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Adaptive Changes in Postsynaptic Dopamine Receptors Despite Unaltered Dopamine Dynamics in Mice Lacking Monoamine Oxidase B

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Abstract: Monoamine oxidase (MAO) B is considered a key enzyme in dopamine metabolism. The present studies, conducted in MAO B knockout mice, show that lack of MAO B does not alter extracellular levels of dopamine in striatum. Similarly, the synthesis, storage, uptake, and release of dopamine are also unaltered. However, autoradiography revealed a significant up-regulation of the D2-like dopamine receptors in the striatum of MAO B knockout mice. Mutant mice also exhibit a functional supersensitivity of D1-dopamine receptors in the nucleus accumbens. Thus, the agonist SKF 38,393-induced c-Fos immunoreactivity was significantly increased in knockout mice as compared with wild-type controls. In view of the apparently normal basal dopamine dynamics observed in MAO B knockout mice, we hypothesize that a dopamine-independent mechanism underlies adaptations in dopamine receptor function that occur as a consequence of MAO B depletion. Finally, these findings suggest that chronic administration of MAO inhibitors, as occurs in the treatment of Parkinson's disease and depression, may be associated with an increased responsiveness of CNS neurons to dopamine receptor ligands.
Key Words: Monoamine oxidase B—Dopamine receptor—Tyrosine hydroxylase—Knockout mice—In vivo microdialysis.
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Monoamine oxidases (MAOs) located in mitochondria metabolize monoamines and contribute to the maintenance of monoamine homeostasis (Youdim and Finberg, 1983, 1991). Biochemical and pharmacological studies have revealed two isoforms of MAO (A and B) (Squires, 1972; Youdim et al., 1988). MAO A preferentially oxidizes serotonin and norepinephrine, whereas MAO B specifically oxidizes β-phenylethylamine (PEA). Both isoforms can metabolize dopamine. Rodent brain shows greater MAO A than MAO B activity, but the reverse is true of human brain (Glover et al., 1977; Garrick and Murphy, 1980). Immunocytochemical studies using antisera against MAO A and B show a differential distribution in cells of the nervous system. MAO A is localized in catecholamine cell groups, particularly in locus ceruleus and substantia nigra. MAO B is expressed in raphe serotonergic neurons as well as in astrocytes and radial glia throughout the brain. By using MAO enzyme histochemistry in MAO A-deficient mice, Ikemoto et al. (1997) described additional MAO B-positive neurons in the striatum, septal nuclei, major island of Calleja, diagonal band, medial forebrain bundle, ventral pallidum, amygdaloid nucleus, thalamus, and hypothalamus.

As MAO B catalyzes the oxidative deamination of dopamine (Youdim and Finberg, 1983, 1991), it has been suggested that the therapeutic effect of selective MAO B inhibitors such as deprenyl in Parkinson's disease may be due to an elevation of dopamine levels within the CNS. However, preclinical data regarding the effects of MAO B inhibitors on dopamine levels are conflicting. Lamensdorf et al. (1996) found that blocking MAO B by either deprenyl or TVP-1012 (a selective MAO B inhibitor that lacks deprenyl's amphetamine-like effect) increased basal dialysate levels of dopamine in rat striatum. A similar finding was described after chronic but not acute administration of rasagiline, a selective MAO B inhibitor that is not metabolized to amphetamine-like derivatives (Finberg et al., 1996). Other laboratories (Butcher et al., 1990; Kaakkola and Wurtman, 1992;

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Abbreviations used: COMT, catechol-*O*-methyltransferase; DOPAC, dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; MAO, monoamine oxidase; 3-MT, 3-methoxytyramine; PEA, β-phenylethylamine; TH, tyrosine hydroxylase.

Brannan et al., 1995), however, found no alteration of dopamine levels following acute administration of deprenyl. Interestingly, studies by Mercuri et al. (1996, 1997) have shown that administration of MAO A or MAO B inhibitors alone caused no long-term prolongation of dopamine-induced electrophysiological responses (depression of firing rate and hyperpolarization) in rat midbrain dopaminergic cells and that the combined administration of MAO A and B inhibitors was required to elicit an effect. Such findings strongly suggest that the inhibition of both MAO A and B enzymes is necessary for the enhancement of dopamine neuronal activity.

In the current study, we have utilized mice genetically deficient in MAO B (Grimsby et al., 1997) to examine the influence of MAO B depletion upon dopamine neurotransmission. In contrast to pharmacological manipulations used in previous studies (Finberg et al., 1996; Lamensdorf et al., 1996), genetic inactivation of the MAO B gene ensures a long-term and complete elimination of the enzyme. Also, the knockout strategy employed provides a degree of enzyme specificity that cannot be achieved by using the "selective" MAO B blockers. It is known that near complete (>90%) blockade of MAO B by selective inhibitors is accompanied by a significant inhibition (10–40%) of MAO A (Lamensdorf et al., 1996). In MAO B knockout animals, however, MAO B was undetectable, whereas MAO A activity was at wild-type levels (Grimsby et al., 1997). Here we show that the complete lack of MAO B in mutant animals does not modify basal dopamine dynamics in striatum, as assessed by the no net flux method of quantitative microdialysis. Similarly, dopamine uptake and levels of dopamine metabolites are unaltered. However, D2-like dopamine receptors are significantly up-regulated in the striatum of MAO B-deficient animals. Furthermore, these animals exhibit D1-dopamine receptor supersensitivity in nucleus accumbens.

MATERIALS AND METHODS

All experiments were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and with an approved animal protocol from the Weill Medical College of Cornell University.

