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Quantification of *S*-Adenosylmethionine-Induced Tremors: A Possible Tremor Model for Parkinson's Disease

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LAMANGO, N. S., R. A. NESBY AND C. G. CHARLTON. *Quantification of* S*-adenosylmethionine-induced tremors: A possible tremor model for Parkinson's disease.* PHARMACOL BIOCHEM BEHAV **65**(3) 523–529, 2000.—Tremor is the most visible symptom of Parkinson's Disease (PD), and should be the appropriate parameter in models for its evaluation. Lack of reliable PD tremor models and methods to distinguish tremors from nontremor movements means that nontremor behavior such as rotation following basal ganglia damage are mostly used. Our laboratory has shown that S-adenosylmethionine (SAM) injections into the brain of rats reliably produced tremors, rigidity, hypokinesia, and abnormal posture. Thus, SAM-induced tremors, when distinguished from nontremor activities, has the potential as a model for testing anti-PD agents. Tremor Monitor-recorded activity profiles of the rats injected with SAM showed low-amplitude signals interlaced with high-amplitude bursts of tremor episodes. Control activities were of low–medium amplitudes with no such patterns. The number of real and apparent episodes detected over 20 min were 92 ± 12 and 84 ± 14 lasting 470 ± 50 and 210 ± 50 s, indicating mean durations of 5.1, and 2.4 s, frequencies of 12 ± 0.1 and 11 ± 0.2 Hz, cycles (waves) per episode of 54 ± 6 and 19 ± 2 and amplitudes of 42.3 ± 5 and 19.8 ± 1 for the SAM-treated and control rats, respectively. The nontremor activities of rats injected with phosphate-buffered saline were distinguished and eliminated by raising the minimum amplitude and number of cycles to 20. This procedure is being enhanced for screening antitremor agents and for elucidating the possible mechanism for Parkinsonism. © 2000 Elsevier Science Inc.

S-Adenosyl-L-methionine Parkinsonism Tremor Hypokinesia Rigidity

THE original name given to Parkinson's disease (PD) is the "Shaking Palsy" because of the prominence of tremors as a symptom of the disorder. The tremors in PD are of the resting type, because they occur when the muscles are in a state of relaxation. Postural tremors, that occur during isometric contraction of skeletal muscles, are also seen during the later stages of PD (17). Resting tremors, and to some extent postural tremors, therefore, are in general the key symptoms of PD and important markers of the disorder.

A pathological feature of PD is the degeneration of dopaminergic neurons, which have their cell bodies in the substantia nigra and their terminals projecting into the neostriatum. Dopamine is significantly depleted in the neostriatum of PD patients. This implies that changes to the substantia nigra and the neostriatal complex are linked to the tremors seen in PD. Therefore, agents that damage the nigrostriatal dopaminergic system and cause hypokinesia, rigidity, and tremors have the potential to be used as models for studying PD. Chemical agents such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxy dopamine (6-OHDA) damage the nigrostriatal dopaminergic neurons, and are most prominently used to produce models of PD. The effectiveness of these agents rely on their ability to cause significant damage to the nigrostriatal dopaminergic system, and the levels of symptoms are apparently dependent on the degree of nigrostriatal damage, which is somewhat difficult to control. As a result, the symptoms produced by these agents are predominantly rigidity, hypokinesia, and poverty of movements, and are not always consistent.

Nontremor movements, such as amphetamine induced ro-

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tation following 6-OHDA basal ganglia damage are routinely used to evaluate agents that may be effective as treatments for PD. This is a useful model but, again, tremors are not the parameter upon which the evaluations are based. Tremorine, the precursor of oxotremorine, an acetylcholinergic agent, has been reported to cause tremors in animals (6,7), and has been used to evaluate agents that may be useful against PD. The acetylcholinergic side effects of tremorine and its profound hypothermic actions, however, has precluded its widespread usage in an animal model of PD and tests based on this model had little or no predictive value in relation to PD (10).

It is clear that the ability to produce a reliable tremor model of PD has been associated with difficulties. For example, out of 10,000 agents, only 10 produced sustained tremors, the best of which was tremorine (6), which eventually was found to be unsuitable for screening potential anti-Parkinson's drugs (10). Tremors, however, remains the major symptom of PD, and it should be targeted as the major and most appropriate parameter used in animal models of PD—models that may find utility in the evaluation of agents with potential to serve in the treatment of PD.

