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Pharmacology, Biochemistry and Behavior 76 (2003) 433–442
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Inhibition mechanism of *S*-adenosylmethionine-induced movement deficits by prenylcysteine analogs

Nazarius S. Lamango*, Lambert T. Ayuk-Takem, Robert Nesby, Wan-Qian Zhao[1], Clivel G. Charlton[1]

College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA

Received 18 June 2002; received in revised form 13 December 2002; accepted 20 August 2003

Abstract

We previously showed that *S*-adenosylmethionine (SAM) induces movement impairments similar to those observed in Parkinson's disease (PD) apparently by prenylated protein methylation; 5 kDa molecules being methylated and the symptoms being inhibited by prenylcysteine (PC) analogs. In the present study, we explore the biochemical mechanism of action of the PC analogs. *N*-acetylgeranylcysteine (AGC), *N*-acetylfarnesylcysteine (AFC), *N*-acetylgeranylgeranylcysteine (AGGC), farnesylthioacetic acid (FTA), farnesyl-2-ethanesulfonic acid (FTE) and farnesylsuccinic acid (FMS), but not farnesylthiotriazole (FTT) and farnesylthiolactic acid (FTL), inhibited the SAM-induced motor impairments. Incubation of the respective analogs with rat brain membranes containing prenylated protein methyltransferase (PPMTase) resulted in the methylation of AGC, AFC and AGGC. FTA, FTE, FMS and FTT, but not FTL, inhibited the enzyme activity. A single injection of the active analogs remained effective for at least 3 days against repeated injections of 1 μmol SAM. Amphetamine-induced hyperactivity in rats was inhibited by SAM but potentiated by FTE. During 60 min, the movement time for amphetamine-treated rats was 1477 s compared with 633 and 1664 s for amphetamine + SAM- and amphetamine + FTE-treated rats, respectively. The total distance for amphetamine + FTE-treated rats was 82% higher than for amphetamine. The horizontal activity was 30,728 (amphetamine), 15,430 (FTE), 18,526 (amphetamine + SAM), 41,736 (amphetamine + FTE) and 7004 (SAM) as compared to the PBS control (4726). The intricate relationship between the actions of SAM, which speeds up prenylated protein methylation and impairs movement, amphetamine, which increases synaptic dopamine levels and movement, and the PC analogs, which prevent the SAM-induced movement impairments, suggests a SAM-induced defect on dopamine signaling as the likely cause of the symptoms. The data reveal that interaction of PC analogs with PPMTase may not be an indicator of anti-PD-like activity.

Keywords: Parkinson's disease; Methylation; Prenylation; Tremor; *S*-adenosyl-L-methionine; Prenylcysteine

1. Introduction

A significant proportion of the proteins assembled in cells are modified at their carboxyl terminal ends by a C15 farnesyl or C20 geranylgeranyl moiety (Anderegg et al., 1988; Lai et al., 1990; Marshal, 1993; Sinensky and Lutz, 1992; Yamane et al., 1991). Some of these proteins are associated with membrane-spanning receptors and relay signals from the ligand-activated receptors into the cell where they interact with various enzymes and ion channels (De Waard et al., 1997; Pumiglia et al., 1995). The prenylated portions of these proteins are thought to be important for functional interactions with cellular targets since non-prenylated isoforms obtained by site-directed mutagenesis show significant loss of activity against phospholipase Cβ and adenylyl cyclases (Hayes et al., 1999; Iniguez-Lluhi et al., 1992; Myung et al., 1999). These proteins eventually undergo methylation reactions on the terminal carboxylic acid group catalyzed by the *S*-adenosylmethionine (SAM)-dependent enzyme, prenylated protein methyltransferase (PPMTase) (Perez-Sala et al., 1998; Volker et al., 1991). It has been shown that methylation increases the hydrophobicity of the proteins and enhances their ability to associate with cellular membranes (Fukada et al., 1994). Parish et al. (1995) showed that methylated G-protein βγ-dimer had a

* Corresponding author. Tel.: +1-850-412-7377; fax: +1-850-599-3347.
E-mail address: nazarius.lamango@famu.edu (N.S. Lamango).
[1] Present address: Department of Pharmacology and Center for Behavioral and Molecular Neuroscience, Meharry Medical College, 1005 DB Todd Blvd., Nashville, TN 37208, USA.

