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Parkinson's Disease-Like Effects of *S*-Adenosyl-L-Methionine: Effects of L-Dopa

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CHARLTON, C. G. AND B. CROWELL, JR. *Parkinson's disease-like effects of* S-*adenosyl-*L-*methionine: Effects of* L-*dopa.* PHARMACOL BIOCHEM BEHAV 43(2) 423–431, 1992.—The major symptoms of Parkinson's disease (PD) are due to degeneration of the nigrostriatal pathway and depletion of dopamine (DA). Tyrosine hydroxylase (TH), norepinephrine (NE), serotonin (5-HT), and melanin pigments are also decreased and acetylcholinergic activity increased. Biochemically, increased methylation can cause the depletion of DA, NE, 5-HT, and melanin pigments and also an increase of acetylcholine; thus, increased methylation can present a biochemical picture that resembles the biochemical changes that occur in PD. During the therapy of PD with L-dopa, it is well known that L-dopa reacts avidly with *S*-adenosyl-L-methionine (SAM), the biologic methyl donor, to produce 3-*O*-methyl-dopa. Correspondingly, L-dopa has been shown to deplete the concentration of SAM, and SAM has been found to induce PD-like motor impairments in rodents; therefore, an excess of SAM-dependent methylation may be associated with Parkinsonism. To further study the effects of methylation, SAM was injected into the lateral ventricle of rats. SAM caused tremors, rigidity, abnormal posture, and dose-related hypokinesia. Doses of 9.38, 50, and 400 nM/rat caused 61.9, 73.4, and 94.8% reduction, respectively, of motor activity. A 200-mg/kg IP dose of L-dopa, given before 50 nM SAM, blocked the SAM-induced hypokinesia. SAM also caused a decrease in TH immunoreactivity, apparent degeneration of TH-containing fibers, loss of neurons, and the accumulation of phagocytic cells in the substantia nigra. These results showed that excess SAM in the brain, probably due to its ability to increase methylation, can induce symptoms that resemble some of the changes that occur in PD.

Parkinson's disease Methylation Tyrosinehydroxylase *S*-adenosyl-L-methionine Dopamine Tremors

PARKINSON'S disease (PD) is best known by a symptom-related degeneration of the nigrostriatal dopaminergic pathway, disappearance of melanin pigments from the pars compacta of the substantia nigra (19,21,44), and depletion of dopamine (DA) in the striatum (25), all of which seem to occur in parallel (36). The primary symptoms are resting tremors, bradykinesia, muscular rigidity, and deficiency in postural reflexes. There seems to be an increase in the metabolic methylation of DA, based upon the finding that a substance with reactive properties similar to 3,4-dihydroxyphenylethylamine (DIMPEA), the 3,4-dimethoxy metabolite of DA, was detected in the urine of PD patients (2), and that the ratio of the levels of homovanillic acid (HVA), an oxidized methylation product of DA, to that of DA (HVA/DA) increased in the neostriatum (47) and urine (5) of patients with PD, although the levels of both DA and HVA were usually decreased, as compared with the controls. Cerebral levels of serotonin (5-HT) (4) and norepinephrine (NE) (15) are also decreased, and the activity of acetylcholine increased in the brain of PD patients.

Although the nigrostriatal degeneration is the major pathology of PD, other neurological damages also occur. Lesions have been identified in the locus coeruleus (1,41), the hypothalamus (27,34,30) the dorsal motor nucleus of vagus (14,45), the sympathetic ganglia (20,26,37,45), and the adrenal medulla (26) of PD cases. These findings mean that the total symptoms of PD may be due to more extensive neurological impairments than those that occur in the nigrostriatum, and may help to explain the cognitive and autonomic nervous system impairments that occur mostly during the later stages of PD. The findings may explain the reasons why a mere destruction of the nigrostriatal pathway cannot reproduce all the symptoms observed in PD (13,22,35,38), and why experiments that caused only the depletion of DA in animals (16,31) do not always significantly mimic PD. Therefore, the nigrostriatum may be more susceptible to the conditions that precipitate PD rather than being the only region involved as originally thought. The wider spectrum of impairments that are seen in PD patients suggest that an agent with toxic potentials, but with inherently marginal specificity, may be involved in the cause of PD. *S*-adenosyl-L-methionine (SAM) may possess such metabolically noxious properties. SAM is the endogenous methyl donor and is a limiting factor in methylation reaction. If SAM is increased in the brain, methylation reac-

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tions also will be increased. Methylation is not a specific and limited process, but if methylation does increase, the nigrostriatum may serve as a primary reaction site. This is because the neostriatum is enriched with biochemicals, for example, the catechols and other biogenicamines that avidly react with SAM, and it also contains processes that depend upon the products of methylation, for example, choline, for the production of acetylcholine.