In vivo microdialysis

Male wild-type and MAO B-deficient mice (6–8 weeks old), both on the 129^{Sv} background (Grimsby et al., 1997), were anesthetized with ketamine (80 mg/kg) and xylazine (8.0 mg/kg) and placed in a stereotaxic apparatus equipped with a mouse adapter (David Kopf, Topanga, CA, U.S.A.) for insertion of a microdialysis guide cannula (CMA 11; CMA, Nagog, MA, U.S.A.) into the dorsal striatum. Stereotaxic coordinates were 0.5 mm anteroposterior, 2.3 mm lateral, and 2.3 mm ventral (Slotnick and Leonard, 1975). Four days after cannula implantation, a microdialysis probe (2-mm membrane, 6,000-mw cutoff; CMA 11) was inserted into the guide cannula. The animals were placed into Plexiglas test chambers, and the inlet tubing of the probe was connected to a microinfusion pump via a single-channel quartz-lined liquid swivel (Instech Laboratories, Plymouth Meeting, PA, U.S.A.). The probe was perfused overnight with filtered artificial cerebrospinal fluid (145 m*M* NaCl, 2.8 m*M* KCl, 1.2 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 0.25 m*M* ascorbate, and 5.4 m*M* D-glucose) at a flow rate of 0.3 μl/min. On the day of experiments, the flow rate of the probe was increased to 0.6 μl/min. After a 120-min equilibration period, perfusate samples were collected every 15 min. The basal extracellular concentration of dopamine was determined by the no net flux method of quantitative microdialysis (Lonnroth et al., 1987). Four concentrations of dopamine (0, 2, 10, and 20 n*M*) were added in random order to the dialysis perfusate to generate a series of points that were interpolated to measure the concentration at which no net flux of dopamine occurred across the dialysis membrane (Parsons and Justice, 1994). After a 30-min equilibration period at each concentration of dopamine (C_{in}), two 15-min dialysis samples were collected for determination of extracellular dopamine levels (DA_{ext}) and the extraction fraction (E_d) of the probe in vivo. Following completion of the no net flux experiments and a 60-min equilibration, six dialysate samples were collected for determination of basal dialysate levels of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), and 5-hydroxyindoleacetic acid (5-HIAA). Animals then received an intraperitoneal injection of the dopamine uptake inhibitor cocaine (15 mg/kg) or saline. Dialysate (5.0 μl) samples were collected for an additional 90 min.

Dialysate samples were analyzed using HPLC coupled to electrochemical detection. Chromatographic separations were performed with a microbore HPLC column (i.d.: 100 × 1 mm) packed with octadecylasilane (C_{18}) on microparticulate (3 μM) silica gel (MF-8949; BAS, West Lafayette, IN, U.S.A.) and a dual-piston pump (PM 80; BAS). The mobile phase consisted of 25 m*M* citrate, 25 m*M* NaH_2PO_4, 100 μM EDTA, 3.9 m*M* sodium octyl sulfate, 8% acetonitrile (vol/vol), 0.6% tetrahydrofuran (vol/vol), and an apparent pH of 3.6. The flow rate of the mobile phase was 100 μl/min. Electrochemical detection was accomplished using a BAS LC-4C amperometric detector. The applied potential was 0.700 V vs. Ag/AgCl. Output from the detector was recorded on a dual-pen chart recorder. Standard curves were constructed for each analyte and used to quantify concentrations in dialysate samples. The ranges of standards used for each analyte were as follows: DOPAC/HVA: 0–500 n*M*, dopamine/3-MT: 0–80 n*M*. Sensitivity was better than 100 n*M* for DOPAC and HVA, 50 n*M* for 5-HIAA, and 1 n*M* for dopamine and 3-MT.

Data analysis

Only data from animals with accurate probe placements as determined by postmortem histological analysis were included in the statistical analysis. Differences in basal extracellular dopamine concentrations between wild-type and mutant mice were estimated using the no net flux method of microdialysis. The amount of dopamine gained or lost from the dialysis probe was plotted as a function of the dopamine concentration added to the perfusate (C_{in}). Linear regression analysis was used to determine (1) DA_{ext}, which equals the C_{in} concentration at which there is no net flux of dopamine through the dialysis probe, and (2) extraction fraction (E_d), which is the slope of the generated line and, in the case of dopamine, has been shown empirically to provide an estimate of dopamine uptake (Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994). Two-way ANOVAs were used to compare DA_{ext} and E_d between wild-type and MAO B-deficient mice. Metabolite dialysate levels during the no net flux determination of dopamine were also measured. The effects of cocaine on dopamine and me-

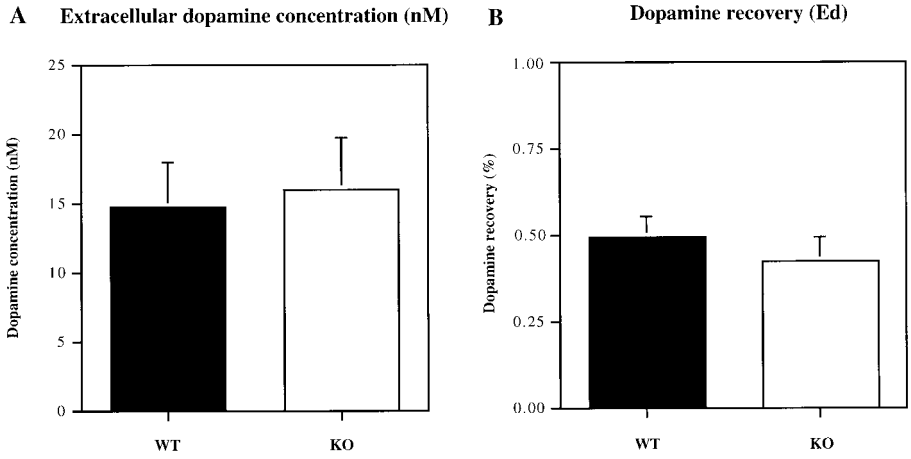

FIG. 1. A: Extracellular dopamine concentration in dorsal striatum of wild-type (WT) and MAO B knockout (KO) mice as measured by the no net flux method of quantitative in vivo microdialysis. **B:** Extraction fraction of the probe in vivo.

tabolites were analyzed over time, thus necessitating the use of a three-factor ANOVA (genotype × challenge × time). The Student–Newman–Keuls test was used for pairwise comparisons. Statistical significance was assumed at $p < 0.05$. The Greenhouse–Geisser correction for multiple time measures was employed, when appropriate.

Dopamine receptor and transporter autoradiography

Mice were killed and their brains were quickly removed, frozen on powdered dry ice, and stored at −70°C. Frozen brains were sectioned to 20-μm thickness, thaw mounted onto gelatin-coated slides, dried, and stored desiccated at −70°C.

Dopamine transporter binding was carried out based on previously published protocols (Boja et al., 1995; Pilotte et al., 1996). In brief, sections were incubated for 60 min at room temperature with 15 pmol of [^{125}I]RTI-121 (2,200 Ci/mmol; DuPont NEN, Boston, MA, U.S.A.), in 10 m*M* phosphate-buffered saline (pH 7.4) with 10 m*M* NaI added to reduce nonspecific binding. Nonspecific binding was measured in the presence of 50 μM cocaine. After the incubation with the radioligand, sections were washed three times for 20 min each in phosphate-buffered saline/NaI at 4°C and dipped into ice-cold distilled water.