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doi:10.1016/j.pbb.2003.08.017

10-fold greater activation potency on phospholipase C and phosphoinositide 3-kinase than the unmethylated form, indicating that methylation may fulfill a more specific role than the mere increase in hydrophobicity. Carboxylmethylation in islets and HIT cells is stimulated by guanosine 5′-[α-thio]triphosphate (GTP-γ-S) while methylation inhibitors inhibit amino acid-induced insulin release from pancreatic islets (Metz et al., 1993).

Prenylcysteine (PC) analogs, which are synthetic mimics of the modified C-terminals of the prenylated proteins, act as competitive substrates or inhibitors of PPMTase (Perez-Sala et al., 1992; Tan et al., 1991). PC analogs also affect a range of cellular and physiological processes. For example, farnesylthiosalicylic acid (FTS) elevated Ca^{2+} concentrations in cells (Tisch et al., 1996) and inhibited Ras-dependent cell growth (Aharonson et al., 1998; Marom et al., 1995). In other studies, PC analogs inhibited capacitative Ca^{2+} entry into cells (Xu et al., 1996), receptor-mediated G-protein activation (Scheer and Gierschik, 1995) and either inhibited or stimulated superoxide production in neutrophils (Ding et al., 1994). GTP-γ-S-induced platelet aggregation was inhibited by *N*-acetylfarnesylcysteine (AFC) (Huzoor-Akbar et al., 1993). In this regard, earlier pharmacological effects of the synthetic analogs were believed to be a result of the inhibition of the methylation of endogenous prenylated protein substrates. Increasing evidence indicates, however, that other targets may be largely responsible for the pharmacological effects of PC analogs since some analogs that do not interact with the enzyme by neither acting as substrates nor inhibitors, do indeed, still invoke pharmacological responses (Ma et al., 1994).

The Gγ subunits of trimeric G-proteins that relay neurotransmitter messages from membrane receptors into cellular effectors (reviewed by Gudermann et al., 1996) undergo prenylation and methylation. The fact that the activities of enzymes such as adenylyl cyclase and phospholipase Cβ are strongly affected by G-βγ dimers implies that improperly regulated methylation/demethylation may result in undesirable physiological consequences. Interestingly, Parkinson's disease (PD) is a neurological disorder that is associated with the progressive degeneration of dopaminergic neurons (Hornykiewicz, 1966). Dopamine, the principal neurotransmitter involved, employs the G-protein-anchored receptor system. In previous studies (Charlton and Crowell, 1992; Crowell et al., 1993; Lamango et al., 2000) we revealed that tremors, hypokinesia, rigidity and postural abnormalities could be induced in rats by intracerebroventricular injections of the endogenous methyl donor, SAM. This induction of symptoms was later correlated to the methylation of small molecular weight proteins that might have resulted in the symptoms (Lamango and Charlton, 2000). Repeated SAM injections also caused the loss of tyrosine hydroxylase immunoreactive neurons in the brains of injected rats (Charlton, 1997). The inhibition of the PD-like symptoms by the PC analogs—AFC, farnesylthiopropionic acid (FTP) and FTS (Lamango and Charlton, 2000), suggested that the symptoms may be due to the methylation of prenylated proteins. In addition, these compounds might have reversed the symptoms by competitively inhibiting the protein methylation process.

In light of the growing evidence that PC analogs may exert pharmacological effects by interacting with molecules other than the methylation enzyme prompted us to synthesize and test various analogs against PPMTase and the SAM-induced symptoms. While most of the analogs tested had inhibitory effects against the methylating enzyme and the motor impairments, FTT inhibited the enzyme activity but not the symptoms and FTL was without effect on both. Amphetamine-induced hyperactive behavior was inhibited by SAM but potentiated by FTE. It could thus be inferred from these data that the motor changes are a consequence of SAM-dependent methylation, which precludes the critical prenyl units from functional interactions with effectors in the cell and that these proteins may be functionally substituted by the appropriate PC analogs.