There is a close relationship between methylation and the biochemical changes that occur in PD. Several important substrates and products of SAM-dependent biologic methylation are identifiable with the chemical pathology of PD. The depletion of DA, NE, and 5-HT that occur in PD could be due to the reaction of these neurotransmitters with SAM. The increased HVA/DA and the presence of a DIMPEA-like substance in PD patients suggest also that there is an appreciable level of reaction between SAM and DA that occurs in PD patients because HVA is an oxidized methylation product of DA and DIMPEA is the 3,4-di-methoxy metabolite of DA. The increased acetylcholine activity, which also occurs in PD, may be related to the reaction of SAM with phospholipids (6) to produce choline, the precursor of acetylcholine. It is also of interest to know that the SAM-dependent methylation of phospholipids (24) and other compounds will produce cytotoxic biochemicals that could cause cell degeneration. In addition, SAM has been shown to be tremorgenic in experimental animals (9,10). The large amount of 3-methyl-dopa which occurs in PD patients undergoing L-dopa therapy (18,23,32,33), is evidence that L-dopa when administered to PD patients reacts with SAM. Because L-dopa is also an effective depletor of SAM (46), it suggests that the pharmacology of L-dopa may involve its role as a methyl acceptor.

Based upon the above reasoning, it is proposed that an excess of methylation may be one of the biochemical impairments contributing to the symptoms of PD. Biologic methylation is tightly regulated, partly by the limiting existence of the methyl donor, SAM. Therefore, if SAM is increased it is expected that methylation reactions will increase, resulting in

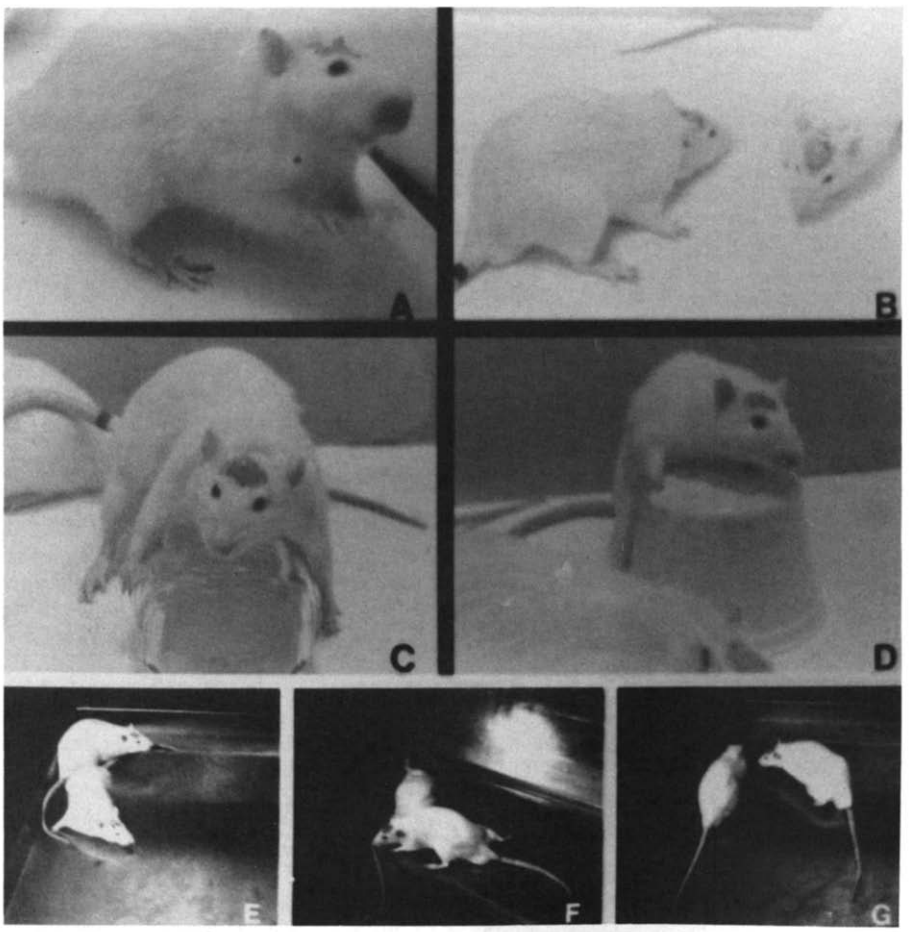

FIG. 1. Motor abnormalities caused by SAM. (A and B) Rats during tremors. Note the blurred forelimbs and snouts in (A). (B) Rat resting on its ventral surface, probably to relieve the shaking. (C and D) "Motor freezing," that is, maintaining a static (frozen) position over an object for an extended period. (E) Rotational behavior is highlighted. (F) Indicates abnormal posture. (G) Recovery of the rat.

biochemical, behavioral, and neurological changes that may show similarity to the symptoms of PD. As an initial approach to determine whether an excess of methylation is involved in Parkinsonism, SAM was injected into the lateral ventricle of rats and their behavior monitored. Changes in tyrosine hydroxylase-like immunoreactivity and degeneration of neurons were also studied.

