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Increased Dopamine Synthesis in Aging Substantia Nigra Neurons

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GREENWOOD, C. E., W. G. TATTON, N. A. SENIUK AND F. G. BIDDLE. *Increased dopamine synthesis in aging substantia nigra neurons.* NEUROBIOL AGING **12**(5) 557–565, 1991.—Striatal dopamine (DA) and metabolite (DOPAC) levels in 8-, 21-, 52- and 104-week-old C57BL mice were compared with those in 11-week-old mice, 20 days after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. DA and DOPAC concentrations expressed relative to striatal wet weight did not change with age. In contrast, DA and DOPAC levels increased almost linearly when values were expressed relative to the proportion of remaining tyrosine hydroxylase-positive (TH+) SNc neurons, reaching a 5–7-fold increase per average remaining TH+ neuron by 104 weeks of age (corresponding to neuronal loss of 70%) relative to that found per average neuron in 8-week-old mice. DA and DOPAC levels per average remaining TH+ SNc neuron following MPTP increased for low doses (neuronal losses less than 42%) but decreased for higher doses (55 and 70% losses) but the DOPAC/DA ratio per SNc neuron increased and was 9-fold higher in the 300 mg/kg MPTP-treated animals in comparison to saline controls. Cytoplasmic TH protein (estimated by somal TH immunodensity) was increased by 45% in SNc somata from mice treated with 150 mg/kg MPTP in comparison to saline controls, and by 63% in 104-week-old mice in comparison to 8-week-old animals. This study provides evidence that an average surviving TH+ SNc neuron compensates for the age-related loss of other SNc neurons by increasing dopamine synthesis similar to younger SNc neurons surviving low levels of toxically induced damage and that the compensation may be in part mediated by increased synthesis of TH.

Aging MPTP Neuronal death Striatum C57BL mice

FOLLOWING exposure to toxins like 6-hydroxydopamine (6-OHDA), catecholaminergic neurons are reported to undergo adaptive changes in synaptic transmission [see (35) for review]. Presynaptically the changes include an increased release of neurotransmitter from the neurons' terminals together with an increase in the neurons' synthesis of neurotransmitter. Using measurements of tyrosine hydroxylase (TH) activity and/or the number of high-affinity reuptake sites in neurons' terminal beds as an index of the relative reduction in neuronal numbers after toxic exposure, it has been proposed that the adaptation was initiated after neuronal loss exceeded 50–60% of normal values (10,34). Although the factors which induce the neurons to initiate the adaptive changes are not known, it has been suggested that the changes represent an attempt to compensate for the neuronal loss induced by the toxin rather than for toxic actions themselves. If so, similar changes should be initiated in any circumstance in which there is loss of a proportion of the neurons projecting to a common target tissue.

Hence similar adaptive changes might be expected with the reductions of numbers of some catecholaminergic neurons that have been reported to accompany aging (15). Yet these compensatory mechanisms may not apply to age-related neuronal loss since the concentrations of catecholamines or their metabolites in rodents show little change or slight decreases throughout the animals' life spans [reviewed in (1,17)]. However, in all those studies, catecholamine concentrations were expressed relative to target tissue wet weight or protein content and were not adjusted for estimates of the proportion of surviving neurons in a similar manner to the studies of toxic action (10,34).

Rather than rely on indirect measures of the numbers of surviving neurons like TH activity or the number of high-affinity uptake sites, target tissue transmitter synthesis can be interpreted using complementary counts of the number of surviving neurons that are responsible for the transmitter synthesis. This has been demonstrated by a recent study comparing the action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on nigrostriatal dopaminergic and cortico-coeruleus noradrenergic neurons (27) in the C57BL mouse at 20 days after treatment with the toxin. In that study, the relationship between MPTP dose and striatal dopamine (DA) concentrations differed from that between MPTP dose and substantia nigra compacta (SNc) neuronal loss. Striatal DA concentrations followed a sigmoidally shaped relationship to MPTP dose while neuronal loss followed an exponentially decreasing relationship. Accordingly, low doses of MPTP produced no changes in striatal DA despite 20–40% SNc neuronal loss while

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higher doses produced decreases in striatal DA similar to SNc neuronal loss. In contrast, neocortical norepinephrine concentrations paralleled locus coeruleus (LC) neuronal loss over the entire MPTP dose range. Hence the relationship between neuronal destruction and the dose of the toxin could not be discerned from target tissue transmitter concentrations alone and joint examination of the transmitter concentrations and the numbers of remaining neurons was required to deduce the magnitudes of transmitter synthesis/release per remaining neuron for any dose.

We have recently investigated the age-related loss of four different immunocytochemically identified subtypes of monoaminergic neurons in brains of C57BL mice. We found that each neuronal subtype demonstrated continuous neuronal loss throughout life according to a decaying exponential relationship and that each subtype displayed different exponential rates of loss (31). The loss of dopaminergic SNc and noradrenergic LC neurons was found to proceed at a much more rapid rate than that observed for dopaminergic retinal amacrine neurons or for serotonergic raphe neurons throughout the life span of the mice. Of interest to the present research, the relatively rapid loss of TH-positive (TH+) SNc and LC neurons resulted in substantial loss of neurons (approximately 75–80% depletion) by two years of age in these two neuronal subtypes which was similar to the extent of loss produced in those same subtypes by high doses of MPTP (27).