Our laboratory has found that the injection of *S*-adenosylmethionine (SAM) into the brain of rodents reliably produced tremors and other movement disorders such as rigidity and hypokinesia (2,4,5). Unlike MPTP and 6-OHDA, the tremors caused by SAM occur acutely. The tremors may be due to biochemical changes, because the tremors are produced within 1 or 2 min after the intraventricular injection of SAM, a time when no tissue damage is evident. SAM reacts avidly with dopamine and results showed that at 15 min SAM depleted dopamine by about 50% in the otherwise normal animals (2). Therefore, the ability to induce tremors by SAM may be related to the diffusion of SAM from the lateral ventricle into the neostriatum and the avidity with which SAM reacts with and depletes dopamine. Histological damage to the substantia nigra occurred over a longer time exposure to SAM (3), indicating that the spectrum of changes caused by SAM resembles the behavioral and pathological symptoms of PD. It follows that SAM-induced parkinsonism, with tremor as an index of effect, has the potential to be developed into a model for testing antitremor agents. To develop such a model, it is important that SAM-induced tremors be distinguished from nontremor activities. To achieve this goal, rats were injected in the lateral ventricle with SAM or phosphate-buffered saline (PBS) and monitored. Signals relating to movements such as tremors, grooming, walking, and rest were all recorded using the Tremor Monitor. Changes in wave amplitude on the recording profile are proportional to the force associated with the movement generating the wave. The SAM-induced tremor episodes appeared on the recording profile as repetitive waves (cycles) of high amplitude signals interlaced with low amplitude recordings that were characterized by other PD symptoms such as abnormal posture and hypokinesia, but lacking the tremor-generated force. The activities detected in the PBS-injected animals, on the other hand, were devoid of such distinct episodes, and were mostly of low to medium amplitudes. These could be filtered out on the basis of their low amplitudes and fewer number of activity waves (cycles). The difference in frequency between the SAM- and PBS-treated rats was insignificant, indicating that frequency is not a distinguishing characteristic for the SAM-induced tremors as are amplitude and duration (cycles or waves per episode) of the high amplitude tremor signals.

METHOD

Materials

Male Sprague–Dawley rats weighing 200–300 g obtained from Harlam Laboratories, OH, were maintained under laboratory conditions of about a 12 L:12 D cycle with water and food supply ad lib, as described before (3). *S*-Adenosyl-L-methionine (SAM, chloride salt) was purchased from Sigma Chemical Co., St. Louis, MO. The tremor recording apparatus (Tremor-Scan Monitor, Accuscan Electronics, Inc., Columbus, OH) consisted of a sensor assembly that generates waves with amplitudes and frequencies determined by the forces and the rate of the movements generating the forces, the interface unit with gain and baseline adjustments, and an IBM compatible personal computer. This apparatus records and stores the activity profile from the sensor assembly in a continuous, time-dependent manner for subsequent analysis.

Monitoring of SAM-Induced Tremors

Rats were cannulated for subsequent injections into the lateral ventricle as previously described (3). Briefly, each rat was anesthetized with 400 mg/kg of chloral hydrate. A stainless steel guide cannula was stereotaxically placed 1.5 mm lateral and 0.6 mm caudal to the bregma, with the tip extended to the inner surface of the cranium, above the dura mater. At least 2 days following cannulation, injections were made through the cannula into the lateral ventricle with a 26-gauge insertion cannula, premeasured to descend 5 mm from the surface of the skull into the lateral ventricle. Each injected rat was put in a cage that was then placed on the sensor assembly of the Tremor-Scan Monitor. The recording parameters were set according to the manufacturer's instructions by adjusting the Gain on the instrument to 4, which ensured that most of the signals with large or small amplitudes remained within the detection limit or not reduced to the baseline respectively, as viewed on the computer monitoring screen. The noise that could interfere with the SAM-induced tremors is usually of low amplitude, and was reduced to baseline levels by this gain setting. This setting was used for all subsequent recording and storage of the vibration signals due to tremor-like activities of the SAM-injected animals as well as the random movement activities of the control animals.