METHOD

Sprague-Dawley male rats, weighing 250–350 g (Harlan Labs, Indianapolis, IN), were used in the experiments. Rats were acclimatized for about 1 week in a colony room with a 12 L : 12 D cycle. Water and food were supplied ad lib. Under chloral hydrate anesthesia (400 mg/kg), a stainless steel guide cannula was stereotaxically placed for injection into the lateral ventricle of each rat. The cannula was affixed with dental cement secured to the skull with two screws. The placement of the cannula, with reference to bregma, was 1.4 mm lateral, 0.5 mm caudal, and the tip extended to the inner surface of the cranium, above the dura mater. Rats were allowed to recover for about 2 days before the tests. Injections were made in the lateral ventricle 5 mm from the surface of the cranium via a premeasured cannula attached by polyethylene tubing (PE20) to a 25-μl Hamilton syringe containing either 5 μl phosphate-buffered saline (PBS) at pH 7.4 or doses of the chloride, iodide, or toluene sulfonate salts of SAM prepared in 5 μl PBS. S-Adenosylhomocysteine (SAH), the demethylated analog of SAM, was also tested. The duration of action of 1 μM/rat of SAM was investigated. Rats were injected and the behavior tested at intervals of 10 min each. The dose-effect relationship of SAM and the ability of L-dopa to block the effects of SAM were also investigated. The histologic and anatomic studies were done in rats that received an injection of SAM (1 μM/rat) or PBS and were sacrificed 1 h or 4 days postinjection. Another group received daily injections for 4 days and animals were killed 6 days after the last injection. In the latter study, 1 μM/rat was given on the first and 2 μM each for the subsequent 3 days. Control animals received PBS.

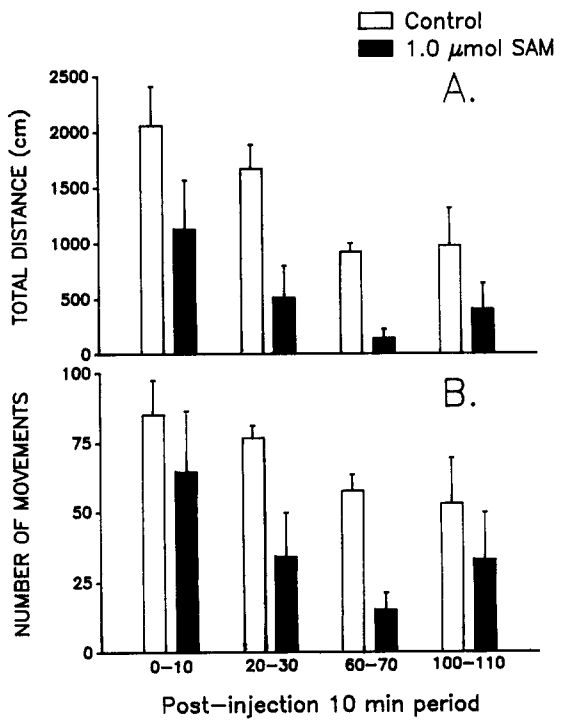

FIG. 2. Effects of SAM (1 μm/rat) on the total distance (TD) rats traveled (A) and on the number of movements (NM) rats made (B) in 10-min periods. SAM significantly decreased both properties of movements for at least 90 min postinjection despite the decreased motor activity in control animals due to acclimitization to the test environment.

Following injections, animals were observed for the presence of tremors and other changes in motor functions. The changes in locomotor activity were measured in a quiet room with reduced lighting using a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). Rats were allowed 30 min to adapt to the test chamber. Rats were injected and returned to their home cages before being placed in the cage of the activity monitor. This was done to reduce the possibility of the rat associating any noxious experience felt during the injections with the activity monitoring process. The distance traveled (DT) and number of movements (NM) made were used to determine the changes in locomotor activity. A rat was evaluated as being cataleptic (frozen) if it remained propped for more than 20 s. Rats were photographed during the sessions.

Groups of rats were studied for the effects of SAM on brain histochemistry. These rats were reanesthetized with chloral hydrate (400 mg/kg) and transcardially perfused with cold PBS followed by 4% paraformaldehyde (PF) in PBS. Brains were removed and placed in cold 15% sucrose, prepared in PBS, and kept at 4°C for about 24 h. Brains were then frozen in powdered dry ice and stored at −78°C. Sections, 30-μm thick, were prepared in a cryostat and mounted on gelatin chrome-alum-coated slides. A set of the slides was stained with cresyl violet or thionin; another was reacted for the determination of TH immunoreactivity (TH-IR).

A modified indirect immunohistochemical procedure (12) was used. The slide-mounted slices were preincubated in 0.3% Triton X-100 in PBS, pH 7.4, for three 5-min periods, then incubated in a similar buffer containing the rabbit anti-TH serum (Pel-Freeze) at 1 : 1,000 dilution, or with nonimmune rabbit serum, as control, for about 24 h at 4°C. The slides were washed in 0.2% Triton X-100–PBS buffer for three 5-min periods and incubated in the buffer containing fluorescent-labeled goat antirabbit serum, 1 : 300 dilution, in reduced light for 30 min. The slides were washed in Triton X-100 buffer for one 5-min period and in PBS for two 5-min periods. The sections were drained and cover slipped using Fluromont and viewed with an epifluorescent-equipped microscope.

At least six rats were used in each experimental group for the behavioral studies. For statistical evaluation, the means ± SE were determined for each group. For significance, paired Student's *t*-values were determined. Probability less than or equal to 0.05 was considered significant. The immunohistochemical and degenerative studies were performed in triplicate.