The similarity of the neuronal loss caused by aging and the toxin MPTP provides an opportunity to compare any adaptive transmitter synthesis engendered by the two different modes of neuronal loss and to determine if the adaptive phenomena found by others for toxically induced loss extends to age-related loss of SNc neurons. Hence, the purpose of this study was to determine the relationship between target tissue transmitter and metabolite concentrations and reductions of TH+ neurons in the SNc in order to determine the transmitter concentrations per average remaining TH+ neuron. The research provides evidence that remaining aged neurons greatly increase their transmitter synthesis to compensate for the loss of other neurons in the same subtype in a manner analogous to that for young neurons surviving toxic insult. The work was presented in part as an abstract (5).

METHOD

Tissue Preparation

Male C57BL/6 mice were examined at 8, 21, 52 and 104 weeks of age (n=3–9 per age). The mice were obtained either from the NIA colony (C57BL/NNia) or from an isogenic colony at the University of Calgary (C57BL/Jbid); both colonies were originally from a common strain of mice from the Jackson Laboratories. The degree to which the strains have diverged from the original Jackson stock is unknown; however, we have previously demonstrated that the rates of age-related neuronal loss for the four monoaminergic neuronal subtypes (dopaminergic SNc and retinal amacrines, noradrenergic LC, and serotonergic raphe neurons) do not differ between the NNia and Jbid C57BL mice (31). Mice were allowed to acclimate to the Toronto animal housing facilities for at least five days prior to sacrifice. In both the Calgary and Toronto animal facilities, mice were maintained in boxes protected from airborne pathogens with filter hoods and semi-sterile procedures were followed so as not to transmit any pathogens from other mouse stocks held in the same facilities. Mice were killed by cervical dislocation, brains removed, striata rapidly dissected and stored at −70°C until HPLC analysis.

Male C57BL mice (Jackson Laboratories), 8–10 weeks of age, were injected intraperitoneally with MPTP (3.75, 7.5, 15.0, 30.0 mg/kg/day) or a saline control solution (n=6 for each MPTP dosage). Injections were given for 10 days so that animals received cumulative dosages of 37.5, 75, 150 or 300 mg/kg [following the schedule described by Heikkila et al. (11), and previously used in our own studies (27)]. Twenty days following the last injection, mice were killed and tissue handled identically to that for the aged mice.

Other mice at the four ages (n=6) or MPTP-treated mice (n=6) were used for immunocytochemistry [see details of treatment and immunocytochemical procedures in (27)]. These animals were given a lethal dose of pentobarbital and perfused with 4% paraformaldehyde/0.1 M sodium phosphate buffer (PB) at 4°C. Brains were transferred to 4°C 0.1 M PB for 1–3 hours and then put in a 30% sucrose/0.1 M PB solution (4°C) overnight or until the brain sunk in the solution. Serial frozen sections, 20 microns in thickness, were cut through the brainstem so as to include all of the SNc.

Analysis of Catecholamine Concentrations

Striata were homogenized in 0.2 N perchloric acid (PCA) containing internal standard 3,4-dihydroxybenzylamine (DHBA), 0.1% sodium metabisulphite and 0.01% sodium EDTA. Tissue homogenate (400 μl) was added to 10 mg alumina and made up to final volume with 1.0 ml Tris buffer (pH 8.6) according to the method of Mefford (16). Catecholamines were desorbed into 400 μl 0.2 N PCA and the samples were centrifuged for 5 min at 13,000 rpm.

DA and its metabolite DOPAC were measured by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection using an LC-4b amperometric detector with glassy carbon electrode and Ag-AgCl reference electrode RE-1 (Bioanalytical Systems, West Lafayette, IN) and an Altex Ultrasphere ODS 5 μ (4.6 mm i.d. × 25 cm length) analytical column (Beckman Instruments, Berkeley, CA). The mobile phase (pH 4.8±0.1) was modified from Mefford (16) and contained 0.1 M sodium acetate, 0.02 M citric acid, 100 mg sodium EDTA, 200 mg/l sodium octyl sulphate and 6% HPLC-grade methanol. The detector potential was +0.60 V vs. Ag-AgCl reference electrode with a flowrate of 1 ml/min. Interrun variability was approximately 5%.

Tyrosine Hydroxylase Immunocytochemistry

Slide-mounted sections were incubated with unlabelled primary TH antisera (Eugene Tech) in 0.2% Triton/0.1 M PB at 4°C overnight. Tissues were washed with phosphate buffer then incubated for 1 hour with biotinylated goat anti-rabbit IgG secondary antibody followed by avidin-HRP incubation. A 0.05% solution of diaminobenzidine (DAB) in 0.01% hydrogen peroxide was used to visualize the immunoreactive somata. For comparative optical density measurements, sections from control and MPTP-treated brains were mounted on the same slide to reduce the effect of slide to slide variability in the assay procedure and were processed for immunocytochemistry. Numbers of TH+ SNc neurons were obtained by counts of alternate serial sections through each nucleus [see (27,31)] for determination of somal counts and examples of TH immunoreactivity in somata of control and MPTP-treated mice.