The Tremor Monitor software, which allows for the determination of [1] the signal amplitude, [2] the number of cycles in each activity or episode, [3] the number of activities, and [4] the duration of each activity or episode, aided in the storage and subsequent retrieval and analysis of the recorded activity profiles. Analysis of the stored data was achieved by altering the minimum and maximum frequency, amplitude, and number of cycles to reflect the unique properties of each kind of movement and, thus, delineate the SAM-induced tremors from nontremor activity signals.

Dose Dependence of SAM-Induced Tremors

The dose-dependent effects of SAM was also determined in cannulated rats injected with 0 (PBS), 0.5, 0.75, and 1.0 μmol of SAM. Their tremor activities were recorded on the Tremor-Scan monitor for 20 min.

Time Dependence of SAM-Induced Tremors

To determine the duration and magnitude of SAM-induced tremors, groups of cannulated rats were injected either with 1 μmol of SAM or with 5 μl of PBS, as a control. The animals

were monitored on the Tremor-Scan Monitor for a duration of 2 h, starting immediately after the injections.

Statistical Analysis

Statistical analysis of the data was conducted using the GraphPad software program (GraphPad Software, Inc., San Diego, CA). A *p*-value, obtained by unpaired *t*-test, of less than 0.05 was considered to be significant.

RESULTS

Characteristics of SAM-Induced Behavior

Although it has been shown previously that SAM induces tremors, hypokinesia, abnormal posture, and rigidity in rats (2,5), a method for quantifying and characterizing the tremors had not been devised. In the present study, we consistently replicated physiological symptoms characteristic of PD in rats using the endogenous methyl donor, SAM. Normal behavioral activities such as grooming and rearing that characterized the PBS-treated animals were never observed in SAM-treated rats until after recovery. Within about 0.5 to 2 min following injection with SAM, rats moved less, the limbs became stiff and spread out, resulting in the animal lying on its ventral surface. The Straub-tail phenomenon, characterized by a stiff curled tail that depicts rigidity, was a common feature. The rigidity of the SAM-treated animals was also assessed as previously done by placing the injected animals with the fore limbs on a pedestal (5). Animals suffering from rigidity typically spend over 2 min before moving off the object. Furthermore, the rats feel rigid to the touch. To calibrate the Tremor-Scan Monitor, a rat treated with 1 μmol SAM and undergoing tremors was placed on the sensory assembly. A gain setting of 4 produced signals with the highest amplitude and minimal truncation at the tips when the animal was observed to be undergoing tremors. Only signals of intermediate amplitude, with no distinct pattern, were observed when the activity of rats treated with PBS and undergoing normal random movements were recorded (Fig. 1A). As shown in Fig. 1B and C, the records for the SAM-injected rats showed distinct episodic cycles of high amplitude tremors punctuated by low-amplitude signals characterizing hypokinesia, rigidity, and abnormal posture. It is worth noting that although activity profiles with low amplitudes could also be recorded in control animals completely at rest, these were distinguishable from the SAM-treated animals, because these were never in combination with any waves of high-amplitude tremor signals. Figure 1D–G shows an identical region of an 11-, 21-, 31-, and 41-fold condensed activity profile, respectively, revealing the wave characteristics of the tremor.