RESULTS

Rats, injected into the lateral ventricle with SAM, showed marked impairment of motor functions. The dominant effects were tremors, hypokinesia, Straub-tail rigidity, and abnormal posture (Fig. 1). Tremor occurred mainly in the snouts and forelimbs following doses of about 0.2–0.5 μM/rat (Figs. 1A and 1B). High doses (about 1 μM/rat and above) caused generalized shaking, which increased when rats were activated to

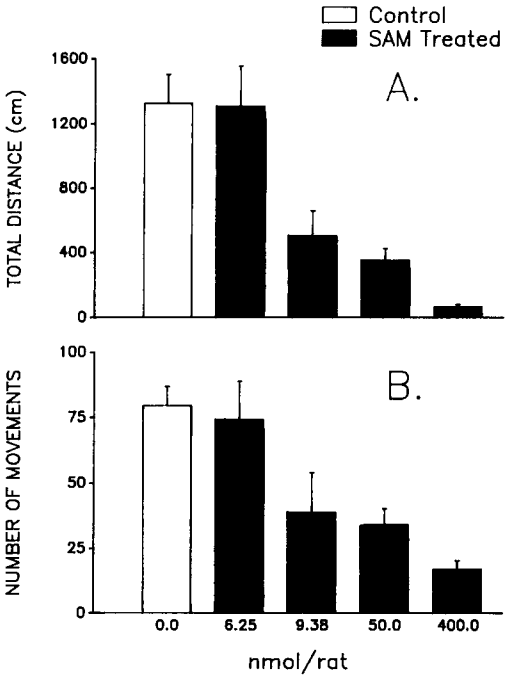

FIG. 3. Dose-effect of SAM on the TD (A) and NM (B). The effective dose was less than 9.38 nM/rat, and 400 nM/rat severely depressed motor activity.

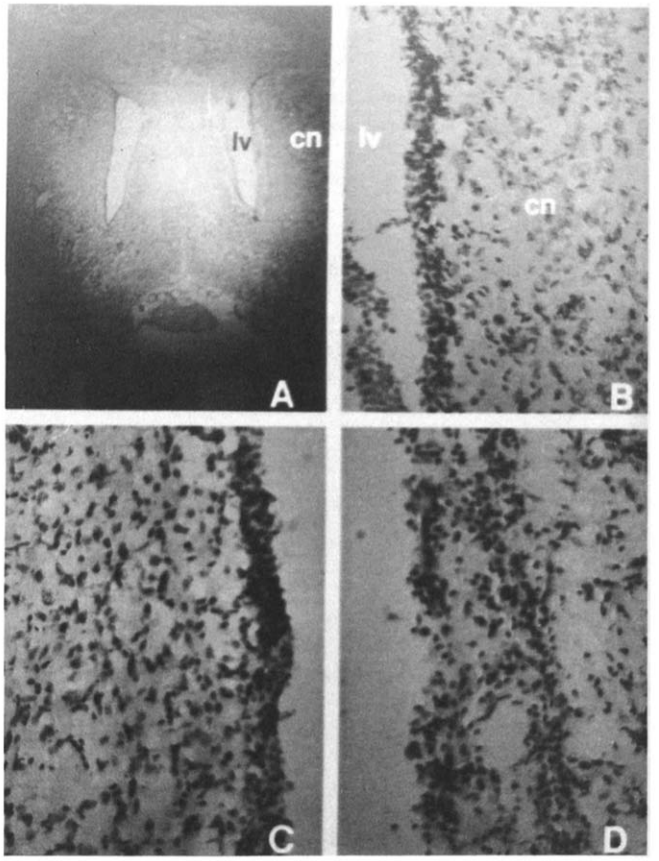

FIG. 4. Nissl-stained caudate nucleus of a rat killed 4 days after injections. (A) Cross section of the brain, highlighting the lateral ventricle of a SAM-injected rat. (B) Medial border of the ipsilateral caudate nucleus (cn) of a PBS-injected rat. (C and D) The contralateral and ipsilateral cn of a SAM-injected rat. Note the disrupted ependymal cell layer of the ventricle (lv) in (D) and the presence of dense Nissal substances in (C) and (D). Bar = 50 μm for (B), (C), and (D).

move. Rats generally showed hesitation before moving, but also exhibited sudden outburst of movement, startled responses, and stimulus-sensitive seizures at the higher dose levels. After about 40 min, the intensity of the tremors subsided and animals exhibited less inclination to move. When propped, rats remained in the abnormal position (Figs. 1C and 1D) for extended periods—in excess of 2 min in some cases. Circling or rotational movements, mainly contralateral to the injection side, were also observed (Figs. 1E and 1F). Rats appeared to be fully recovered within about 2 h following injection of SAM (Fig. 1G), but showed more tolerance to a subsequent dose given 24 h later.