A Javelin TV camera and a Coreco frame grabber together with an IBM 386-based densitometry system was used to estimate the amount of TH immunoreactivity in 30–60 randomly chosen TH+ somata from two pairs of 8-week-old and 104-week-old mice and two pairs of saline- and MPTP-treated (150 mg/kg) mice. The system provides for 512×512 images, digitized to

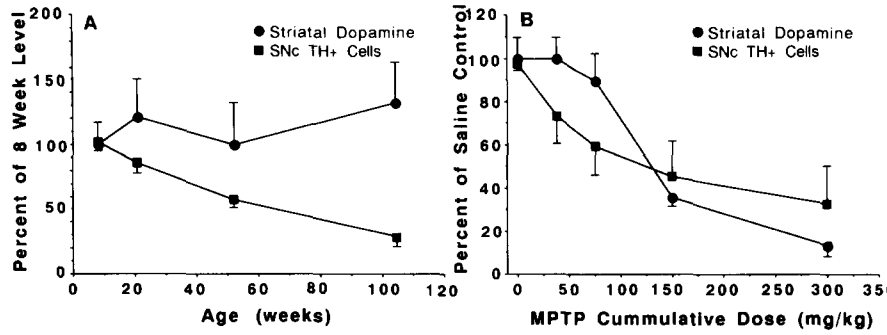

FIG. 1. Percentage loss of striatal DA concentrations expressed relative to wet weight of striatal tissue and SNc TH+ soma (mean ± S.D.). Panel A provides data for C57BL mice ranging from 8 to 104 weeks of age (n = 3–9/age group). Data provided in panel B is from C57BL mice measured 20 days after receiving doses of MPTP ranging from 37.5 to 300 mg/kg (n = 6/MPTP dose).

0–127 for each pixel. A mouse was used to outline a 3-micron square area for density measurements. Density levels were chosen so as to differentiate cell cytoplasm from underlying coverslip substratum using interference-contrast microscopy [see (32) for a detailed description of the computer densitometry system, the convolution-deconvolution techniques to remove noise from the images in the plane of section and the averaging techniques to reduce the amount of noise introduced by the solid state electronics of the camera and (24) for a justification for optic density measurements of relative immunoperoxidase concentrations as estimates of TH concentrations within individual neurons]. Optical density was measured in the somata to determine average cytoplasmic optical density per unit area for the TH+ neurons. Optical density was then measured in the immediately adjacent tissue to each TH+ neuron to determine the average background optical density per unit area. For each soma, the background optical density in the immediately adjacent area was subtracted from the somal optical density to obtain the TH cytoplasmic optical density above background optical density (OD_{cyt}).

Background values for young and old mice were normalized against the mean background optical density value for the 8-week-old animals while those for the MPTP-treated mice were normalized against the mean background value for the saline-treated mice. OD_{cyt} values for the 104- and 8-week mice and for the MPTP- and saline-injected mice were normalized against the mean OD_{cyt} values for 8-week-old and saline-injected mice, respectively. The neuron to neuron variation in the immunoperoxidase OD_{cyt} for the TH antibody in control animals is similar in extent to that found for LC neurons using comparable quantitative approaches (24). The distribution of normalized optical densities for TH+ somata measured in control and MPTP-treated mice were compared by analysis of variance.

Estimation of Relative Monoamine Concentrations in Surviving Neurons

We determined the relative neurotransmitter concentration in the terminal areas per average remaining TH+ neuron in SNc as a means of assessing the degree of adaptive transmitter synthesis that had occurred at graded levels of neuronal loss in both the aging and MPTP-treated animals. The neurochemical values obtained from HPLC analysis from the aging mice were normalized relative to the mean eight-week-old value and then corrected for the mean proportion of remaining TH+ neurons at each age. An identical approach was taken for the MPTP-treated animals only neurochemical values were normalized relative to the mean concentration in mice treated with saline at 8 weeks. Values for the proportion of remaining SNc neurons were shared with our study of MPTP-treated mice (27). Variance of the ratio (normalized neurochemical value/proportion of remaining TH+ neurons) was determined according to the equation: Y/X Variance = $(Y_{mean}/X_{mean})^2 \times [(\text{variance } Y/(n_y) \times Y_{mean}^2) + (\text{variance } X/(n_x) \times X_{mean}^2)]$.

RESULTS

Panel A in Fig. 1 presents the change in target tissue neurotransmitter concentrations and the loss of TH+ SNc neurons throughout the life span of the mice. Striatal DA concentrations appeared to increase slightly with increasing age but in fact did not show any statistically significant change ($p > 0.05$) despite the progressive marked loss of TH+ neurons in the SNc [see (31) for further details]. Similarly, panel B in Fig. 1 presents changes in target tissue transmitter concentrations and the accompanying SNc neuronal loss associated with graded doses of MPTP [see (27) for further details]. These data demonstrate that the previously reported maintenance of DA concentrations in C57BL mice with aging (3, 21, 28) may have occurred upon a background of extensive neuronal loss.

Panels A–C in Fig. 2 present DA and DOPAC concentrations relative to striatal wet weight and therefore represent the DA and DOPAC contributed by the entire remaining SNc neurons at each age. In these figures the error bars are standard deviations rather than standard errors. The standard deviations were used to match the neurochemical data to the counts of neuronal numbers which typically show lower variance. Expression of neurochemical values in this manner suggests little or no change in estimated DA synthesis or release (estimated by the DOPAC/DA ratio, panel C). However, the measurement of overall target tissue concentrations by themselves does not allow for the fact that these striatal neurochemical concentrations are being supported by progressively decreasing numbers of TH+ SNc neurons illustrated in Fig. 1A. Therefore, when DA and DOPAC levels are expressed relative to the mean proportion of remaining TH+ neurons at each age examined (panels D and E), it can be clearly seen that the concentrations of both DA and DOPAC per average remaining TH+ neuron are increased in the terminal areas in a graded fashion throughout the age span examined. For example, striatal DA concentration per surviving SNc neuron is on average 5-fold