Raw data was obtained by combining the analysis parameters to ensure the registration of most of the tremors when the recordings from SAM-treated animals. For this initial analysis, a frequency window of 8 to 20, minimum amplitude of 10, and a minimum of 10 cycles per activity or episode were employed. Although tremors were never observed in PBS-treated animals, activity was registered in the recordings of both SAM- and PBS-treated rats. However, a comparison between these raw sets of data for the SAM- and the PBS-treated rats revealed significant differences. The PBS-treated animals displayed a lower number of cycles (waves) per activity, 19 ± 2 compared to 54 ± 6 for the SAM-injected animals (Fig. 2A). The average amplitude for both groups followed a similar pattern, 19.8 ± 1 and 42.3 ± 5 for the PBS- and SAM-treated rats, respectively (Fig. 2B). Similar frequencies were observed when recordings of PBS-treated (11 ± 0.2) and SAM-treated (12 ± 0.1) animals were analyzed. The raw data show that SAM-treated rats underwent tremors for a total duration of 470 ± 50 s within the 20-min monitoring period, whereas the PBS-treated animals underwent activities lasting 210 ± 50 s (Fig. 3A). the number of activities measured, however, was not significantly different for both groups, being 84 ± 14 for the PBS-injected rats and 92 ± 12 for the SAM-treated rats (Fig. 3B). Thus, nontremor related activities of control animals were also detected using the minimum amplitude and minimum cycles per activity of 10. These nontremor activities were eliminated when the experimentally derived values (illustrated in Fig. 2) of 20 for both the minimum amplitude and cycles per activity was used in reanalyzing the recorded data. These parameters registered most of the tremor activity in the recordings of the SAM-treated rats (360 ± 18 s) and almost none in the PBS controls (6 ± 4 s, Fig. 3C). Similarly, the number of tremor activities exhibited by the SAM-treated group was 70 ± 8 compared to only 2 ±1 detected for the PBS controls (Fig. 3D). It is worth noting that these more stringent analysis criteria underestimated the number and duration of the tremors, because tremor signals with amplitudes and number of cycles of less than 20 were eliminated from the data of the SAM-treated animals. The activities with amplitudes above 20 were practically nonexistent in the control animals. We observed that the activities recorded in the PBS-treated animals were, in fact, due to random movements and grooming.

Dose Dependence of SAM-Induced Tremors

SAM induced tremors in a dose-dependent manner, showing a steep dose response for both the number and duration of tremors. The total duration of tremors was minimal when rats were injected with 0.5 μmol of SAM but increased 9- and 12-fold when injected with 0.75 and 1 μmol of SAM, respectively, compared to the 0.5 μmol injection (Fig. 4A). The number of tremors recorded was minimal when rats were injected with 0.5 μmol of SAM but increased seven- and eightfold when the rats were injected with 0.75 and 1 μmol of SAM, respectively (Fig. 4B). This disproportionate increase in the duration and number of tremors when the dose of SAM was increased indicates that higher doses increased not only the number but also the duration of the episodes.

Time Dependence of SAM-Induced Tremors

A 1-μmol dose of SAM or PBS was administered to rats and their activities recorded over a 2-h period. The data was analyzed as stated above, and the total duration and number of tremors occurring within successive 10-min intervals following injection were registered. The total duration of tremors peaked within the first 10 min accounting for a fifth of the recording period (120 ± 20 s). In the successive 10-min periods, the total time that the rats underwent tremors declined in a first order fashion (Fig. 5A). As shown in Fig. 5B, the highest number of tremor episodes occurred during the first 10 min, at a rate of about two episodes per min. The number of tremors decreased in a first order fashion from the first 10-min interval value of 19 ± 4. The background noise seen in the control rats is apparently due to rapid and active grooming soon after handling. Although the severity of the tremors