The locomotor activity, measured as the total distance (TD) traveled and the number of movements (NM) made in a 10-min period, was decreased in rats treated with 1 μM/rat of SAM (Fig. 2). This reduction was statistically significant for about 90 min postinjection. Recovery was evident during the 100- to 110-min period (Figs. 2A and 2B). At 0–10, 20–30, 60–70, and 100–110 min postinjection, the TD traveled by SAM-injected rats was 54.9, 30.8, 15.6, and 41.6% of control, respectively (Fig. 2A), and the NM made were 76.0, 44.5, 26.5, and 62.3% of control, respectively (Fig. 2B). A gradual reduction in activity was also seen in control rats, but such slowing down in rats normally occurs and is due to familiar-

TABLE 1

EFFECT OF PRETREATMENT OF L-DOPA (200 mg/kg, IP) ON THE HYPOKINESIA CAUSED BY 50 nM/RAT OF SAM

			L-Dopa (200 mg/kg, IP)		
	Control (PBS)	SAM (50 nM)	1 h	2 h	4 h
A.	1028 ± 99*	284 ± 88*†	270 ± 93	363 ± 85	943 ± 156†
B.	83 ± 11*	24 ± 70*†	30 ± 11	37 ± 70	85 ± 12†

The effects were significant at 4-h postinjection test period. A, total distance (TD) in centimeters; B, number of movements (NM).

Statistical significance: *Controls from SAM. †non-L-dopa from L-dopa-treated rats.

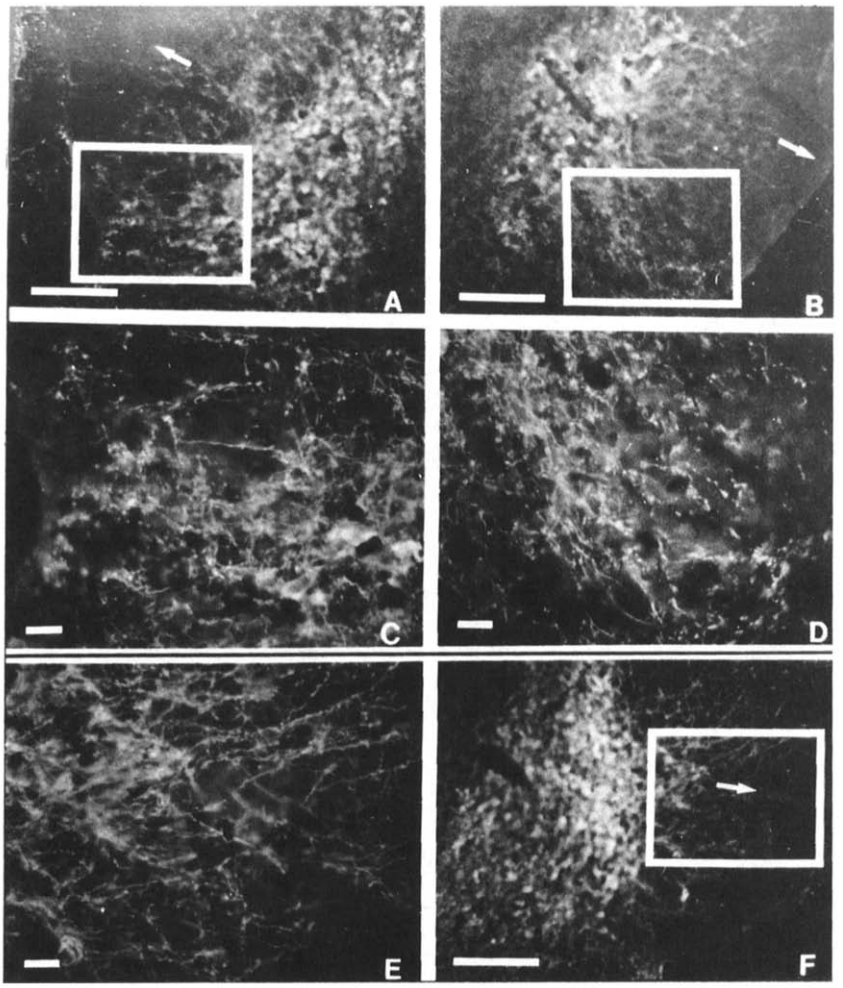

FIG. 5. Decrease in tyrosine hydroxylase immunoreactivity (TH-IR) in the substantia nigra (SN) of a SAM-injected rat compared to a PBS-injected rat. A low magnification of the contralateral (A) and ipsilateral (B) ventral par compacta of the SN of a SAM-injected rat and the ipsilateral (F) SN of a PBS-injected rat is shown. Parts (C), (D), and (E) highlight the boxed areas in (A), (B), and (F), respectively. The TH-IR is shown to be lower in the ipsilateral (B) than the contralateral (A) SN of the SAM-injected rat, and both SNs showed lower levels of TH-IR than the ipsilateral SN of the PBS-treated rat (F). Notice the progressive disruption of the profile of the TH-IR-containing fibers in (D) and (C) (SAM treated) compared with the intact fibers in (E) (PBS control). Arrows point toward the ventrolateral. Bar = 50 μm for (C), (D), and (E) and 312 μm for (A), (B), and (F).

ization with the test environment. SAM has a relatively greater effect on reducing the TD traveled than on reducing the NM that rats made. This means that rats traveled a shorter distance per movement. A dose of 6.25 nM/rat of SAM did not affect the TD traveled by rats, but 9.38, 50, and 400 nM/rat reduced the TD to 38.1, 26.6, and 5.19% of the PBS control rats, respectively (Fig. 3A). The same doses decreased the NM to 92.5, 48.8, 42.5, and 21.25% of the control values, respectively (Fig. 3B). A dose of 200 mg/kg L-dopa, administered 4 h before SAM, blocked the hypokinesia (both TD and NM) caused by a 50-nM/rat dose of SAM (Table 1). At 1 and 2 h before SAM, the same dose of L-dopa was without a significant effect (Table 1). A control dose of L-dopa (200 mg/kg) without SAM did not influence (1,302 ± 224 cm) the TD.