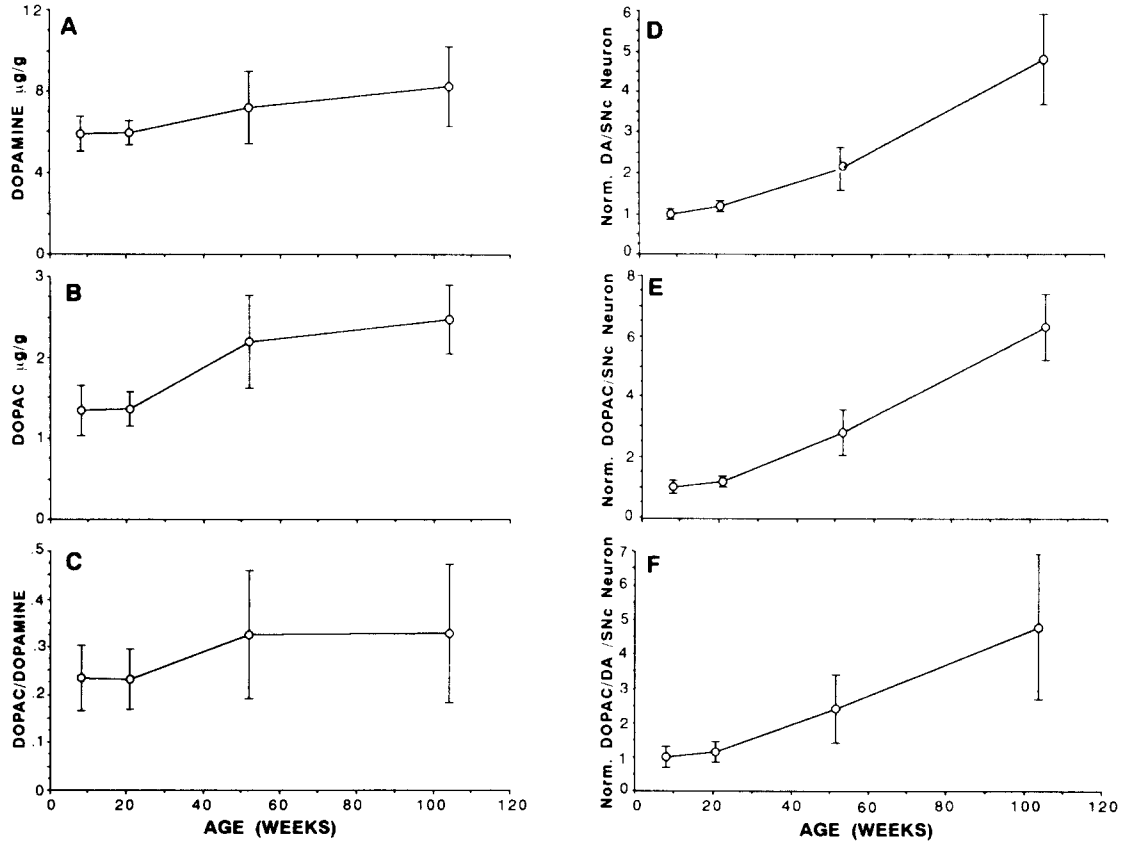

FIG. 2. DA and metabolite (DOPAC) concentrations (mean ± S.D.) in striatum measured in C57BL mice ranging from 8 to 104 weeks of age (n = 3–9/age group). Panels A–C provide neurochemical values expressed relative to wet weight of striatal tissue. Panels D–F provide neurochemical values normalized to mean value at eight weeks of age and expressed relative to the proportion of surviving SNc neurons at each of the age groups examined. Values for SNc survival at each age were calculated from our regression line of SNc age-related neuronal death in this strain of mice (31). Variance of the ratio was calculated as described in the Method section.

higher in 104-week-old mice than in 8-week-old mice. Furthermore, the DOPAC/DA ratio per average remaining TH+ neuron (panel F) shows a continual increase throughout the life span, thereby suggesting that the remaining SNc neurons increase their turnover and release of DA as the animals age.

Although other studies had reported decreasing striatal DA concentrations with increasing MPTP dosages in the mouse (2, 8, 12, 19, 20, 30), we found at 20 days after MPTP treatment, striatal DOPAC concentrations fell less precipitously than the DA levels resulting in an increased DOPAC/DA ratio for MPTP dosages exceeding 100 mg/kg (panels A–C, Fig. 3). Despite overall decreases in DA and DOPAC concentrations, a similar increase in the ratio of DOPAC/DA concentrations in striatum following high doses of MPTP administered to mice has been previously reported (9,30).

Panels D to F of Fig. 3 present the normalized neurochemical values corrected for the proportion of remaining TH+ neurons at each dose of MPTP. At low doses of MPTP, an average remaining SNc neuron increases its DA production such that DA concentrations per neuron rise by approximately 50% at 75 mg/kg MPTP. A comparable increase in DOPAC concentrations/neuron is observed at low doses of MPTP such that only a slight increase in the DOPAC/DA ratio occurs. Therefore, for low levels of MPTP, it appears that surviving neurons are capable of increasing their synthesis of DA to the extent that both release and total terminal DA content are maintained at a level which more than compensates for the loss of fellow neurons. At higher doses of MPTP, both DA and DOPAC concentrations per neuron gradually decrease. Since DOPAC values/neuron fall more slowly than DA concentration/neuron, DOPAC/DA per average neuron increases markedly (Fig. 3). Other work in our laboratory has shown that the SNc neurons have not effected full compensation of the decreased TH synthesis found after MPTP exposure at 20 days which accounted for the decreased striatal DA and DOPAC values at the higher doses (unpublished observations). Yet the increase in DOPAC/DA per remaining neuron is similar for the toxic and age-related neuronal loss and parallels that reported for SNc loss produced by 6-OHDA but based upon indirect estimates of neuronal numbers (10,34).