2. Materials and methods

2.1. Materials

Geranyl chloride, *trans*,*trans*-farnesyl bromide, all *trans*-geranylgeraniol, DL-thiolactic acid, mercaptosuccinic acid, thioacetic acid, 2-mercaptoethanesulfonic acid, 1*H*-1,2,4-triazole-3-thiol and phosphorus tribromide were purchased from Sigma-Aldrich. Male Sprague–Dawley rats weighing 200–300 g were obtained from Harlam Laboratories, Ohio, and maintained under laboratory conditions as previously described (Charlton and Crowell, 1995). Animal care and use was approved by the Florida A&M University Animal Care and Use Committee, which ensures that the NIH Health Guide for Care and Use of Laboratory Animals is adhered to.

2.2. Synthesis of PC analogs

All *trans*-geranylgeranyl bromide was synthesized from all *trans*-geranylgeraniol and phosphorus tribromide according to the method of Fukuda et al. (1981). The resulting all *trans*-geranylgeranyl bromide was reacted with *N*-acetylcysteine to form *N*-acetyl all *trans*-geranylgeranylcysteine (AGGC) according to the method of Tan et al. (1991). *N*-acetylgeranylcysteine (AGC) was synthesized from geranyl chloride and *N*-acetyl cysteine as previously described (Tan et al., 1991). Farnesyl-2-mercaptoethanesulfonic acid (FTE), farnysylthioacetic acid (FTA), farnesylmercaptosuccinic acid (FMS), DL-farnesylthiolactic acid (FTL) and farnesylthiotriazole (FTT) were each synthesized from *trans*, *trans*-farnesyl bromide and 2-mercaptoethanesulfonic acid, thioacetic acid, mercaptosuccinic acid, DL-thiolactic acid and 1,2,4-triazole-3-thiol, respectively, according to the procedure of Tan et al. for the synthesis of FC analogs. The resulting analogs (Fig. 1) were then purified by silica gel chromatography and analyzed by thin layer chromatography.

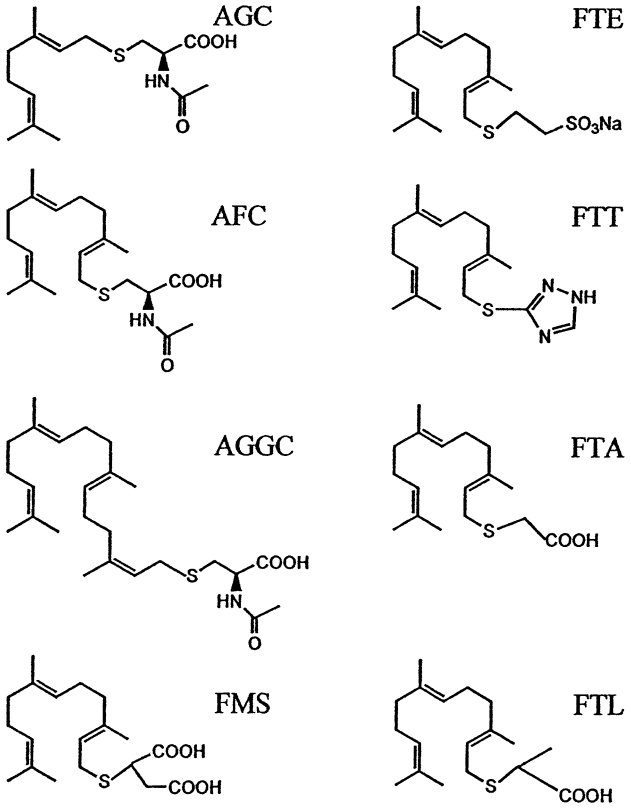

Fig. 1. Structures of the PC analogs.