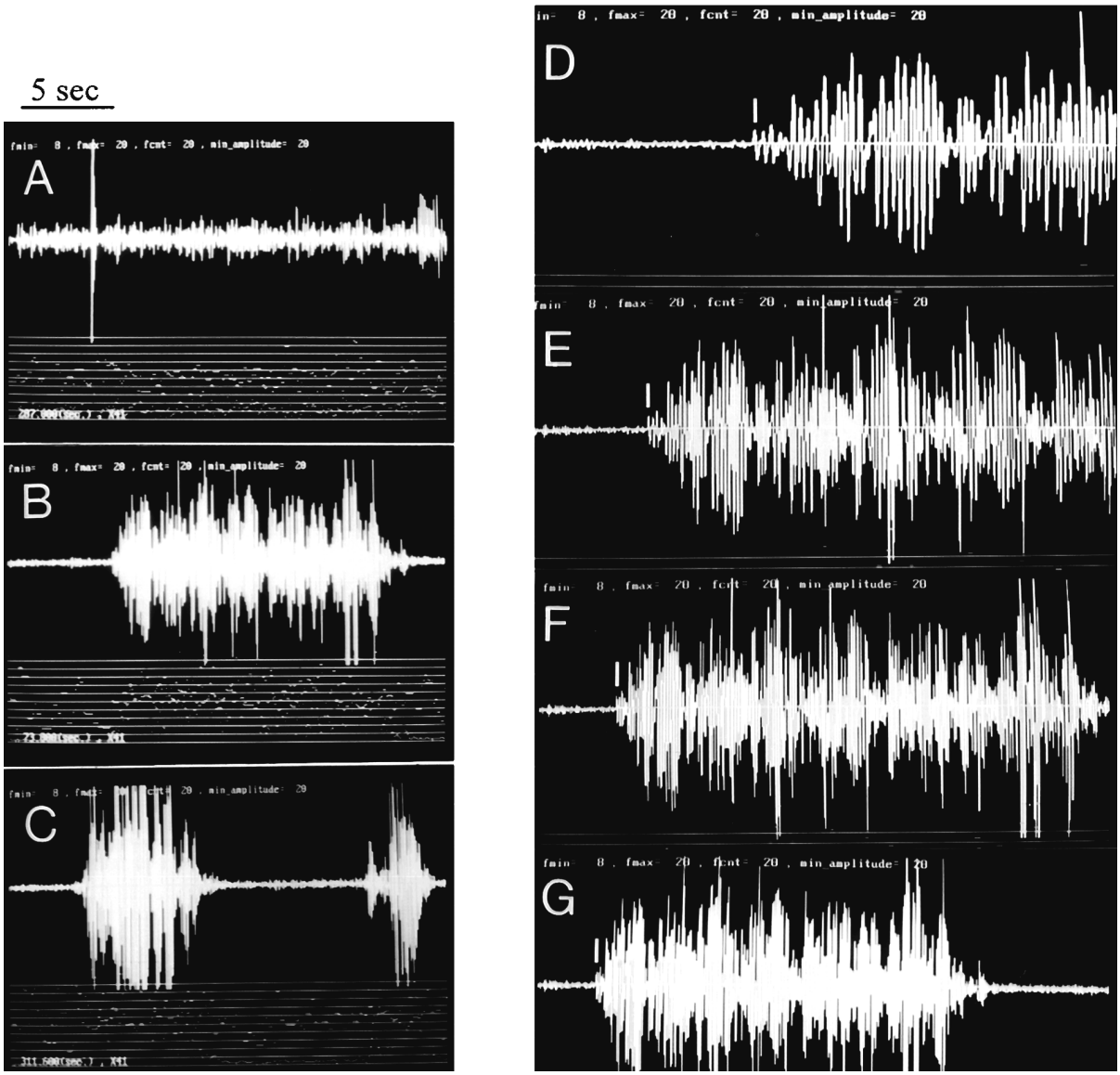

FIG. 1. Segments of Tremor Monitor activity profiles. Rats were cannulated as described in the Method section. At least 2 days after cannulation, they were injected with either PBS or 1 μmol of SAM and their movements recorded using the Tremor Monitor. Recorded sections from animals injected either with PBS (A) or 1 μmol SAM (B and C). In each case, the time axis of the activity profile has been condensed 41-fold. Recorded section from an animal injected with 1 μmol of SAM after the time axis of the activity profile was condensed 11- (D), 21- (E), 31- (F) and 41-fold, respectively. The onset of the tremor is indicated by |.

was almost reduced to control levels within 40 to 50 min following injection, abnormal posture, hypokinesia, and rigidity remained a characteristic feature for about 90 min. The recovery usually commenced with a gradual increase in mobility coupled with gradual raising of the ventral surface from the floor of the cage as the rigidity decreased and the animals regained their normal posture.

DISCUSSION

The effect of SAM in inducing tremors, hypokinesia, abnormal posture, and rigidity has been reported in rats and mice (2,4,5); however, the quantification and characterization of tremors has not yet been done. Tremors are the most identifiable symptom of PD; therefore, distinguishing tremors from nontremor activities and quantifying the SAM-induced tremors may enable the development of a SAM-induced tremor model for screening potential antitremor drugs that may find utility in the therapy of PD.