Accordingly, we have plotted the normalized DOPAC/DA ratio per remaining SNc neuron relative to age-related percentage loss (Fig. 4A) and MPTP-induced percentage loss (Fig. 4B) of neurons for individual animals on a semi-logarithmic scale in order to estimate the relationship between these variables. We were able to fit the age-related loss to a single exponential relationship while the MPTP-induced loss seemed to be best represented by two exponential relationships, one for losses less than 40% and another for losses of 40–70%. It is interesting to note that others have reported that no change in DA metabolism is observed with low levels of neuronal loss and that an increase in the striatal

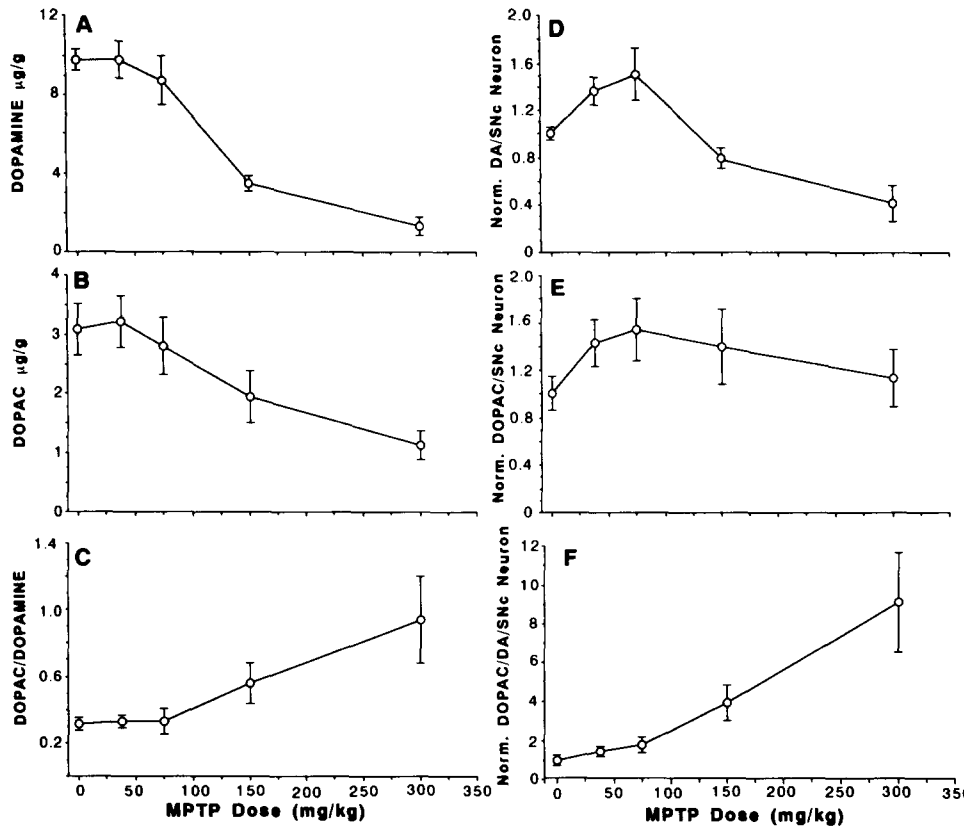

FIG. 3. DA and DOPAC concentrations (mean ± S.D.) in striatum measured in C57BL mice 20 days after receiving doses of MPTP ranging from 37.5 to 300 mg/kg (n = 6/MPTP dose). Panels A–C provide neurochemical values expressed relative to wet weight of striatal tissue. Panels D–F provide neurochemical values normalized to mean value for saline-treated control animals and expressed relative to the proportion of surviving SNc neurons at each dose of MPTP administered. Values for SNc neuronal survival were obtained from a previous study of SNc neuronal death using comparable experimental paradigms (27). Variance of the ratio was calculated as described in the Method section.

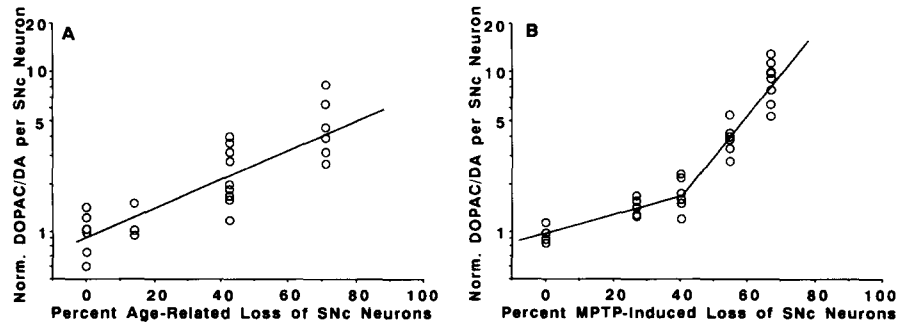

FIG. 4. Normalized striatal DOPAC/DA ratios per surviving SNc TH+ neuron for individual animals relative to the proportion of SNc loss. Note that the DOPAC/DA ratio is plotted on a logarithmic scale. Panel A presents data for C57BL mice ranging from 8 to 104 weeks of age. The DOPAC/DA ratio at each age was normalized relative to the mean value obtained at 8 weeks of age. Values from C57BL mice 20 days after receiving doses of MPTP ranging from 37.5 to 300 mg/kg are provided in panel B. The DOPAC/DA ratios at each dose of MPTP were normalized relative to the mean value obtained with saline control. Neurochemical values were taken from panel F of Figs. 2 and 3, respectively and reexpressed relative to SNc loss to allow for direct comparison between these two groups of animals.