2.3. Methylation of PC analogs

Rat brain membranes were prepared as previously described (Lamango and Charlton, 2000). To determine whether the PC analogs could be methylated by PPMTase present in the membrane preparation, the respective analogs were dissolved in DMSO and incubated with rat brain membranes (400–500 μg of protein) in the presence of [^{3}H-methyl]-SAM (0.1 μM, 0.5 μCi) containing 100 mM Tris–HCl, pH 7.4, 5 mM $MgSO_4$ and 0.1 M NaCl at 37 °C. Reactions were stopped at the appropriate time intervals with 5% TFA and extracted with 200 μl of hexane. After centrifugation (4000 × *g* for 5 min), the upper hexane layer was removed and analyzed by reversed-phase (RP)-HPLC and radiochromatographic detection as previously described (Lamango and Charlton, 2000). PC analogs that were not methylated were tested for their ability to interact with the PPMTase by measuring their ability to inhibit the methylation of AFC. For this purpose, the methylation of AFC was conducted in the presence of each PC analog (200 μM).

2.4. Effect of PC analogs on SAM-induced motor impairments

Male Sprague–Dawley rats were cannulated for subsequent injection into the lateral ventricle as previously described (Crowell et al., 1993). Briefly, each rat was anaesthetized with 400 mg/kg chloral hydrate. A stainless steel 21-gauge 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. Injections were conducted through a sharp-pointed stainless steel 26-gauge needle that was premeasured to descend into the lateral ventricle via the cannula.

Studies were conducted during afternoon periods under dim light and at room temperature. Each PC analog (1 μmol/5 μl) was either dissolved or suspended (AGGC and FTT) in PBS. The pH of each solution was determined to be 7.4 and each was allowed to warm up to room temperature before injection. Unless stated otherwise, each PC analog was injected 5 min prior to the injection of 1 μmol SAM (1 μmol/5 μl). Each compound was injected at a rate of about 2 μl/min using a sharp-pointed 26-gauge needle through a 21-gauge cannula. After each injection, the animals were immediately placed in a Tremor Monitor (AccuScan Electronics, Columbus, OH) and their tremor activity recorded over a 20-min period. The Tremor Monitor uses a force transducer interfaced with a personal computer to detect and store forces generated by the movements of the animals. Signature parameters are subsequently used to decipher and quantify tremor signals from nontremor activity. The long-term ability of some of the PC analogs to inhibit the SAM-induced symptoms was studied by subjecting animals previously treated with the respective PC analogs with repeated injections of 1 μmol SAM (1 μmol/5 μl) at 0.08, 2, 4, 24 and 48 h post-PC analog injection. These were then immediately monitored for tremors for 20 min after each SAM injection. Our previous study with PC analogs (Lamango and Charlton, 2000) revealed that the side of injection (left or right) of PC analogs did not affect its potentiation of intraperitoneally-administered amphetamine-induced ipsiversive rotation of 6-hydroxydopamine-lesioned rats. Apart from AGGC and FTT that were not soluble in PBS, all the other PC analogs and SAM were completely soluble at the concentrations used and were therefore expected to diffuse easily in the cerebrospinal fluid.

2.5. Effect of FTE and SAM on amphetamine-induced hyperactivity

Cannulated male Sprague–Dawley rats were injected intracerebroventricularly with either PBS, FTE or SAM (1 μmol of each in 5 μl of PBS) followed by an intraperitoneal injection of PBS or amphetamine dissolved in PBS (5 mg/ml, 5 mg/kg). Various movement parameters of the animals (movement time, total distance, horizontal activity and the number of movements) were then studied in an Animal Activity Monitor (AccuScan Instruments, Columbus, OH) over a 60-min period as previously described (Lamango and Charlton, 2000). The Animal Activity Monitor uses a grid of invisible infrared beams to track the location of animals and compute their various movement parameters.

2.6. Statistical analysis

Tremor and movement behavior data were analyzed by one-way ANOVA followed by Newman–Keuls multiple comparisons using the GraphPad Prism computer software program (GraphPad Software, San Diego, CA). A *P* value of less than .05 was considered to be significant.