The mean wave frequency for the SAM-induced tremors was the same as the mean frequency for the activities exhibited by the control animals indicating that frequency was not an identifying characteristic for the SAM-induced tremors. This suggests that although frequency is generally used as an identifying characteristic for various types of tremors, the fre-

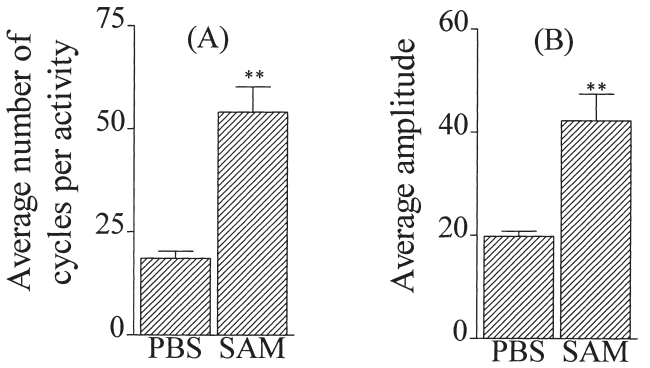

FIG. 2. Characteristic movement differences between PBS- and SAM-treated rats monitored. Twenty-minute activity profiles recorded and stored immediately following injection were analyzed with parameters set at 8, 20, 10, and 10, respectively, for minimum and maximum frequency, minimum amplitude, and minimum number of cycles (waves) so as to register the maximum number and duration of the observed tremors in the SAM-treated animals. Analysis of the recorded data from the control (PBS) and treated (SAM) animals yielded on average distinctly different number of cycles per tremor (A) and amplitude (B), $^{**}p < 0.01$ by unpaired *t*-test. The results are the means ± Sem, $n = 4$.

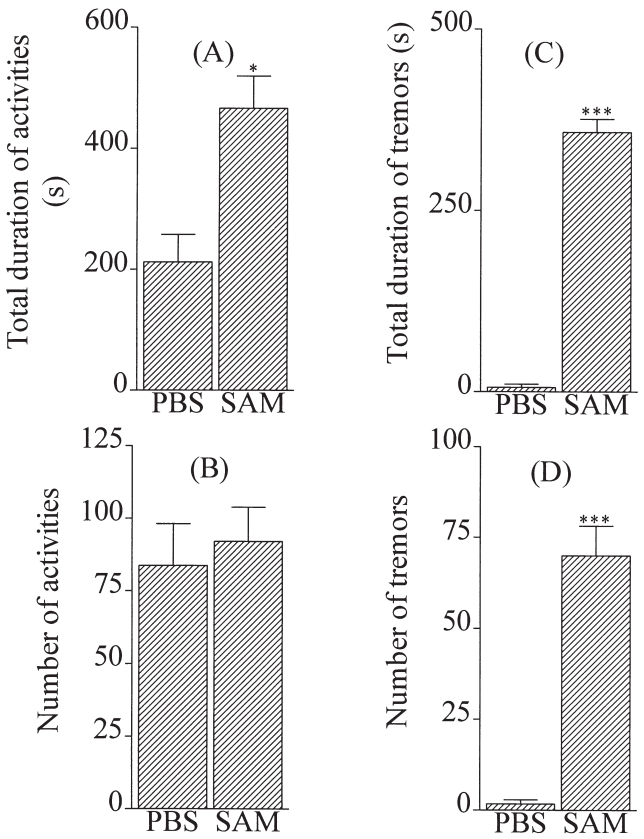

FIG. 3. Total duration and number of tremors. The recorded data for the animals described for Fig. 2 when analyzed with minimum and maximum frequencies, amplitude, and minimum number of cycles (waves) per tremor set at 8, 20, 10, and 10, respectively (A and B) or 8, 20, 20, and 20 to take into account the experimentally derived characteristic differences between SAM and PBS-injected rats as described in Fig. 2 (C and D), $^{*}p < 0.05$, $^{***}p < 0.001$ by unpaired *t*-test. The results are the means ± Sem, $n = 4$.

quency of the waves may not be a very relevant marker in rats. This is because the frequency band for the repertoire of activities exhibited by the normal rats may overlap the frequency at which tremors occur. In this study, the identifying characteristics for tremors were (a) waves of high amplitude, and (b) the number of such waves per episode. These parameters were 19 ± 2 and 19.8 ± 1 for the control rats and 54 ± 6 and 42.3 ± 5 for the SAM-injected rats, respectively. Setting the minimum number of waves per episode of 20 and a minimum amplitude of 20 eliminated the background noise and the nontremor activities.