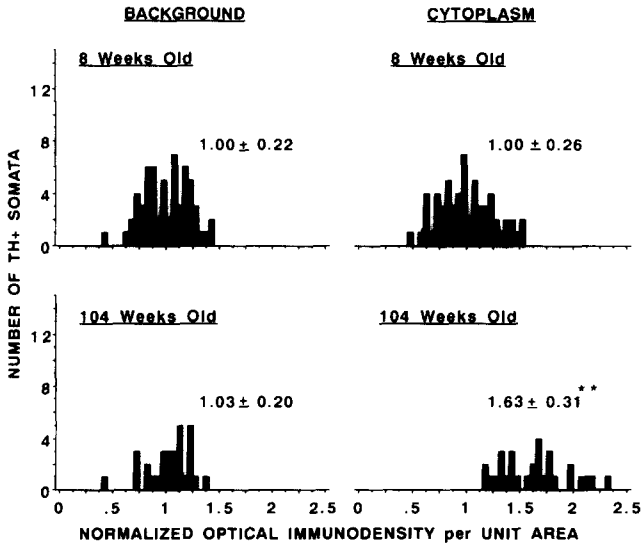

FIG. 5. Normalized optical density per unit area of TH immunoreactive product in SNc somata measured after treatment of tissue slices with TH antibody. Values were taken from randomly selected SNc somata in 2 mice either 8 weeks (N = 60) or 104 weeks (N = 30) of age. Background optical immunodensity was normalized relative to the mean background optical density for 8-week-old animals. Cytoplasmic (Cyt) optical immunodensities were determined by subtracting the background optical density in the immediately adjacent tissue from the somal optical density for each soma. Data were then normalized relative to the mean cytoplasmic optical density for 8-week-old mice. Background optical densities did not differ between groups (18.8 ± 4.1 versus 19.4 ± 3.7 for 8- and 104-week-old animals, respectively). Mean cytoplasmic optical immunodensity was 3.6-fold above background (68.4 ± 12.1) in 8-week-old mice. Mean optical density in 104-week-old SNc somata was significantly greater ($p>0.001$) than that observed in 8-week-old animals. Increased optical density using this procedure has been previously shown to represent an increase in TH protein (24).

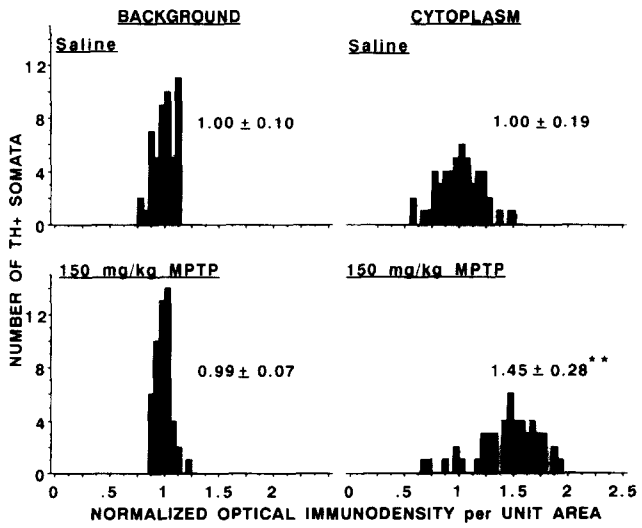

FIG. 6. Normalized optical density per unit area of TH immunoreactive product in SNc somata measured after treatment of tissue slices with TH antibody. Values were taken from 50 randomly selected SNc somata in 2 mice treated with either saline or 150 mg/kg MPTP. MPTP animals were killed 20 days after toxin administration. Background optical immunodensity was normalized relative to the mean background optical density for saline-treated animals. Cytoplasmic optical immunodensities were determined by subtracting the background optical density in the immediately adjacent tissue from the somal optical density for each soma. Data were then normalized relative to the mean cytoplasmic optical density for the saline-treated group. Background optical densities did not differ between groups (22.5 ± 2.3 versus 22.2 ± 1.6 for saline- and MPTP-treated animals, respectively). Mean cytoplasmic optical immunodensity was 2-fold above background (42.2 ± 3.0) in saline-treated group. Mean optical density in SNc somata surviving MPTP treatment was significantly greater ($p>0.001$) than that observed in control somata.

DOPAC/DA ratio is not observed in 6-OHDA-treated rats until neuronal loss exceeds 50–60% using indirect measures of neuronal loss (10,34), our data shows that single or double exponential relationships between DA synthesis and numbers of remaining neurons may account for an apparent breakpoint at 50–60%.

Immunocytochemistry

In order to obtain an indication as to whether the increase in DA synthesis in remaining TH+ neurons following MPTP-induced or age-related loss of SNc neurons can be in part explained by increased concentrations of the rate-limiting enzyme, the relative optical density of TH immunoreactivity in the somal cytoplasm of individual SNc neurons was examined in two pairs of mice treated either with saline or 150 mg/kg MPTP and two pairs of mice at 8 and 104 weeks of age. The average optical densities for background TH immunoreaction immediately surrounding the somata were not statistically different in either the young and old or control and MPTP-treated animals (18.8 ± 4.1 versus 19.4 ± 3.7 mean ± S.D. for 8-week- and 104-week-old mice and 22.5 ± 2.3 versus 22.2 ± 1.6 for saline and MPTP mice, respectively). Mean TH somal cytoplasmic optical densities were 3.6- or 2-fold above mean background values for the 8-week-old and saline-treated control mice, respectively.