3. Results

3.1. Methylation of PC analogs

When the individual PC analogs were incubated with rat brain membrane preparations in the presence of [^{3}H-methyl]-SAM followed by RP-HPLC with radiochromatographic detection, peaks of radioactivity were detected in samples that included AGC, AFC and AGGC as the putative substrates (Fig. 2). The radioactive peaks indicate the presence of [^{3}H-methyl]-esters of the PC analogs. On the contrary, no such peaks of radioactivity were detected in samples that included FTA, FTE, FMS, FTT and FTL, indicating the absence of PC analog methylation. A time-dependent study of the methylation of AGC, AFC and AGGC revealed a rapid rate of methylation that peaked within 1 h of the start of incubation (Fig. 3). The time taken for the methylation level to peak depended on the amount of enzyme used, decreasing with increasing amount of membrane preparation (results not shown). As shown in Figs. 2 and 3, prolonged incubation also resulted in a decrease of the methylation products.

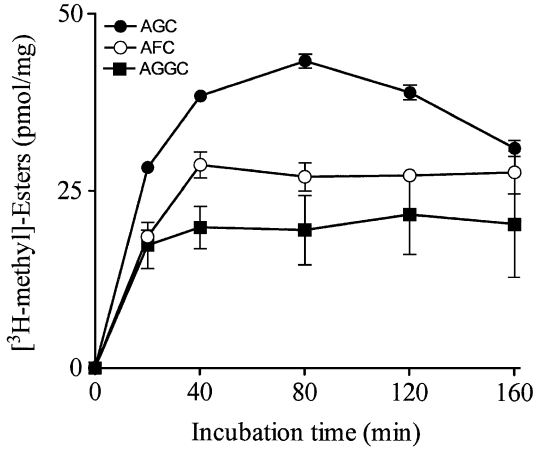

Fig. 3. Time course of the methylation of PC analogs. Incubations were conducted as described in Fig. 1 and in the Materials and methods section. Following RP-HPLC analysis, the peaks of radioactivity were quantified and the amount of radioactive methyl incorporation was computed with the help of a calibration curve obtained by RP-HPLC analyses of the initial radioactivity.

The PC analogs that are not substrates of PPMTase may interact with the enzyme as competitive inhibitors of the methylation process. For this reason, the possible inhibitory