Brains of rats that were killed 1 day after receiving a single injection of SAM (1 μM/rat) showed no significant damage, but at 4 days postinjection there were cellular disruptions in areas proximal to the injected lateral ventricle. The ependymal cell layer of the ipsilateral (Fig. 4D) SAM-injected lateral ventricle was disrupted and the cells of both caudates (Figs. 4C and 4D) of SAM-injected rats showed dense Nissl substances when compared to control (Fig. 4B). The intensity of TH-IR in the substantia nigra (SN) of the same group of rats (Figs. 5A and 5B) was less than the intensity in the SN of control rats (Fig. 5F). The decrease in the intensity of the TH-IR was the pronounced in the ipsilateral SN (Fig. 5B) than in the contralateral side (Fig. 5A) of SAM-treated animals. The TH-containing fibers in the SN of SAM-injected rats appeared

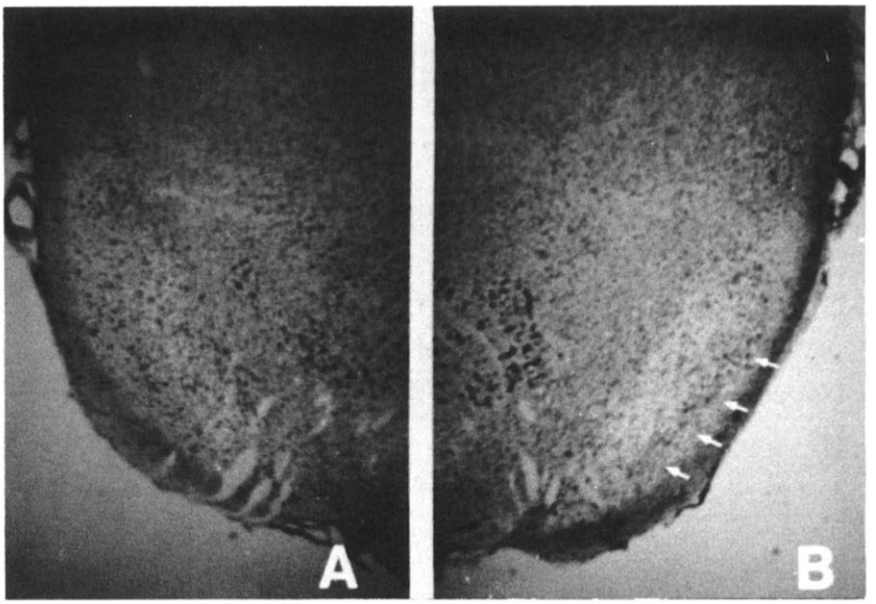

FIG. 6. Substantia nigra (SN) of a SAM-injected rat. The accumulation of phagocytic cells (arrows), the relative absence of larger neurons and the denuded and hypochromatic appearance of the SN, ipsilateral to the injection (B), as compared to the contralateral side (A), are shown.

degenerated—evidenced by a significant disruption (more bead-like) of the fiber profile of the contralateral side (Fig. 5C) and the ipsilateral side (Fig. 5D), as compared to the ipsilateral side (Fig. 5E) of PBS-injected rats. To more thoroughly examine the tissue degeneration caused by SAM, rats were injected for 4 consecutive days and killed 6 days after the last injection. In these animals, SAM caused major tissue disruptions at and proximal to the injection site and involved the caudate nucleus. The substantia nigral region showed a reduction in area and a decrease in the population of the larger neurons (Fig. 6B). The ipsilateral SN of SAM-injected rats appeared denuded and hypochromatic and contained an accumulation of phagocytic cells (Fig. 6B, arrows). These findings are indicative of degeneration in the SN.

DISCUSSION

In a previous study, it was shown that injection of SAM into the lateral ventricle of mice caused tremors and impaired motor functions (10). The onset, intensity, and duration of the effects were dose dependent and were antagonized by L-dopa (10). The results from the present study show that SAM can induce tremors and other abnormal motor functions in rats. SAM decreased both the TD that rats traveled and the NM made. SAM has a relatively greater effect on reducing the TD than on reducing the NM. This means that in the presence of SAM rats traveled a shorter distance per movement; this is seen as a decrease in the ratio of the TD to the NM (TD/NM) for SAM-injected rats as compared to the TD/NM for controls. Therefore, SAM may serve to decrease the motivation or capability to move, as well as the ability to continue moving once a move is initiated.

The patterns of the SAM-induced motor impairments, for example, the postural tremors, seen in rats may be different from the Parkinsonian types of hypokinesia that occur in humans. This may be due to the fact that humans are supported by two hindlimbs whereas rats are supported by all four limbs. This means that tremors of the forelimbs would likely cause postural shaking in rats. Despite the anatomic differences, however, there are marked physiological similarities between the two impairments. For example, following injection of SAM tremors occur mainly in the snouts and forelimbs of rats that may be compared to the occurrence of tremors mainly in the lips, hands, and fingers of human PD patients. Furthermore, the immobility demonstrated in rats, evidenced by them remaining propped for extended periods, may be similar to the motor freezing that occurs in human PD patients.

