpine-induced reduction in locomotor activity was significantly greater at all doses in coloboma mice than wild-type mice ($P<.01$; post-hoc Scheffe), except 2 mg/kg. Data are expressed as mean ± S.E.M. with $n=6$ at all doses, except $n=1$ at 2 mg/kg, which is lethal in these mice.

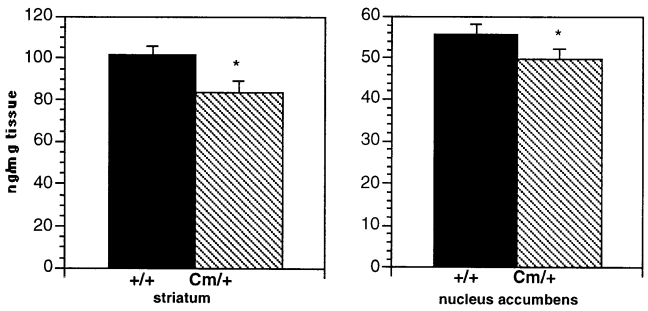

Fig. 4. Effects of NSD-1015 in control ($n=10$) and coloboma ($n=10$) mice. L-DOPA accumulation was measured in the striatum and nucleus accumbens by HPLC 40 min after 150 mg/kg sc administration of NSD-1015. L-DOPA was not detectable in either control or coloboma saline-treated group (not shown). L-DOPA accumulation was significantly lower in the striatum [$F(3,41)=1.92$, $P<.05$] and nucleus accumbens [$F(3,47)=4.37$, $P<.05$], ANOVA. * Indicate significant differences between coloboma and wild-type mice.

assessed after vehicle administration to provide baseline locomotor activity and then after drug treatment in a repeated-measures design. Mice were challenged with apomorphine 1 week after vehicle treatment. Reserpine was delivered 24 h after vehicle injection. Drug effects were expressed as percent of baseline activity where appropriate.

3. Results

3.1. Presynaptic effects

Apomorphine is a mixed D_1/D_2 DA receptor agonist, with both pre- and postsynaptic sites of action. At low doses, apomorphine preferentially acts at D_2-like DA autoreceptors to produce a sedative response by reducing the release of DA (Masuda et al., 1987). Both coloboma mice and wild-type littermates showed a decrease in locomotor activity after a subcutaneous injection of 0.05 mg/kg apomorphine, a low dose that preferentially stimulates presynaptic DA receptor sites (Fig. 1). Despite the reduction in SNAP-25, the reduction in coloboma mutant activity (44% ± 11) was remarkably similar to the reduction in normal mice (35% ± 10) after apomorphine stimulation of presynaptic DA receptor sites (Fig. 2).

Reserpine depletes the vesicular releasable pool of monoamines, which may be affected by the SNAP-25 deficit. The reserpine treatment produced a clear dose-dependent decrease in the locomotor activity of wild-type and coloboma mice. The dose-dependent reduction in coloboma mouse locomotor activity was significantly greater than the wild-type response ($P<.01$). After the low dose of 0.1 mg/kg reserpine, coloboma mice locomotor activity was reduced to 24% while wild-type mice retained 64% of their control activity (Fig. 3).

The rate of L-DOPA accumulation in the striatum and nucleus accumbens following inhibition of aromatic amino acid decarboxylase by NSD-1015 was used as an indirect measure of the in vivo rate of tyrosine hydroxylation. L-DOPA was not readily detectable in vehicle-treated wild-type or coloboma mice but accumulated rapidly after NSD-1015 treatment. L-DOPA accumulation in coloboma mice was significantly lower than controls both in striatum ($P<.001$) and nucleus accumbens ($P<.05$), suggesting that TH activity is reduced in coloboma mice (Fig. 4). The L-DOPA accumulation in the cerebellum was normal, suggesting a region-specific reduction in TH activity (data not shown).

3.2. Neurochemical effects

The catecholamine precursor tyrosine was assessed because coloboma are generally about 75–80% normal body weight and may exhibit dietary deficiencies. There was no difference in the tyrosine concentration between wild-type and coloboma mice in any brain region tested