The TH cytoplasmic optical density above background optical density (OD_{cyt}) was increased 1.63-fold in 104-week-old mice in comparison to 8-week-old animals ($p>0.0001$; Fig. 5). Similarly, the OD_{cyt} was increased 1.45-fold in an average SNc neuronal soma in the MPTP-treated mice in comparison to controls ($p>0.0001$; Fig. 6). These data suggest an average 63% and 45% increase in TH concentration per unit area in these somata.

DISCUSSION

The results of this study indicate that DA synthesis and turnover is increased in aged TH+ SNc neurons following age-related neuronal loss. This study is the first to combine neurochemical measurements with direct determinations of the numbers of remaining TH+ neurons in order to calculate neurochemical indices per average remaining neuron. The logarithmic increase in DOPAC/DA per average remaining neuron is almost linearly related to the degree of neuronal loss observed in the SNc, with an estimated 4-fold increase in DA turnover rate (based on increased DOPAC/DA ratios) in surviving neurons when approximately 75% of the TH+ SNc neurons have been lost due to aging. The increase in the DOPAC/DA ratio occurs over the entire range of neuronal loss studied, that is up to 70–75%. Total striatal concentrations of DA and DOPAC were maintained at similar levels throughout life despite progressive neuronal loss. Hence, the compensatory increase in DA turnover and likely synthesis by an average remaining neuron appears to explain the unchanged values throughout life found by others (3, 21, 28). We have no data to determine whether the increase would continue for more extensive neuronal loss.

The findings differed for the age-related and MPTP-induced SNc neuronal loss in that the neurons were apparently unable to

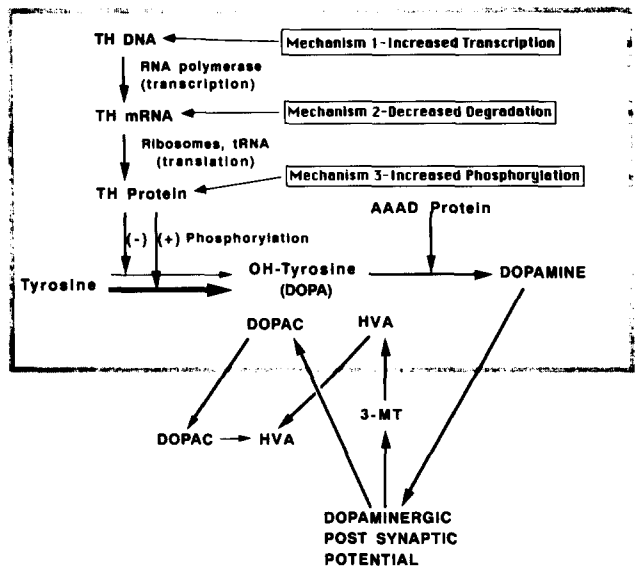

FIG. 7. Three postulated mechanisms whereby surviving SNc neurons could increase their synthesis of DA. Mechanism 1 represents an increase in TH gene expression. Mechanism 2 represents a decrease in degradation of either TH mRNA or TH protein. Both of these mechanisms would result in an increased amount of TH protein/SNc neuron. Mechanism 3 represents posttranslational phosphorylation of existing TH protein.

maintain DA and DOPAC concentrations per remaining neuron within the normal range for higher MPTP dosages at 20 days following exposure to the toxin. The differences in response between age-related and MPTP-induced neuronal loss may in part be due to the time frame used in our MPTP studies. That is, mice were killed 20 days following the last injection of MPTP. This time frame was chosen based on our previous results demonstrating that maximal SNc cell loss had occurred by this time (27). However, the TH+ SNc neurons remaining after MPTP-induced axonal damage may have been at varying stages of a repair/compensatory sequence, such that maximal induction of DA synthesis may not have occurred in all neurons [see (32) for a more detailed discussion of axonal repair mechanisms and return of TH+ soma following MPTP damage of dopaminergic retinal amacrine cells]. A time-dependent increase in DA metabolism in SNc neurons surviving 6-OHDA or MPTP exposure has been previously demonstrated (30,35). In these former studies, striatal DA levels did not reach control values even when animals had been allowed to survive for greater periods of time posttoxic exposure; however, the estimated degree of SNc loss (usually greater than 80–90%) exceeded that observed for the highest dose of MPTP used in this present study.

The data presented here suggest that DA synthesis and release per average surviving neuron increase following neuronal loss associated with either aging or toxin induced damage. This interpretation is based on steady-state measures of DA, DOPAC and the DOPAC/DA ratio coupled with counts of numbers of TH+ neurons. However, it is important to recognize that these steady-state measures likely provide nonlinear estimates of DA turnover and release. Other factors such as alterations in DA compartmentalization, DA reuptake sites and intraneuronal catabolism of DA not undergoing release may all contribute to the results observed in this study so that the 4-fold increase we found in DOPAC/DA ratios may overestimate the increase in DA synthesis. Yet the magnitude of the changes we have found make it unlikely that the other factors account for most of the changes in the ratio. For example, preliminary observations in our laboratory using a comparable approach to that reported here suggest that DA synthesis and turnover per average remaining TH+ SNc neuron is increased approximately 3-fold in 104-week-old animals in comparison to 8-week-old mice when assessed following the administration of either NSD-1015 or pargyline (6). It will be worthwhile to determine whether other measures of DA release, such as the in vivo dialysis approach used in studies of 6-OHDA loss of SNc neurons (25), parallel the results reported in this study.

A variety of mechanisms may be involved in the apparent increase in DA synthesis and release per average remaining neuron with aging or MPTP-induced neuronal loss reported in this study. Most evidence relating to compensatory mechanisms comes from studies examining the impact of toxic insult to younger animals [see (35) for review]. The degree to which these mechanisms may be involved in the age-related increase in DA synthesis reported here is unknown.