D1-Dopamine receptors were labeled with [^{3}H]SCH 23390 (70.7 Ci/mmol; DuPont NEN) as described (Knable et al., 1994). In brief, sections were preincubated in binding buffer containing 20 m*M* Tris-HCl (pH 7.4), 120 m*M* NaCl, and 2 m*M* $MgCl_2$ at 4°C for 5 min. Total binding was determined by incubating sections in binding buffer containing 2 n*M* [^{3}H]SCH 23390 and 20 n*M* mianserin (to block 5-HT_{2A} and 5-HT_{2C} receptors) at 4°C for 30 min. Slides were rinsed twice for 10 min each in binding buffer and dipped in distilled water. Nonspecific binding was determined in the presence of 10 μM (+)-butaclamol.

D2-Dopamine receptor autoradiography was performed as described previously (Yokoyama et al., 1995). Sections were preincubated in binding buffer containing 50 m*M* Tris-HCl (pH 7.6), 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$ for 5 min at room temperature. Then, sections were incubated in binding buffer containing 0.5 n*M* [^{3}H]YM-09151-2 (85.5 Ci/mmol; DuPont NEN) for 2 h to label D2 receptors. Nonspecific binding was defined by adding 10 μM (+)-butaclamol. Sections were washed twice (10 min each) and dipped in ice-cold distilled water.

Sections were dried under a stream of cool, dry air and apposed to ^{3}H-Hyperfilm (Amersham) together with either ^{125}I-polymeric or tritium-impregnated acrylic standards (Amersham). Exposure time for the dopamine transporter was 2–3 days at room temperature, whereas for the D1 and D2 binding, it was 5 weeks at 4°C. Autoradiographic films were developed using photographic chemicals from Eastman Kodak. Quantification of the images was obtained by using the MCID image analysis system (Imaging Research, St. Catharines, Canada). ANOVA was used to analyze the effects of genotype on each brain region followed by Fisher's PLSD test for post hoc comparison. Differences were considered to be significant at $p < 0.05$.

c-Fos and tyrosine hydroxylase (TH) immunohistochemistry

The assay was performed as previously described (Weiser et al., 1993). In brief, 2 h after drug injection, animals were anesthetized with Nembutal (150 mg/kg) and perfused intracardially with 4% paraformaldehyde. Free-floating sections (40 μm) were incubated with a rabbit c-Fos antiserum (Fos and related antigens, 1:8,000 dilution; Cambridge Research) or a rabbit TH antiserum (1:25,000 dilution) (Min et al., 1994). The antigen was visualized with the ABC Vector Elite kit. Immunoreactive nuclei were counted with a bright-field microscope at 10× magnification. The average number of positive nuclei per field (0.02 mm^2) was calculated from various brain regions from three adjacent sections of each mouse. Data analysis was by independent *t* test. Differences were considered to be significant at $p < 0.05$.

RESULTS

Basal dopamine dynamics in mice lacking MAO B

In our previous study (Grimsby et al., 1997), tissue levels of dopamine did not differ in the CNS of wild-type and MAO B-deficient mice. However, these measures reflect total tissue dopamine content and do not preclude changes in extracellular dopamine levels or dopamine uptake. Therefore, the no net flux method of quantitative microdialysis was used to assess basal dopamine dynamics in MAO B mutant animals. Figure 1A shows that the basal extracellular concentration of dopamine in dorsal striatum did not differ in wild-type and knockout animals. Extracellular levels were 15.9 ± 3.8 n*M* (MAO B

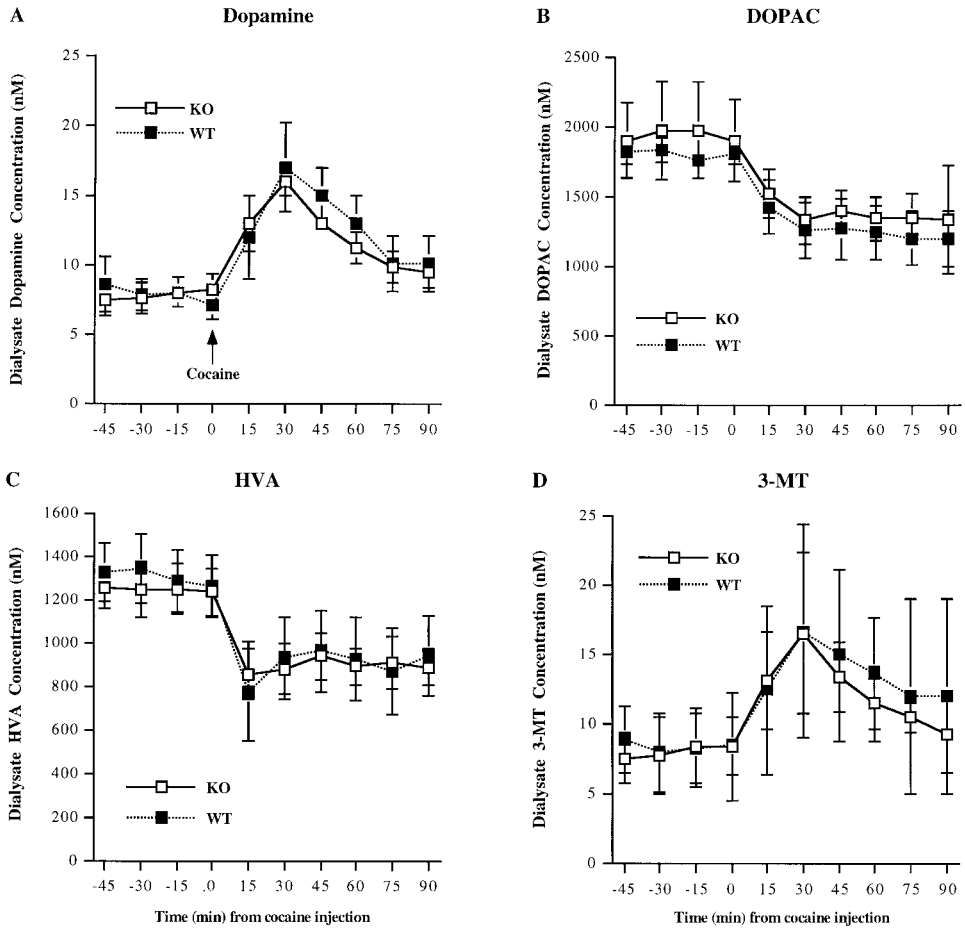

FIG. 2. Dopamine (**A**), DOPAC (**B**), HVA (**C**), and 3-MT (**D**) concentrations in striatal dialysate of wild-type (WT) and MAO B knockout (KO) mice before and after an intraperitoneal injection of 15 mg/kg cocaine.

knockout, n = 6) and 14.7 ± 3.3 n*M* (wild type, n = 6). Similarly, the E_d, which has previously been shown to provide an index of dopamine uptake (Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994), did not differ in mutant and wild-type mice (Fig. 1B).