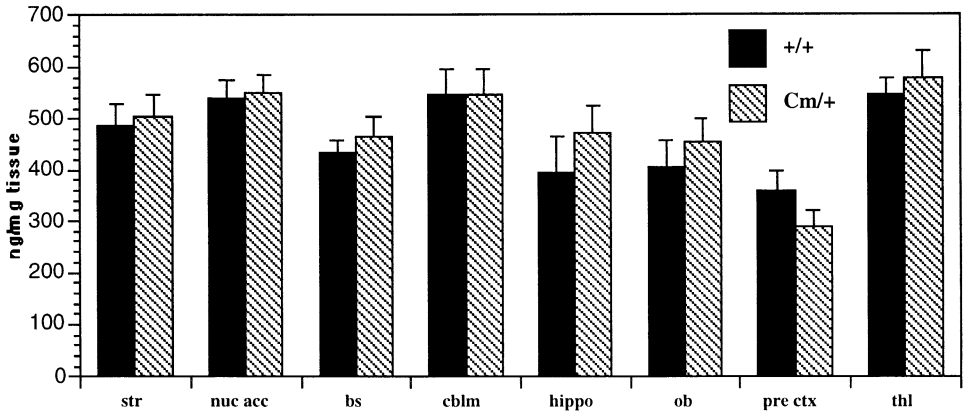

Fig. 5. Regional analysis of tyrosine in wild-type ($n=15$) and coloboma ($n=15$) mice. The catecholamine precursor tyrosine (tyr) was measured in the caudoputamen (str), nucleus accumbens/olfactory tubercle (nuc acc), brainstem (bs), cerebellum (cblm), hippocampus (hippo), olfactory bulb (ob), prefrontal cortex (pre ctx), and thalamus (thl). Results are expressed in ng/mg wet weight of tissue ± S.E.M. There were no significant differences between normal and mutant mice in any brain region.

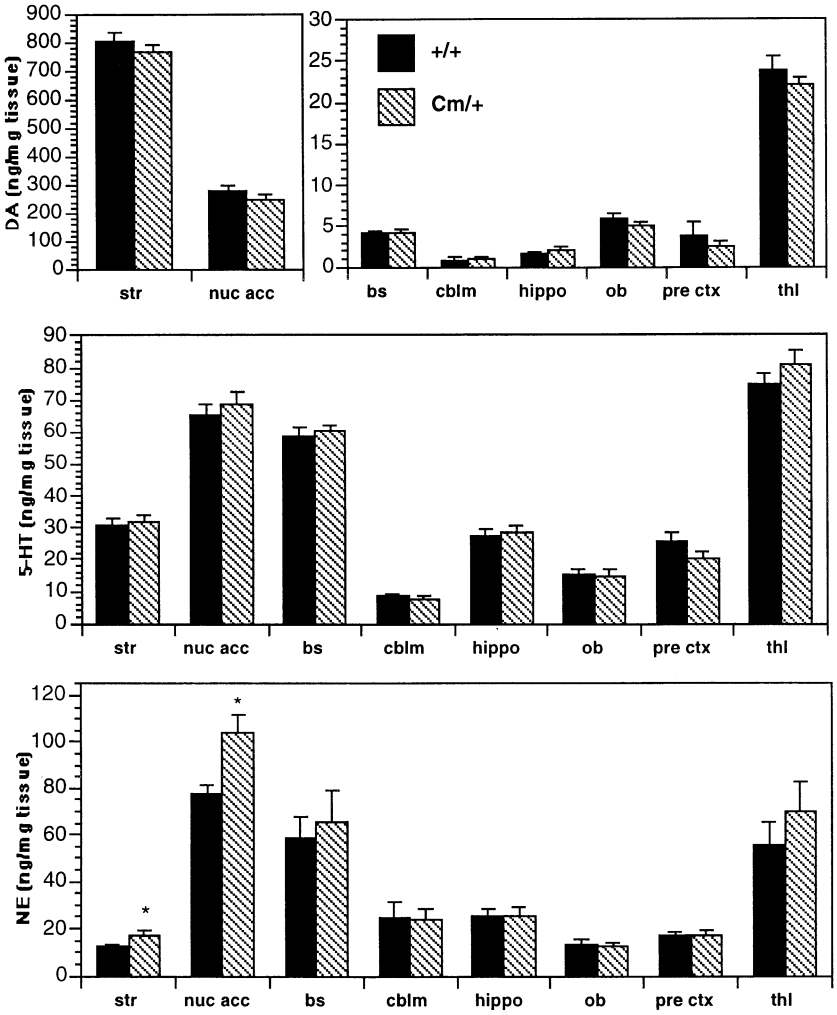

Fig. 6. Regional analysis of monoamines (DA, serotonin, and NE) in wild-type ($n=15$) and coloboma ($n=15$) mice. Monoamines were analyzed in the caudoputamen (str), nucleus accumbens/olfactory tubercle (nuc acc), brainstem (bs), cerebellum (cblm), hippocampus (hippo), olfactory bulb (ob), prefrontal cortex (pre ctx), and thalamus (thl). Results show average values in ng/mg wet weight of tissue ± S.E.M. Statistical analysis was performed on each region with separation by compound and group using ANOVA. For DA and serotonin, there were no significant differences across brain regions. Statistically significant effects were observed for NE in str [$F(1,23)=4.40$, $P<.05$] and in nuc acc [$F(1,29)=4.52$, $P<.05$]. * Denote significant differences between normal and mutant animals in particular brain regions.