The computational reevaluation of the stored data using the 8, 12, 20, and 20 setting for the minimum and maximum frequency, minimum number of cycles (waves) per episode, and signal amplitude, respectively, almost completely eliminated the background activities, registering tremors that occurred only in the SAM-treated animals. These parameters were used to determine the dose–effect relationship of SAM on inducing tremors and the duration of SAM-induced tremors. This study, which shows that SAM-induced tremors can be quantitatively measured, raises the possibility that the procedure may be useful for antitremor drug testing.

Such a model, which is based on the tremors induced by SAM, may offer certain advantages because it is centered around an endogenous biochemical of which the totality of its effects resemble the symptoms of PD. The model is quantitative, i.e., the degree to which a potential antitremor agent will inhibit the duration and number of tremors caused by SAM can be determined. In addition, the model offers economy in time, because the effects of SAM can be evaluated within 20 min after injection, and a single parameter—tremor—is used in the evaluation. Data generated from such a model would, therefore, be more precise. Furthermore, challenging the SAM-induced effects with agents with known pharmacological actions may help in elucidating the mechanism by which the tremors and the other PD symptoms are induced.

In the present study, we consistently replicated physiological symptoms characteristic of PD in rats, using the endogenous methyl donor, SAM; therefore, the precipitation of tremors is reliable and reproducible. In addition, the symptoms resemble the changes seen in PD and to some extent those reported for MPTP; but unlike the MPTP and the 6-OH-dopamine models, SAM is endogenous, and may, in fact, be involved with the symptoms of PD in humans. In addition to the observed tremors, there was marked rigidity and hypokinesia, especially with the higher dose levels of SAM. The behavioral and physiological changes manifested by SAM-treated rats are characteristic of the symptoms described by James Parkinson and widely reported as the hallmarks of PD (9,13,19).

The mechanism of SAM-induced toxicity is not known, but because the symptoms were reversible, it is possible that during the experiments reported here, SAM induced a reversible aberration of the normal homeostasis of vital biochemical processes that regulate neuromuscular functions. Based on the known role of SAM and the aberrations that

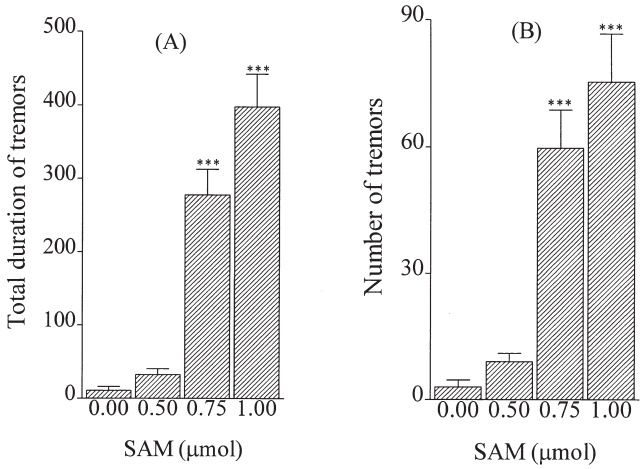

FIG. 4. Dose-dependence of SAM-induced tremors. Cannulated rats were injected either with PBS or varying doses of SAM and all movements recorded for the following 20 min. Data analysis with minimum and maximum frequencies, minimum amplitude, and minimum number of cycles (waves) per tremor set at 8, 20, 20, and 20, respectively, revealed that the total duration (A) and number of tremors (B) both increased with increasing doses of SAM injected between 0.5 and 1 μmol, $***p < 0.001$ when compared to PBS controls by unpaired *t*-test. The results are the means ± Sem, $n = 4$.