Conventional microdialysis techniques were used to examine the influence of acute blockade of the dopamine transporter by cocaine (15 mg/kg i.p.) upon dialysate levels of dopamine and its metabolites in dorsal striatum (Fig. 2B–D). At baseline, no difference in the levels of dopamine and its metabolites was seen between wild-type and mutant animals. The acute administration of cocaine resulted in an approximately twofold increase in dialysate dopamine levels in both wild-type and MAO B mutant mice. There was no difference between genotypes in the magnitude of this effect. As expected, blocking the reuptake of dopamine decreased dialysate levels of DOPAC and HVA (produced by MAO intracellularly) and increased levels of the dopamine metabolite 3-MT [produced extracellularly by catechol-*O*-methyltransferase (COMT)] (Kopin, 1985; Trendelenburg, 1990; Mannisto et al., 1992) (Fig. 2B–D). No difference in the levels of these metabolites was found between wild-type and mutant animals (Fig. 2B–D).

TH immunoreactivity is unchanged in MAO B knockout mice

As TH is the rate-limiting enzyme in dopamine synthesis, changes in dopaminergic function are often accompanied by alterations in TH levels (Giros et al., 1996; Fukamauchi et al., 1997). Comparison of TH immunoreactivity in dorsal striatum, substantia nigra, ventral tegmental area, and olfactory tubercle of wild-type and MAO B knockout mice revealed no genotype difference (data not shown). This finding is consistent with the normal dopamine dynamics of MAO B knockout mice.

Characteristics of dopamine transporter in mice lacking MAO B

To further examine the dopamine system in MAO B-deficient animals, we studied if the level of the dopamine transporter was altered in knockout mice. Evaluation of [^{125}I]RTI-121 binding in striatum, nucleus accumbens, and olfactory tubercle showed no difference in dopamine transporter levels in MAO B knockout and

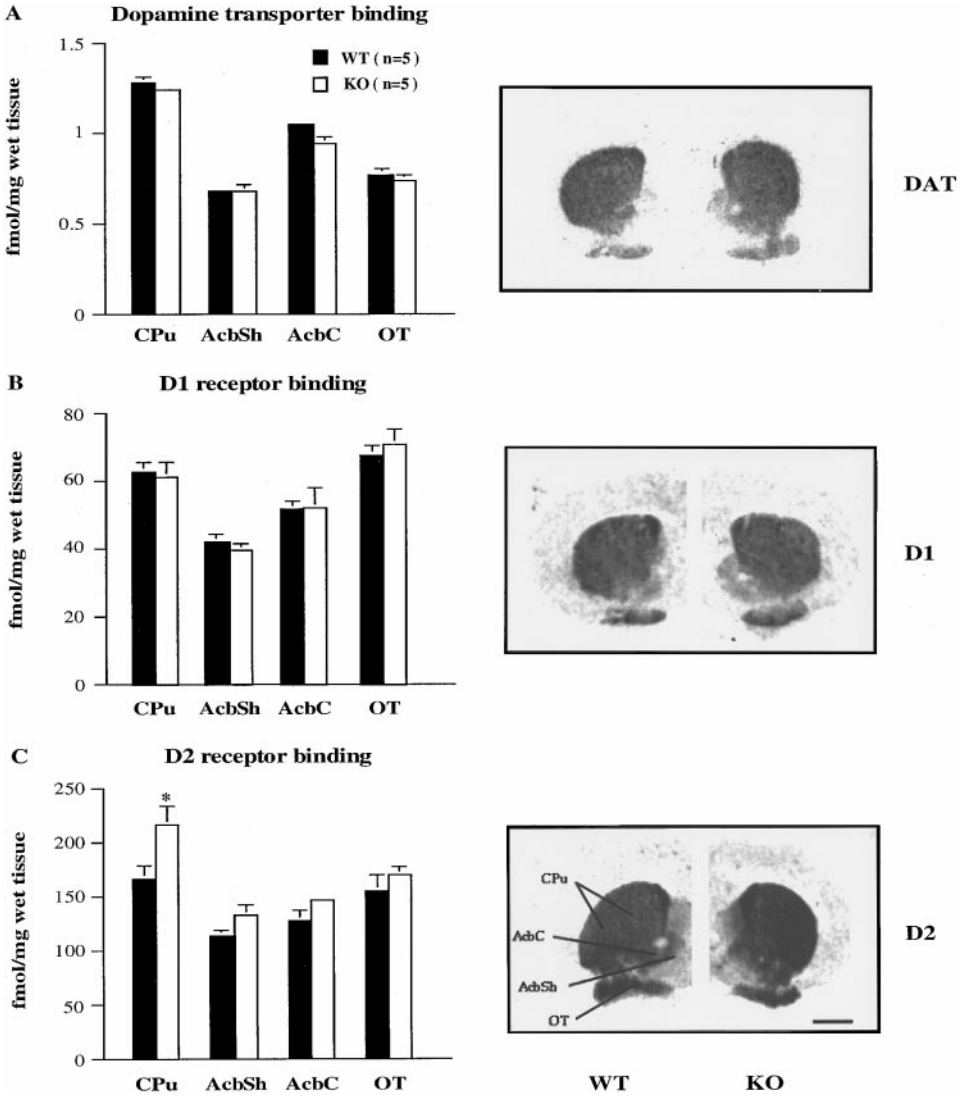

FIG. 3. Determination of dopamine transporter binding using [^{125}I]RTI-121 (**A**), D1-receptor binding using [^{3}H]SCH 23390 (**B**), and D2-like receptor binding using [^{3}H]YM-09151-2 (**C**) by autoradiography. **Left:** Means of specific binding in five wild-type (WT) and five knockout (KO) animals in striatum (caudate–putamen; CPu), nucleus accumbens shell (AcbSh), nucleus accumbens core (AcbC), and olfactory tubercle (OT). *$p < 0.05$. **Right:** Representative coronal sections showing total binding in CPu, AcbSh, AcbC, and OT from WT and KO animals. Bar = 1 mm.

wild-type animals (Fig. 3A). This finding is also consistent with the normal dopamine dynamics found in knockout mice (Figs. 1 and 2).