(Fig. 5). Surprisingly, given its well-established role in locomotor behavior, the concentration of DA was comparable in all brain regions of control and coloboma mice (Fig. 6 and Table 1). However, the DA metabolites DOPAC and HVA were significantly reduced in the nucleus accumbens in coloboma mice, as shown in Table 1. This was a region-specific reduction, as DOPAC and HVA were normal in other brain regions tested including brain stem, cerebellum,

Table 1
Tissue concentration of DA, HVA, and DOPAC in +/+ and Cm/+ mice

Genotype	*n*	HVA	DOPAC	DA
Striatum				
+/+	13	78.40 ± 3.3	73.55 ± 3.2	805.50 ± 30.9
Cm/+	12	69.45 ± 3.8	65.23 ± 2.6	767.30 ± 29.4
Nucleus accumbens				
+/+	16	36.60 ± 1.3	47.39 ± 1.7	285.28 ± 12.9
Cm/+	15	30.01 ± 1.5**	39.58 ± 1.7**	251.82 ± 14.9

Monoamine content (ng/mg of wet weight of tissue) was measured by HPLC with electrochemical detection. Data are expressed as mean ± S.E.M.

** Statistically significant differences were observed for DA metabolites in the nucleus accumbens of coloboma mice, HVA [$F(1,29)=10.20$, $P<.01$] and DOPAC [$F(1,29)=10.40$, $P<.01$].

hippocampus, olfactory bulb, and thalamus (data not shown). Although not statistically significant, there was a trend toward decreased HVA and DOPAC in the striatum. The other DA metabolite, 3-MT, was not significantly different between control and coloboma mice (data not shown). Unexpectedly, the NE concentration was significantly increased in both striatum ($P < .05$) and nucleus accumbens ($P < .05$) of coloboma mice (Fig. 6). The 42% (striatum) and 35% (nucleus accumbens) increase in NE concentrations of coloboma mice as compared to wild-type mice was region specific, as NE concentrations in all other brain regions were normal. Serotonin (Fig. 6) and its metabolite 5-HIAA were normal in coloboma mice in all brain regions assessed (data not shown).

4. Discussion

Catecholamine neurochemistry was examined in the hyperactive mouse mutant coloboma to determine the biochemical consequences of expressing only 50% of normal quantities of SNAP-25 (Hess et al., 1996). Previous research (Raber et al., 1997) has demonstrated decreased DA release in the coloboma mouse, suggesting that catecholamine regulation may be perturbed. In fact, depletion of catecholamines with reserpine treatment lead to a dramatic decrease in coloboma mouse locomotor activity. This increased sensitivity to reserpine also suggests abnormal catecholamine function. Although the reserpine response was exaggerated in coloboma mice, presynaptic apomorphine responses are intact. At low doses, apomorphine selectively activates both presynaptic and somatodendritic DA autoreceptors (Masuda et al., 1987). Stimulation of DA autoreceptors in the cell bodies and dendrites within areas A9 and A10 slows the firing rate of DA neurons, while stimulation of nerve terminal autoreceptors inhibits DA synthesis and release. It has been demonstrated that peripheral administration of low doses of apomorphine decreases the release of DA by nearly 50% (Masuda et al., 1987; Winkler and Weiss, 1986). Behaviorally, low doses of apomorphine result in sedation due to the reduction in synaptic DA concentrations. Both control and coloboma mice responded to apomorphine with comparable reductions in motor activity. The coloboma mouse response to apomorphine suggests that vesicular DA release occurs despite the deficit in SNAP-25. Thus, while vesicular release may be perturbed in these mice, the presynaptic mechanisms regulating exocytotic DA release appear intact.

SNAP-25 is expressed panneuronally (Oyler et al., 1989) but catecholamine dysregulation was region-specific in mutant mice. Reductions in in vivo tyrosine hydroxylation and the DA metabolites HVA and DOPAC were observed only in the striatum and nucleus accumbens. Similarly, increases in NE concentrations were confined to the striatum and nucleus accumbens. All other brains regions tested were normal. The specificity of the effect is somewhat surprising, given the distribution of SNAP-25; however, the basal ganglia appear to be extremely sensitive to insult in both man and mouse. Several disorders in human including Huntington's disease and Lesch–Nyhan disease cause specific abnormalities of the basal ganglia despite the expression of the mutant gene throughout the rest of the brain and body. Coloboma mice may be useful for identifying features of the basal ganglia that promote its susceptibility to insult.