The SAM-induced motor aberrations in rats have many of the features reported for the subacute toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (28,39), an agent used to produce animal models of Parkinsonism. The substantial occurrence of postural tremors that is activated by external stimuli, freezing episodes, Straub-tail phenomenon (28), the stimulus-sensitive action myoclonus, the exaggerated startled response or acuity to sensory stimulation, and the hesitation prior to movement that were reported for MPTP (39) are all reminiscent of the impairments caused by SAM.

The observation that L-dopa blocked the SAM-induced hypokinesia is another indication that the hypokinetic effects of SAM may be similar to the hypokinesia observed in Parkinsonism because L-dopa is used to counteract the symptoms of PD. The relatively long preinjection time of 4 h that was required for L-dopa to block the effects of SAM may be related to the slow absorption of L-dopa from the peritoneal cavity. The effects of L-dopa in this study may involve its conversion to DA in the brain. The newly increased DA levels would offset the depletion of DA, known to occur after the cerebral ventricular injection of SAM (9). L-Dopa may also react directly with SAM, thereby decreasing the levels and reactivity of SAM in the brain. The depletion of SAM by L-dopa has been demonstrated (43,46), and such reaction may be responsible for the production of 3-*O*-methyldopa in laboratory ani-

mals and PD patients following the administration of L-dopa (18,23,32). Accordingly, the suggestion that SAM may be increased in PD also infers that L-dopa, during its use as treatment for PD, may play a key role as a depletor of SAM.

Several biochemical events in PD can be explained by an increase in the SAM-dependent methylation process. A SAM-dependent increase in the methylation of DA, NE, and 5-HT may help to explain the depletion of DA, NE, melanin (Fig. 7), and 5-HT that occur in PD. The methylation of DA may also explain the observed increase in the HVA : DA ratio (HVA/DA) in the striatum (47) and urine (5), and the appearance of a DIMPEA-like product in the urine (2) of PD patients, noting that HVA is formed principally from 3-methoxytyramine, the monomethyl analog of DA (42) and DIMPEA is the di-methyl analog of DA. The depletion of melanin in PD may be due to the fact that tyrosine and dopa, the likely precursors for melanin, will be shunted from melanin synthesis toward the catecholamine methylation pathway (Fig. 7).

The methylation of biogenicamines will occur during the course of the disease as well as during therapy with L-dopa. The production of methylated biogenicamines may contribute to the therapy-related problems observed in some PD patients following L-dopa therapy because high levels of plasma 3-*O*-MD were shown to correlate with the incidence of L-dopa-induced dyskinesia (23,32), and the simultaneous administration of 3-*O*-MD with L-dopa to Parkinsonian patients produced clinical deterioration (8,11,33). The reports showing that increases in methylated biogenicamines occur following L-dopa administration (43,46), and that various methylated amines (17), including DIMPEA (3,17), cause hypokinesia in experimental animals, suggest a direct action for the methylated biogenicamines in antagonizing the action of L-dopa in addition to the role of 3-*O*-MD as a competitor for the transport mechanism of L-dopa.

The decrease in TH immunoreactivity in the substantia nigra following injection of SAM may be related to the neuronal damage that was also observed in the substantia nigra. The