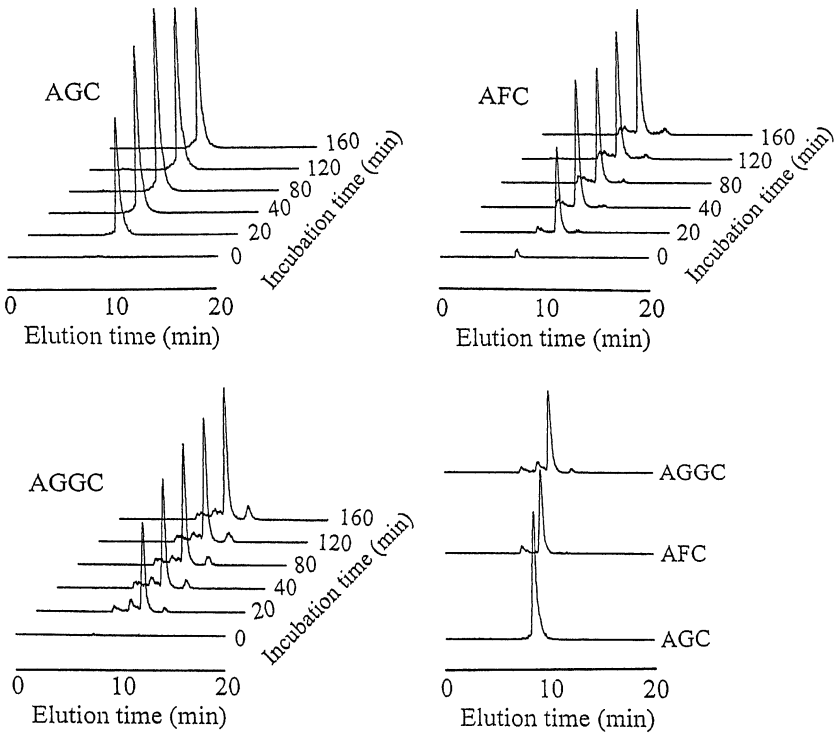

Fig. 2. Methylation of PC analogs. Rat brain membranes (400–500 μg of protein) were incubated with [^{3}H-methyl]-SAM (0.1 μM, 0.5 μCi) containing 100 mM Tris–HCl, pH 7.4, 5 mM $MgSO_4$, 0.1 M NaCl and the respective PC analogs (200 μM) at 37 °C. At the indicated time intervals, the reactions were stopped with 5% TFA and extracted with 200 μl of hexane. The hexane extracts were then analyzed by RP-HPLC with online radiochromatographic detection. The relative elution times of AGC, AFC and AGGC are depicted in the lower right panel.

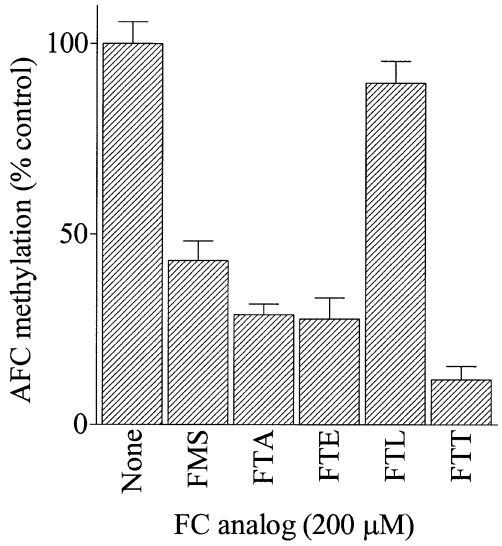

Fig. 4. Inhibition of AFC methylation by PC analogs. AFC (200 μM) was incubated with rat brain membranes, 200 μM of the indicated PC analog and [^{3}H-methyl]-SAM as described in the Materials and methods section. After 20 min, the reactions were stopped with 5% TFA, extracted with hexane and analyzed by RP-HPLC with online radiochromatographic detection. The radioactive peak areas were quantified and the relative methylation in the presence of the various PC analogs was computed as a percentage of the activity in the controls (± S.E.M., $N=3$).

effects of the nonsubstrate PC analogs on the methylation of AFC were investigated. The respective analogs were incubated with rat brain membranes in the presence of AFC and [^{3}H-methyl]-SAM. As shown in Fig. 4, FTA, FTE, FMS and FTT, but not FTL, inhibited the methylation of AFC by rat brain membranes. Identical results were observed with different enzyme preparations.

3.2. Effect of PBS and SAM on body temperature

Cannulated rats were injected either with 5 μl of PBS or 1 μmol of SAM contained in 5 μl of PBS. At about 5 min when all the SAM-injected animals were undergoing severe tremors in conjunction with rigidity, hypokinesia and abnormal posture, their body temperatures were measured with a temperature probe inserted through the anus. The body temperatures of the PBS-treated animals were measured at about the same 5-min postinjection period. The PBS-treated rats displayed a body temperature of 37.77 ± 0.07 °C compared with 38.04 ± 0.10 °C for the SAM-treated animals. Prior to any injections, the cannulated rats displayed a body temperature of 37.88 ± 0.08 °C. The difference between these values was insignificant as determined by one-way ANOVA followed by Newman–Keuls multiple comparisons ($N=5$).

3.3. Inhibition of SAM-induced motor impairments by PC analogs

We previously showed that SAM-induced tremors, hypokinesia, rigidity and abnormal posture in the rat could be blocked by AFC, FTP or FTS. Accordingly, we studied the