Although the mechanism(s) underlying the regional specificity of the effect remain largely unknown, the differential regulation of catecholamines within the basal ganglia is consistent with the phenotypic expression of hyperactivity in coloboma mice. Both the striatum and nucleus accumbens play a central role in the control of motor activity. Injection of DA agonists into the 6-OHDA-lesioned nucleus accumbens or specific lesions of the nucleus accumbens reproduced the hyperactivity and have implicated the nucleus accumbens as the site mediating this hyperactivity (Jackson et al., 1975; Joyce and Koob, 1981; Koob et al., 1981). The motor activity of rats following bilateral application of DA to nucleus accumbens increases the activity of normal as well as reserpine-treated rats (Costall and Naylor, 1975; Wachtel et al., 1979). Spontaneous hypertensive rats (SHR), a proposed model of hyperactivity, also show selective alterations in catecholaminergic function in the caudate putamen and nucleus accumbens (Russell et al., 1995).

Hyperactivity is most often associated with an increase in synaptic DA concentrations (Giros et al., 1996; Levy and Hobbles, 1988; Russell et al., 1995; Shaywitz et al., 1984). However, NE, not DA, was significantly increased in coloboma mice, while DA metabolites were significantly reduced in the nucleus accumbens with a similar trend observed in the striatum. Further, there was a clear trend toward reduced DA concentrations in both striatum and nucleus accumbens in coloboma mice. This is consistent with the observed reduction in L-DOPA accumulation in the striatum and nucleus accumbens, which likely reflects a reduction in DA synthesis because the vast majority of TH in the basal ganglia is present in dopaminergic terminals with only a small proportion contributed by noradrenergic terminals. Overall, these results suggest a decreased utilization of DA in contrast to the increase that might be expected with a hyperactive phenotype. The decreased DA utilization is consistent with previous findings suggesting a region-specific decrease in DA release (Raber et al., 1997). The dopaminergic reductions may actually result from the increase in NE, particularly in the nucleus accumbens where noradrenergic innervation is dense. Stimulation of presynaptic α_2-adrenergic receptors inhibits the release and synthesis of DA, presumably by acting through heteroreceptors located on dopaminergic terminals (de Villiers et al., 1995). Therefore, high levels of NE would lead to a decrease in DA utilization similar to that observed in the nucleus accumbens of coloboma mice. In fact, chronic

alteration in noradrenergic transmission in mice lacking the NE transporter produces changes in dopaminergic postsynaptic responses and enhanced locomotor behavior after administration of psychostimulants (Xu et al., 2000), consistent with the notion that an abnormal NE regulation may affect DA regulation.

The hyperactive phenotype is difficult to explain in light of the neurochemical data. Reduced or normal DA concentrations are most often associated with hypoactive or normoactive states, respectively (Koob et al., 1978, 1981; Zhou and Palmiter, 1995; Zigmond and Stricker, 1989). The role of NE in the regulation of locomotor activity is more obscure, but results from both animal models and human disorders suggest that it should not be overlooked, particularly in pathologic states (Segal and Mandell, 1970; Swerdlow and Koob, 1989; Trovero et al., 1992). In mice treated with reserpine to deplete monoamines, clonidine, a mixed α_1/α_2-adrenergic agonist, markedly potentiates locomotor activity (Pichler and Kobinger, 1981). Similarly, rats treated with 6-OHDA to deplete brain catecholamines exhibit a significant increase in locomotor activity in response to intracerebroventricular or intranucleus accumbens challenge with NE. In contrast, NE has no effect on locomotor activity in catecholamine-intact rats (Swerdlow and Koob, 1989). In these models, it is likely that the actions of clonidine and NE were postsynaptic, as autoreceptor regulation of synthesis and release was preempted by catecholamine depletion. It appears that DA deficits, and perhaps the accompanying DA receptor supersensitivity, are necessary to unmask the locomotor-activating properties of NE. In ADHD and Tourette's syndrome, both multifactorial disorders of hyperactivity, clonidine is an effective treatment in a subset of patients (Leckman et al., 1982, 1991; Silverstein et al., 1985). At the doses prescribed, it is thought that clonidine acts preferentially as an α_2-adrenergic agonist at autoreceptors to suppress noradrenergic function (Anden et al., 1976; Silverstein et al., 1985); a reduction in noradrenergic transmission appears to ameliorate hyperactivity in humans.

The results of the present experiments support the hypothesis that abnormal region-specific changes in monoaminergic transmission regulate the hyperactivity in the mutant mouse. The specific role of NE in hyperactivity can now be tested at the preclinical level using coloboma mice as a model.

Acknowledgments

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