Increased D2-like but not D1-receptor levels in MAO B knockout mice

The density of D1-like receptors, as measured by [^{3}H]SCH 23390 binding, was normal in knockout mice (Fig. 3B). However, levels of D2-like receptors (D2, D3, and D4), measured by [^{3}H]YM-09151-2, were increased by ~30% ($p = 0.03$, t test) in striatum (Fig. 3C). A smaller increase (17%) in D2-like receptor binding in nucleus accumbens shell was also observed, but it did not reach statistical significance ($p = 0.07$). D2-like receptors, representing somatodendritic autoreceptors, are also located in the ventral midbrain (substantia nigra and ventral tegmental area). In contrast to striatum, receptor autoradiography showed no difference in D2-like autoreceptor density between knockout and wild-type mice in the substantia nigra pars compacta (119.5 ± 26.2 and 101.8 ± 8.7 fmol/mg of wet tissue in knockout versus wild-type mice, n = 5 animals, three slices per animal), substantia nigra pars reticulata (47.5 ± 11.7 and 49.1 ± 8.4 fmol/mg of wet tissue in knockout versus wild-type mice, n = 5 animals, three slices per animal), and ventral tegmental area (103.0 ± 12.9 and 87.1 ± 12.2 fmol/mg of wet tissue in knockout versus wild-type mice, n = 5 animals, three slices per animal).

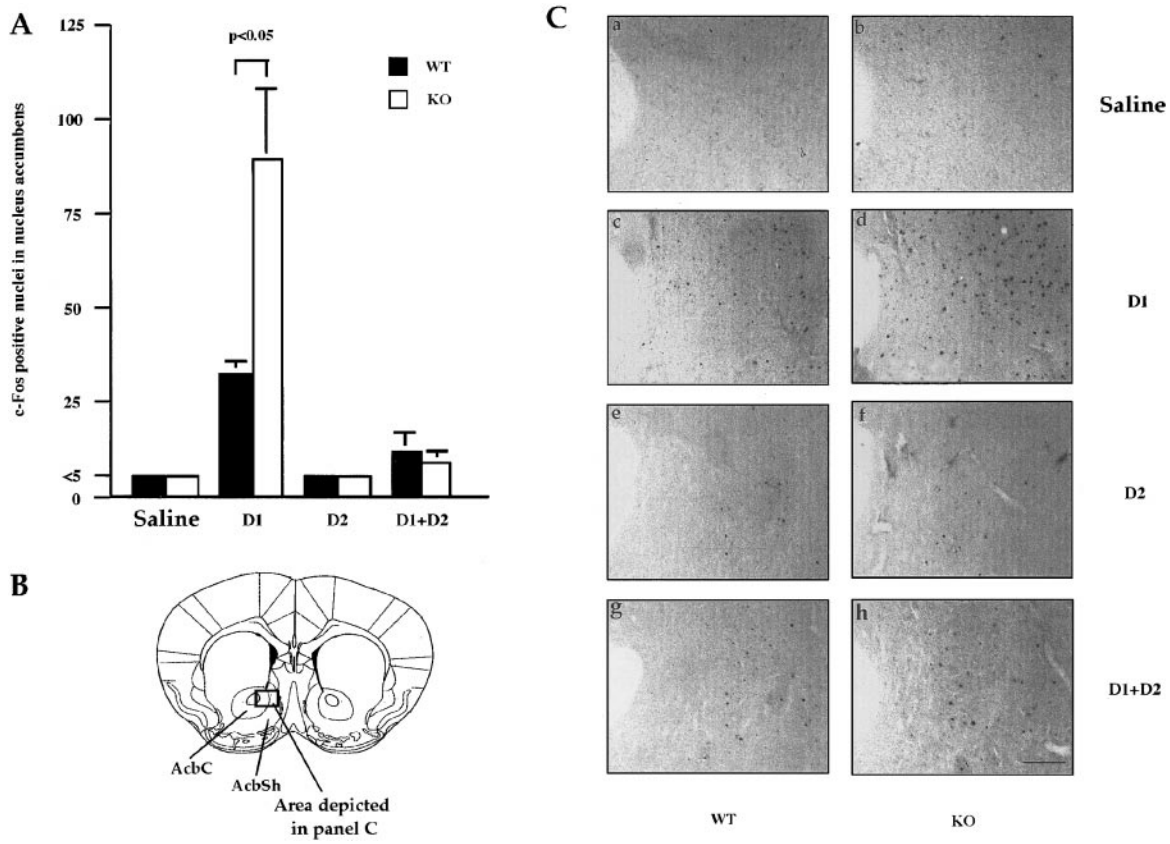

FIG. 4. c-Fos expression in the nucleus accumbens of wild-type (WT) and MAO B knockout (KO) mice following intraperitoneal injection of saline, 8 mg/kg SKF 38,393, a selective D1 agonist (D1), 1 mg/kg quinpirole, a D2 agonist (D2), and 8 mg/kg SKF 38,393 and 1 mg/kg quinpirole together (D1 + D2) (WT, n = 6; KO, n = 6). **A:** Number of c-Fos-positive nuclei in nucleus accumbens. **B:** Region within nucleus acumbens displayed in C. **C:** Representative sections from individual WT and KO mice treated with the drugs. AcbC, nucleus accumbens core; AcbS, nucleus accumbens shell. Bar = 1 μm.

Functional D1-receptor supersensitivity in nucleus accumbens of MAO B mutant mice

As a further step in characterizing the dopamine system, we probed the function of the D1 receptor and its signaling in MAO B-deficient animals by measuring the level of c-Fos immunoreactivity following agonist stimulation. Administration of the selective D1-agonist SKF 38,393 (8 mg/kg i.p.) resulted in approximately three times more c-Fos-immunopositive cells in the nucleus accumbens of mutant mice than in wild-type animals (Fig. 4). In agreement with published data, SKF 38,393 failed to induce c-Fos in striatum and olfactory tubercle (Arnauld et al., 1993). The D2-agonist quinpirole (1 mg/kg) did not induce c-Fos. Rather, quinpirole caused a suppression of the D1 agonist-induced c-Fos response and resulted in near baseline c-Fos levels in nucleus accumbens of both wild-type and MAO B mutant animals (Fig. 4).

DISCUSSION

The no net flux dialysis study demonstrates that basal extracellular levels of dopamine do not differ in the dorsal striatum of MAO B knockout mice and their wild-type littermate controls (Figs. 1 and 2A). These studies also show that dopamine uptake is virtually identical in wild-type and knockout mice (Fig. 1B). Previous studies employing the no net flux method of quantitative microdialysis have shown that the E_d of the microdialysis probe provides an estimate of dopamine clearance by the dopamine transporter (Parsons and Justice, 1994). Consistent with the unaltered uptake in MAO B knockout mice, the density of the dopamine transporter measured by autoradiography was also unchanged in these animals (Fig. 3A). Taken together, these findings strongly suggest that there is no disruption of presynaptic dopamine neuronal function in MAO B-depleted mice.