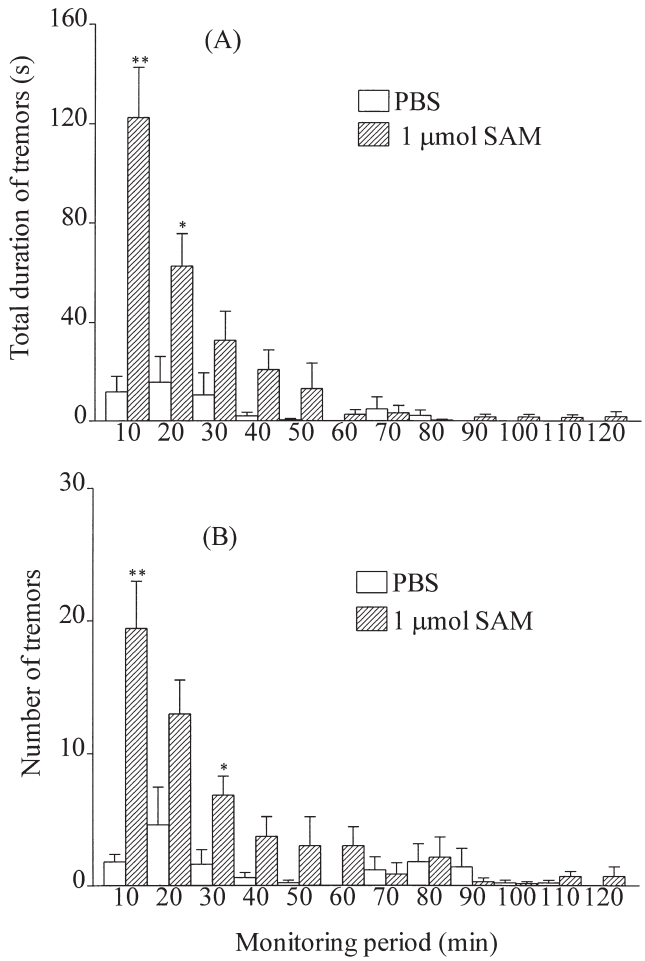

FIG. 5. Time-dependence of SAM-induced tremors. Cannulated rats were injected either with PBS or SAM and their movements recorded over a 2-h period. The total duration (A) and number of tremors (B) for successive 10-min intervals were computed using the same parameters as in Fig. 4, $*p < 0.05$, $**p < 0.01$ when compared to PBS controls for the respective time interval by unpaired *t*-test. The results are the means ± Sem, $n = 7$.

cause PD, it is likely that the biochemical effects of SAM include the methylation of catecholamine, such as dopamine and L-DOPA. This is of interest, because, dopamine and its precursor, L-DOPA, are avid methyl acceptors. SAM injections followed by a radioenzymatic assay have been shown to deplete ipsilateral DA levels in the caudate nucleus by about 50%, while L-DOPA at 200 mg/kg reversed the SAM-induced hypokinesia by 87% (2). Depletion of DA in the neostriatum is the major impairment associated with PD (11,16). SAM-induced tremors typically occur within seconds or minutes after injection, but repeated daily administration of SAM caused over 50% reduction of tyrosine hydroxylase (TH) immunoreactivities, TH-containing nerve fibers, and substantia nigra neurons (1,2,3,5).

The rapid onset of action of SAM-induced tremors may dispel a nucleic acid/gene expression-related process, but nucleic acid methylation may be involved in the nonacute effects. SAM also plays a role in the recently proposed hypothesis of apoptosis, which involves the methylation of Ras-related GTP-binding proteins (18). Other proteins that are candidates for methylation include the G-protein γ-subunit that is an important component of receptor and signaling systems of such critical neurotransmitters as dopamine whose levels are depleted in PD. Furthermore, SAM-induced methylation of proteins at the isoprenylated C-terminals represents a reversible biochemical process (12,20) essential for the biological activity of these proteins (8,14,15). A mechanism involving the signal transduction pathway is consistent with the rapid tremor-inducing effects of SAM.

Although the symptoms reported here lasted only for about 90 min, given that they were induced in otherwise normal animals with functional mechanisms to counteract the effects of a temporary excess of SAM, they, nevertheless, raise the interesting questions as to what may be the case in individuals with an upregulated SAM synthesis and/or downregulated apparatus for counteracting the effects of excessive methylation.

In summary, the study demonstrated that the injection of SAM into the lateral ventricle of rats induced tremors that could be quantified, and other motor deficits that resemble the changes seen in PD. Although the biochemical basis of SAM-induced PD symptoms merit further investigation, excessive SAM-dependent methylation hypothesis is credible in view of the experimental data presented, the reproducibility of the effects, the biochemical importance, and the endogenous nature of SAM, and the fact that SAM activity is increased in aging.

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