As depicted in Fig. 7, activity of TH, and hence DA synthesis, can be modulated either by changes in the amount of TH protein available, mediated via alterations in TH gene expression (mechanisms 1 and 2), or by posttranslational modification of existing protein (mechanism 3; i.e., TH phosphorylation). TH activity has been shown to increase in SNc neurons surviving 6-OHDA administration (34), with an initial increase in TH activity proposed to represent increased TH phosphorylation and a sustained increase in the TH activity proposed to represent increased TH protein synthesis (33,34).

The finding of increased TH immunolabelling density in synaptosomes isolated from SNc neurons after 6-OHDA lesions (33) may be similar to our observation that TH immunodensity is increased in the somata of SNc neurons remaining after MPTP exposure. The estimated 45% increase in somal TH concentration per unit volume provided by the optical density measurements could provide a reflection of the TH concentration throughout the neuron. Yet care must be taken in interpreting the data since we do not know how TH is distributed throughout the neuronal cytoplasm, particularly how somal concentrations relate to that in the terminal axons. Furthermore, MPTP may produce transient somal atrophy (7) and therefore somal concentrations might not reflect an increase in total somal TH content. More importantly, we have evidence for sprouting of SNc terminal axons at 20 days after MPTP treatment (unpublished observations) so that given uniform TH concentration throughout the neuron, somal concentrations might underestimate the total TH in the terminal axons.

Our data show a comparable increase in TH immunodensity in the soma of SNc neurons surviving age-related neuronal death indicating that TH concentration was increased by 63% in 104-week-old mice in comparison to 8-week-old animals. Preliminary measurements in our laboratory suggests that most of the TH+ somata hypertrophy by 10–30% in the aged animals (unpublished observations) so that total TH content is likely increased in aged somata. Similar to the MPTP-treated SNc neurons the somal increases are not adequate to provide measures of TH content in other portions of the neurons particularly the terminal axons.

These results suggest that TH gene expression could be increased in the remaining TH+ soma and may be consistent with a recent report of little or no change with aging in TH mRNA levels in SNc of Long-Evans rats at two years of age (13). While we do not know the magnitude of SNc TH+ neuronal loss in their aged rats, assuming that mRNA measurements were made against a background of progressive decline in SNc neuronal numbers, the data would suggest that TH mRNA levels per surviving neuron increased throughout the life span of these animals.

Our finding of increased TH protein density in SNc soma of MPTP-treated mice is somewhat at variance with a report of decreased SNc somal TH mRNA abundance measured nine months

after 6-OHDA lesion to the SNc (22). The reason for the apparent discrepancy between these studies is unclear but may, in part, relate to mechanisms of action of the dopaminergic toxins employed. That is, MPTP results primarily in damage to the terminal axons, mediated through interference with mitochondrial oxidative respiration (18,23). Thus, changes in the soma are secondary to axon damage, with little direct somal damage occurring in response to MPTP. In contrast, Pasinetti et al. (22) observed extensive atrophy of the SNc soma persisting as long as nine months after 6-OHDA administration. Thus the degree of somal damage may play an important role in the ability of surviving neurons to increase TH gene expression.

Our data also indicate that the striatal DA concentration per average remaining neuron is increased since neuronal loss occurs in the absence of striatal DA depletion both with aging and low doses of MPTP. An expansion in axonal terminal arborizations per surviving neuron, increased DA concentration per terminal, or a combination of the two could serve to accommodate the increased DA per surviving neuron. It has been previously argued that the DA content per nerve terminal does not change substantially as a result of toxically induced loss of neighboring SNc neurons (33). Thus, data may suggest that the remaining neurons have undergone sprouting of their neurites. We have preliminary evidence for sprouting of aged SNc neurons' terminal axons in the striatum (unpublished observations) and for neurites of TH+ amacrine cells who do not loose their TH immunoreactivity by 20 days after MPTP treatment (32). It will be interesting to determine whether terminal axon sprouting is sufficient to account for the increased DA content in the aging neurons' terminal fields and if an increase in the number of arbors, an increase in DA content per arbor, or a combination of the two explains the overall increase in DA content per average remaining neuron.

The underlying mechanism resulting in increased DA synthesis in TH+ SNc neurons following either age-related or MPTP-induced neuronal loss is not known. However, alterations in extracellular DA levels may provide an essential signal to the surviving neurons. It is well known that dopaminergic presynaptic autoreceptors sense changes in extracellular DA concentrations and modulate both action potential activity and DA synthesis [see (29) for review]. Furthermore, studies in both invertebrates and mammals provide evidence that extracellular transmitter concentrations can either retard or induce axonal sprouting [see (14) for a general review and (4,26) for the roles played by DA and NE in striatal and cortical sprouting]. It may be that neurons continue to be responsive to extracellular neurotransmitter levels and that these extracellular signals provide the neuron with information regarding its maximal space allocation not only during development but also throughout the life span. While this postulate is at present highly speculative, our results of increased DA synthesis reported here and sprouting in dopaminergic retinal amacrine neurons following MPTP exposure (32) and in dopaminergic SNc neurons with aging (unpublished data), is consistent with the above hypothesis.

In conclusion, the results of this study indicate that DA synthesis and turnover are increased in proportion to the degree of SNc TH+ neuronal loss throughout the life span of C57BL mice and that increased TH protein per remaining TH+ neuron may, in part, explain the increased neurotransmitter synthesis. Therefore, these results do not support the posit that aged neurons lose their capacity for adaptive protein synthesis and therefore are not capable of dynamic change.

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