In addition, no change was found between MAO B knockout and wild-type mice in the basal dialysate levels of DOPAC, HVA, and 3-MT (Fig. 2B–D). DOPAC and 3-MT are dopamine metabolites produced by MAO and COMT, respectively. HVA is produced by MAO and COMT from 3-MT and DOPAC, respectively. These data demonstrate that COMT does not compensate for the loss of MAO B and that MAO A alone is sufficient to metabolize dopamine. Consistent with the normal

dopamine dynamics of MAO B knockout mice, TH immunoreactivity in regions comprising the mesostriatal (striatum and substantia nigra) and mesoaccumbens (ventral tegmental area) dopamine systems of wild-type and MAO B knockout mice revealed no genotype difference. As immunocytochemistry assesses protein levels, we cannot exclude a change in TH enzyme activity in MAO B knockout animals. However, in light of the normal dopamine dynamics, a change in TH enzyme activity in MAO B knockout mice is unlikely.

The finding that dopamine levels are unaltered in the absence of MAO B is in accord with several studies that have used a pharmacological approach to probe the role of MAO B in dopamine neurotransmission (Butcher et al., 1990; Kaakkola and Wurtman, 1992; Brannan et al., 1995; Mercuri et al., 1996). However, increases in dialysate dopamine levels following MAO B inhibition have also been reported (Finberg et al., 1996; Lamensdorf et al., 1996). These disparate findings may be due to the limited selectivity of MAO B inhibitors used in the previous studies. Thus, a near complete blockade of MAO B cannot be achieved unless MAO A is inhibited to a certain degree. For example, in the study of Lamensdorf et al. (1996), a 90% inhibition of MAO B by deprenyl and TVP-1012 was also associated with 15 and 40% reductions in MAO A, respectively. As dopamine can be catabolized by both MAO A and MAO B, the drug effect on both isoforms could produce the increase in the extracellular level of dopamine. Indeed, consistent with this hypothesis, electrophysiological studies by Mercuri et al. (1996, 1997) have shown that the administration of both MAO A and MAO B inhibitors is required for the long-term prolongation of the dopamine-induced responses in rat midbrain dopaminergic cells.

Only one other study (Jones et al., 1998) has characterized basal extracellular dopamine levels in mice. Dialysate dopamine levels in dorsal striatum of wild-type mice were approximately two times lower than those observed in the present study. In this regard, however, it is important to note that mice with different genetic backgrounds were used in these two studies. Jones et al. (1998) used the mixed genetic background C57BL/129^{Sv}, whereas MAO B knockout mice were bred on the pure 129^{Sv} background. Pharmacogenetic studies (Bosy and Ruth, 1989; Erwin et al., 1993) in inbred strains of mice have revealed genotype-dependent differences in basal and drug-evoked dopamine neurotransmission. Evidence that basal dopamine uptake in the striatum differs significantly in C57BL and 129^{Sv} strains of mice has also recently been obtained (M. He and T. Shippenberg, unpublished results). Therefore, strain-related differences in basal dopamine dynamics may underlie the different values obtained in these two studies.

The present findings do not exclude the possibility that MAO B has some role in regulating dopamine levels in humans. It has been suggested that MAO B has a greater contribution than MAO A in dopamine metabolism in human compared with mouse (Garrick and Murphy, 1980; O'Carroll et al., 1983). However, patients with the deletion of the MAO B gene (atypical Norrie disease) have normal levels of dopamine metabolites in plasma (Lenders et al., 1996). Only when both MAO A and B genes are deleted (classic Norrie disease) are abnormal levels of dopamine metabolites seen in plasma. This suggests that lack of MAO B alone does not cause significant changes in dopamine turnover in humans either. As MAO B deficit does not elicit a compensatory up-regulation of MAO A in either mice (Grimsby et al., 1997) or humans (Lenders et al., 1996), it is likely that the available level of MAO A is generally sufficient to maintain a physiological rate of dopamine catabolism.

Although dopamine dynamics appeared normal, receptor autoradiography studies showed an increase in D2-like receptor binding to [^{3}H]YM-09151-2 in striatum of MAO B knockout mice. [^{3}H]YM-09151-2 labels D2-like receptors localized both presynaptically and postsynaptically in the striatum. The absence of significant changes in dopamine synthesis, release, and extracellular dopamine levels, which are known to be modified by dopamine autoreceptors (Saller and Salama, 1984; El Mestikawy and Hamon, 1986; Strait and Kuczenski, 1986; Goldstein et al., 1990), suggests no alteration in presynaptic D2 receptors. Presynaptic D2-like autoreceptors are located not only at dopaminergic terminals in the striatum but also in the somatodendritic compartment of dopamine neurons in the ventral midbrain. We could directly measure the density of these somatodendritic D2-like autoreceptors in the ventral midbrain (substantia nigra pars compacta, substantia nigra pars reticulata, and ventral tegmental area) by receptor autoradiography. This study showed no difference in receptor density between knockout and wild-type mice. Taken together, these studies suggest that the increased [^{3}H]YM-09151-2 binding in the striatum of MAO B mutant animals is probably due to an up-regulation of D2-like receptors at the postsynaptic site.

No change in D1-receptor number was measured in MAO B-deficient mice as compared with wild-type animals. However, MAO B mutant mice displayed an increased c-Fos response to the D1-receptor agonist SKF 38,393 in nucleus accumbens, indicating a D1-receptor supersensitivity. The importance of the receptor changes in the dopamine system of MAO B knockout mice is that similar changes could occur during chronic administration of nonselective MAO inhibitors or selective MAO B blockers, such as in depression and Parkinson's disease. These receptor changes in turn could result in an altered responsiveness to dopaminergic drugs. As MAO B deficiency apparently does not alter dopamine dynamics, the changes in receptor density and function are likely induced by a dopamine-independent mechanism. The only MAO substrate that shows abnormally high levels in MAO B knockout mice is PEA, raising the possibility that it is responsible for the up-regulation of D2-like receptors and the supersensitization of D1 receptors.