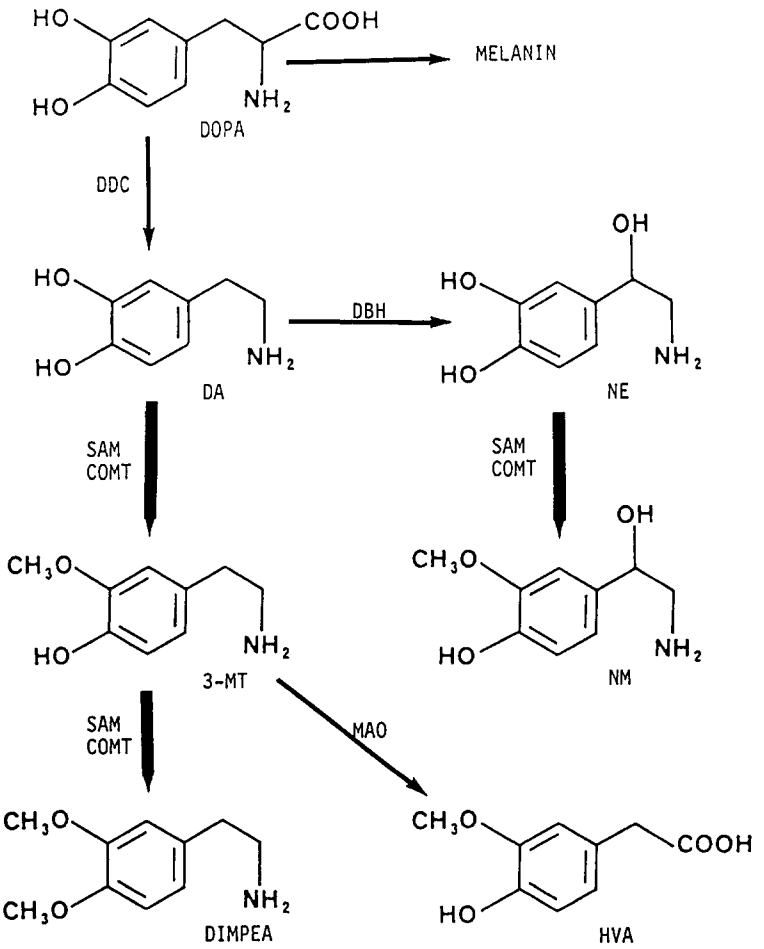

FIG. 7. Reaction (heavy arrows) of SAM with DA, 3-methoxytyramine (3-MT), and NE. It shows how it is possible for excessive methylation to deplete DA and NE and increase DIMPEA and HVA/DA. Increased methylation may also cause the depletion of melanin merely by shunting L-dopa toward the methylation of DA and NE and away from the production of melanin. DOPA, L-dopa; DDC, dopa decarboxylase; DA, dopamine; SAM, *S*-adenosylmethionine; COMT, catechol-*O*-methyltransferase; 3-MT, 3-methyoxytyramine; DIMPEA, dimethoxyphenylethylamine; DBH, dopamine betahydroxylase; MAO, monoamine oxidase; NE, norepinephrine; HVA, homovanillic acid.

results also show that SAM causes disruption in the striatum. Although degeneration in the striatum is not regarded as an important occurrence in PD, it is clear that losses of striatal dopaminergic terminals occurred during the disease.

Similar to the anatomic effects of SAM the toxic effects of MPTP and 1-methyl-4-phenylpyridinium (MPP^+), the active metabolite of MPTP, are seen preferentially (7) but not exclusively (39) in the nigrostriatal dopaminergic system. Like MPTP, 6-hydroxydopamine also preferentially affects the dopaminergic system, but high doses will interfere with the noradrenergic systems as well. The most important biochemical effect of MPTP and 6-hydroxydopamine is a depletion of DA and an increase in HVA/DA. These biochemical changes are significant findings in PD, and we recently found that SAM caused an increase in the HVA : DA ratio (unpublished results) as well as depletion of DA (9); therefore, there are significant similarities between the effects of MPTP, 6-hydroxydopamine, and SAM.

It should be noted that similar to the proposed effects of SAM and the reported effects of MPTP all the symptoms of PD patients are not due to the impairments of the basal ganglia. The basal ganglia are primarily affected and are accountable for the major symptoms that are seen in PD, but lesions have been identified, also, in the other nervous tissues (1, 14,20,27,30,34,37,45), as well as in the adrenal medulla (26) of PD patients. The involvement of various non-nigrostriatal brain regions may help to explain the occurrence of depression, cognitive impairments, anorexia, orthostatic hypotension, sweating, and other autonomic dysfunctions observed in some PD patients.

The proposed selective effects of excess methylation on the basal ganglia may be explained only if the specialized features of the nuclei that composed the basal ganglia are considered, for example, the physical, chemical and functional properties of the caudate nucleus (CN). The CN possesses a large surface area of exposure to the ventricle and contains the terminals for the substantia nigral dopaminergic cells. It is enriched with DA and L-dopa, both of which are potent methyl acceptors. The CN also maintains a high metabolic activity (40). SAM is a polar agent and will accumulate in the cerebrospinal fluid (CSF), thus placing SAM in close proximity to the CN, separated only by a thin barrier of ependymal cells. SAM disrupts the ependymal cell layer (Fig. 4); therefore, SAM will gain access to the CN. The accessibility of SAM to the CN, the high metabolic activities in the CN (40), and the presence of the reactive substrates, DA and L-dopa, will cause the CN to serve as an important reaction site for SAM. Another important feature that may allow SAM to show a dominant effect on the functions of the CN is the finely tuned, precise, and sensitive nature of the motor functions that the basal ganglia control, so slight interference with the order of operation of the basal ganglia by SAM, as compared to the interference with other neuronal areas, will be seen as impaired motor functions.

An in-depth and analytic examination of the symptoms and therapy of PD shows a striking similarity to the reactivity of SAM and the effects reported following its introduction into the brain. Thus, excess methylation may be related to the symptoms of PD. Excess methylation, though, may not be a singular causative factor in PD but may superimpose a primary condition that predisposes a person to PD. The predisposition could be due to the quantity of nigrostriatal DA cells with which a person is endowed. Therefore, in a person with a small number of nigrostriatal DA cells excess methylation may cause Parkinsonism by depleting DA and accelerating the reduction of DA cells to the critical level at which PD symptoms occur. Another person with the normal population of DA cells may not show the symptoms because the critical level of DA cells at which symptoms occur would not be reached. It is of interest to know that methylation reactions increase during aging, and there is also an obvious similarity between PD symptoms and the signs of aging, for example, the poverty of movements, postural defects, and a reduction in emotional expressions are seen in both cases. The increased methylation that occurs during aging may cause age-related onset of PD, in direct relationship to the population of substantia nigra DA cells, the predisposing factor, and may lend support also to the description of PD as a premature rapid aging of the striatal dopaminergic system (29).

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