PEA is co-synthesized with dopamine (from L-phenylalanine) by aromatic L-amino acid decarboxylase, the enzyme that catalyzes the conversion of L-DOPA to

dopamine (Juorio, 1988; Paterson et al., 1990). In the presence of MAO B, the half-life of PEA is very short (20 s) (Durden and Philips, 1980), and the concentration of this trace amine is ~20 n*M* in brain. In the absence of MAO B, PEA's half-life is increased and its level reaches 0.1–0.2 μM (Grimsby et al., 1997). PEA is not stored in vesicles and its release is not regulated. However, PEA is highly lipophilic, so there is a buildup of the extracellular concentration of this trace amine in the absence of MAO B. Because it is produced by dopaminergic cells, PEA (when its catabolism is impaired) could reach especially high concentrations in the dopamine system, including striatum and nucleus accumbens, and high PEA levels may induce receptor changes in these brain areas. How may PEA elicit changes in dopamine receptors? High concentrations of PEA are known to have amphetamine-like behavioral effects (Sabelli et al., 1975; Risner and Jones, 1977; Vaupel et al., 1978). This suggests that PEA, like amphetamine, could alter dopamine efflux via the dopamine transporter that may indirectly cause abnormalities in dopamine receptor function. However, PEA, up to 10^{-3} *M*, had no effect on dopamine release in a cell line overexpressing the human dopamine transporter (L. Chen and M. Toth, unpublished data). This result is in agreement with the unaltered dopamine dynamics of MAO knockout mice. Another possibility is that PEA acts on the D1-dopamine receptor as an antagonist, causing receptor supersensitivity. Antagonists often induce disuse supersensitivity, a state characterized by an increased receptor response to agonists. Although PEA is similar in structure to dopamine, it is not likely an antagonist on the D1 receptor. Our experiments with a cell line overexpressing the D1-dopamine receptor show that PEA has no effect on the dopamine-induced activation of adenyl cyclase (the enzyme functionally linked to the D1 receptor) (L. Chen and M. Toth, unpublished data). PEA alone did not activate adenyl cyclase. Finally, PEA could act on a specific receptor to modulate an intracellular signaling pathway, leading to changes in dopamine receptors. However, membrane receptors for PEA have not been identified yet. Alternatively, PEA may modulate an intracellular signaling pathway directly because it is lipophilic and thus can enter cells readily.

REFERENCES

Arnauld E., Arsaut J., and Demotes-Mainard J. (1993) Functional heterogeneity of the caudate–putamen as revealed by c-fos induction in response to D1 receptor activation. *Brain Res. Mol. Brain Res.* **18,** 339–342.

Boja J. W., Cadet J. L., Kopajtic T. A., Lever J., Seltzman H. H., Wyrick C. D., Lewin A. H., Abraham P., and Carroll F. I. (1995) Selective labeling of the dopamine transporter by the high affinity ligand 3β-(4-[^{125}I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester. *Mol. Pharmacol.* **47,** 779–786.

Bosy T. Z. and Ruth J. A. (1989) Differential inhibition of synaptosomal accumulation of [^{3}H]-monoamines by cocaine, tropococaine and amphetamine in four inbred strains of mice. *Pharmacol. Biochem. Behav.* **34,** 165–172.

Brannan T., Prikhojan A., Martinez-Tica J., and Yahr M. D. (1995) In vivo comparison of the effects of inhibition of MAO-A versus MAO-B on striatal L-DOPA and dopamine metabolism. *J. Neural Transm.* **10,** 79–89.

Bungay P. M., Morrison P. F., and Dedrick R. L. (1990) Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sci.* **46,** 105–119.

Butcher S. P., Fairbrother I. S., Kelly J. S., and Arbuthnott G. W. (1990) Effects of selective monoamine oxidase inhibitors on the in vivo release and metabolism of dopamine in the rat striatum. *J. Neurochem.* **55,** 981–988.

Durden D. A. and Philips S. R. (1980) Kinetic measurements of the turnover rates of phenylethylamine and tryptamine *in vivo* in the rat brain. *J. Neurochem.* **34,** 1725–1732.

El Mestikawy S. and Hamon M. (1986) Is dopamine-induced inhibition of adenylate cyclase involved in the autoreceptor-mediated negative control of tyrosine hydroxylase in striatal dopaminergic terminals? *J. Neurochem.* **47,** 1425–1433.

Erwin V. G., Womer D. E., Campbell A. D., and Jones B. C. (1993) Pharmacogenetics of cocaine II. Mesocorticolimbic and striatal dopamine and cocaine receptors in C57BL and DBA mice. *Pharmacogenetics* **3,** 189–196.

Finberg J. P., Lamensdorf I., Commissiong J. W., and Youdim M. B. (1996) Pharmacology and neuroprotective properties of rasagiline. *J. Neural Transm. Suppl.* **48,** 95–101.

Fukamauchi F., Mataga N., Wang Y. J., Sato S., Yoshiki A., and Kusakabe M. (1997) Tyrosine hydroxylase activity and its mRNA level in dopaminergic neurons of tenascin gene knockout mice. *Biochem. Biophys. Res. Commun.* **231,** 356–359.

Garrick N. A. and Murphy D. L. (1980) Species differences in the deamination of dopamine and other substrates for monoamine oxidase in brain. *Psychopharmacology (Berl.)* **72,** 27–33.

Giros B., Jaber M., Jones S. R., Wightman R. M., and Caron M. G. (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379,** 606–612.

Glover V., Sandler M., Owen F., and Riley G. J. (1977) Dopamine is a monoamine oxidase B substrate in man. *Nature* **265,** 80–81.

Goldstein M., Harada K., Meller E., Schalling M., and Hokfelt T. (1990) Dopamine autoreceptors: biochemical, pharmacological, and morphological studies. *Ann. NY Acad. Sci.* **604,** 169–175.

Grimsby J., Toth M., Chen K., Kumazawa T., Klaidman L., Adams J. D., Karoum F., Gal J., and Shih J. C. (1997) Increased stress response and beta-phenylethylamine in MAOB-deficient mice. *Nat. Genet.* **17,** 206–210.

Ikemoto K., Kitahama K., Seif I., Maeda T., De Maeyer E., and Valatx J. L. (1997) Monoamine oxidase B (MAOB)-containing structures in MAOA-deficient transgenic mice. *Brain Res.* **771,** 121–132.

Jones S. R., Gainetdinov R. R., Jaber M., Giros B., Wightman R. M., and Caron M. G. (1998) Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc. Natl. Acad. Sci. USA* **95,** 4029–4034.

Juorio A. V. (1988) Brain β-phenylethylamine: localization, pathways, and interrelation with catecholamines, in *Progress in Catecholamine Research, Vol. 42B: Central Aspects. Neurology and Neurobiology* (Sandler M., Dahlstrom A., and Belmaker R. H., eds), pp. 433–437. Liss, New York.

Justice J. B. Jr. (1993) Quantitative microdialysis of neurotransmitters. *J. Neurosci. Methods* **48,** 263–276.

Kaakkola S. and Wurtman R. J. (1992) Effects of COMT inhibitors on striatal dopamine metabolism: a microdialysis study. *Brain Res.* **587,** 241–249.

Knable M. B., Hyde T. M., Egan M. F., Tosayali M., Wyatt R. J., and Kleinman J. E. (1994) Quantitative autoradiography of striatal dopamine D1, D2 and reuptake sites in rats with vacuous chewing movements. *Brain Res.* **646,** 217–222.

Kopin I. J. (1985) Catecholamine metabolism: basic aspects and clinical significance. *Pharmacol. Rev.* **37,** 333–364.

Lamensdorf I., Youdim M. B. H., and Finberg J. P. M. (1996) Effect of long-term treatment with selective monoamine oxidase A and B inhibitors on dopamine release from rat striatum in vivo. *J. Neurochem.* **67,** 1532–1539.

Lenders J. W., Eisenhofer G., Abeling N. G., Berger W., Murphy D. L., Konings C. H., Wagemakers L. M., Kopin I. J., Karoum F., van

Gennip A. H., and Brunner H. G. (1996) Specific genetic deficiencies of the A and B isoenzymes of monoamine oxidase are characterized by distinct neurochemical and clinical phenotypes. *J. Clin. Invest.* **97,** 1010–1019.

Lonnroth P., Jansson P. A., and Smith U. (1987) A microdialysis method allowing characterization of intercellular water space in humans. *Am. J. Physiol.* **253,** E228–E231.

Mannisto P. T., Ulmanen I., Lundstrom K., Taskinen J., Tenhunen J., Tilgmann C., and Kaakkola S. (1992) Characteristics of catechol *O*-methyltransferase (COMT) and properties of selective COMT inhibitors. *Prog. Drug Res.* **39,** 291–350.

Mercuri N. B., Bonci A., Siniscalchi A., Stefani A., Calabresi P., and Bernardi G. (1996) Electrophysiological effects of monoamine oxidase inhibition on rat midbrain dopaminergic neurones: an in vitro study. *Br. J. Pharmacol.* **117,** 528–532.

Mercuri N. B., Scarponi M., Bonci A., Siniscalchi A., and Bernardi G. (1997) Monoamine oxidase inhibition causes a long-term prolongation of the dopamine-induced responses in rat midbrain dopaminergic cells. *J. Neurosci.* **17,** 2267–2272.

Min N., Joh T. H., Kim K. S., Peng C., and Son J. H. (1994) 5′ upstream DNA sequence of the rat tyrosine hydroxylase gene directs high-level and tissue-specific expression to catecholaminergic neurons in the central nervous system of transgenic mice. *Mol. Brain Res.* **27,** 281–289.

O'Carroll A. M., Fowler C. J., Phillips J. P., Tobbia I., and Tipton K. F. (1983) The deamination of dopamine by human brain monoamine oxidase. Specificity for the two enzyme forms in seven brain regions. *Naunyn Schmiedebergs Arch. Pharmacol.* **322,** 198–202.

Parsons L. H. and Justice J. B. Jr. (1994) Quantitative approaches to in vivo brain microdialysis. *Crit. Rev. Neurobiol.* **8,** 189–220.

Paterson I. A., Juorio A. V., and Boulton A. A. (1990) 2-Phenylethylamine: a modulator of catecholamine transmission in the mammalian central nervous system? *J. Neurochem.* **55,** 1827–1837.

Pilotte N. S., Sharpe L. G., Rountree S. D., and Kuhar M. J. (1996) Cocaine withdrawal reduces dopamine transporter binding in the shell of the nucleus accumbens. *Synapse* **22,** 87–92.

Risner M. E. and Jones B. E. (1977) Characteristics of β-phenethylamine self-administration by dog. *Pharmacol. Biochem. Behav.* **6,** 689–696.

Sabelli H. C., Vazquez A. J., and Flavin D. (1975) Behavioral and electrophysiological effects of phenethanolamine and 2-phenethylamine. *Psychopharmacologia* **42,** 117–125.

Saller C. F. and Salama A. I. (1984) Dopamine synthesis in synaptosomes: relation of autoreceptor functioning to pH, membrane depolarization, and intrasynaptosomal dopamine content. *J. Neurochem.* **43,** 675–688.

Slotnick B. M. and Leonard C. M. (1975) *A Stereotaxic Atlas of the Albino Mouse Forebrain.* DHEW Publication, pp. 75–100. U.S. Government Printing Office, Rockville.

Squires R. F. (1972) Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: a comparison of eight mammalian species. *Adv. Biochem. Psychopharmacol.* **5,** 355–370.

Strait K. A. and Kuczenski R. (1986) Dopamine autoreceptor regulation of the kinetic state of striatal tyrosine hydroxylase. *Mol. Pharmacol.* **29,** 561–569.

Trendelenburg U. (1990) The interaction of transport mechanisms and intracellular enzymes in metabolizing systems. *J. Neural. Transm. Suppl.* **32,** 3–18.

Vaupel D. B., Nozaki M., Martin W. R., and Bright L. D. (1978) Single dose and cross tolerance studies of β-phenethylamine, α-amphetamine and LSD in the chronic spinal dog. *Eur. J. Pharmacol.* **48,** 431–437.

Weiser M., Baker H., Wessel T. C., and Joh T. H. (1993) Differential spatial and temporal gene expression in response to axotomy and deafferentation following transection of the medial forebrain bundle. *J. Neurosci.* **13,** 3472–3484.

Yokoyama C., Okamura H., and Ibata Y. (1995) Dopamine D2-like receptors labeled by [^{3}H]YM-09151-2 in the rat hippocampus: characterization and autoradiographic distribution. *Brain Res.* **681,** 153–159.

Youdim M. B. H. and Finberg J. P. M. (1983) Monoamine oxidase inhibitors, in *Psychopharmacology, Part 1: Prelinical Psychopharmacology* (Grahame-Smith D. G. and Cowen P. E., eds), pp. 37–51. Excerpta Medica, Amsterdam.

Youdim M. B. H. and Finberg J. P. M. (1991) New directions in monoamine oxidase A and B selective inhibitors and substrates. *Biochem. Pharmacol.* **41,** 155–162.

Youdim M. B. H., Finberg J. P. M., and Tipton K. F. (1988) Monoamine oxidase, in *Handbook of Experimental Pharmacology, Vol. 90* (Trendelenburg U. and Weiner N., eds), pp. 119–192. Springer, Berlin.