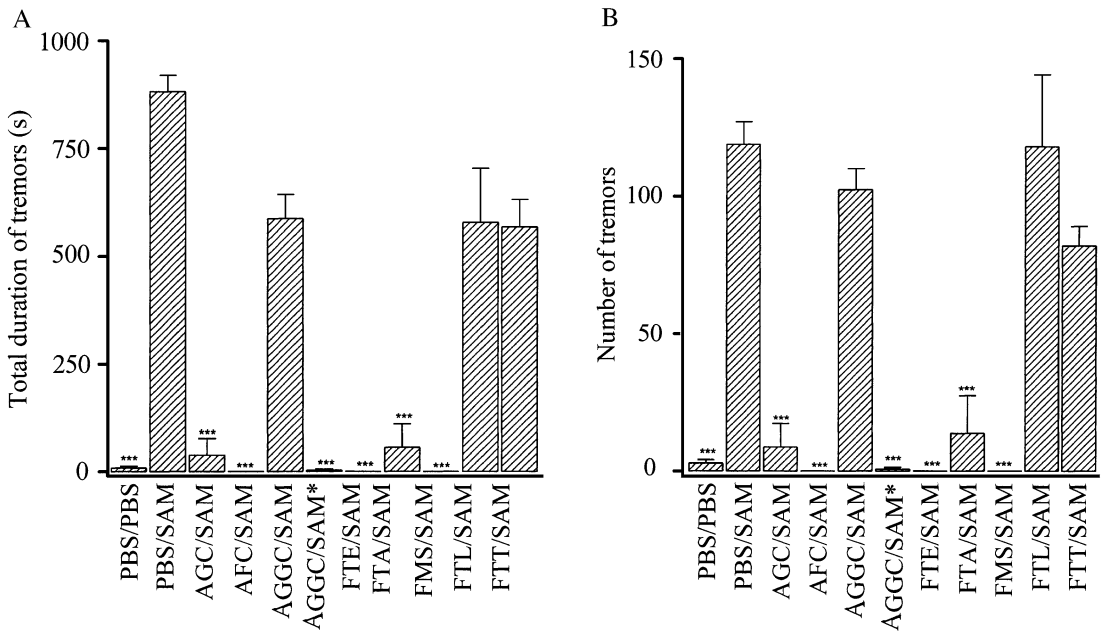

Fig. 5. Inhibition of SAM-induced symptoms by PC analogs. Cannulated rats received intracerebroventricular injections of either PBS (5 μl) or the indicated PC analog (1 μmol dissolved in 5 μl of PBS) 5 min prior to injection with 1 μmol of SAM. The animals were then monitored for tremors on a Tremor Monitor over a 20-min period. The total duration of tremors (panel A) and number of tremors (panel B) were obtained by analyses of the raw data using the data analysis software supplied with the instrument as previously described (Lamango et al., 2000), * * * $P<.001$ when compared to the PBS/SAM samples by one-way ANOVA followed by Newman–Keuls multiple comparisons. The results are the means ± S.E.M., $N=6$, AGGC/SAM* denotes a repeated injection of 1 μmol SAM 3 days after the injection of 1 μmol AGGC and SAM.

effects of structural modifications of the PC analogs on their ability to reverse the SAM-induced effects. The short-term and long-term (0.08, 2, 4, 24 and 48 h) inhibitory effects of a single PC analog administration against the SAM-induced motor deficits were studied by repeated injections of 1 μmol of SAM. Each injection of SAM was followed immediately by a 20-min monitoring period during which each animal was analyzed for tremors on the Tremor Monitor. Cannulated rats injected with PBS (5 μl) followed 5 min later by an injection of 1 μmol of SAM showed the cardinal symptoms of PD within 2 min. However, when the SAM injections were preceded by an injection of the C10 (AGC) or the C15 (AFC) PC derivatives, no tremors, hypokinesia, rigidity and abnormal posture were observed (Fig. 5). AGGC (the C20 PC derivative) did not block the severe symptoms if SAM was injected 5 min after AGGC treatment. Injection of the same AGGC animals with 1 μmol of SAM 3 days later revealed neither tremors nor the other motor impairments (Fig. 5). FTE, a farnesyl derivative in which the *N*-acetyl-L-cysteine of AFC is replaced by 2-mercaptoethanesulfonate, also blocked the SAM-induced motor impairments. FTA and FMS, but not FTL and FTT, inhibited the SAM-induced PD-like symptoms, significantly reducing both the number and duration of the tremors.

Because AGGC blocked the SAM-induced symptoms only after significant prior exposure to the PC analog, tests were conducted to see whether such prior exposure might also be required for FTL and FTT to inhibit the SAM-

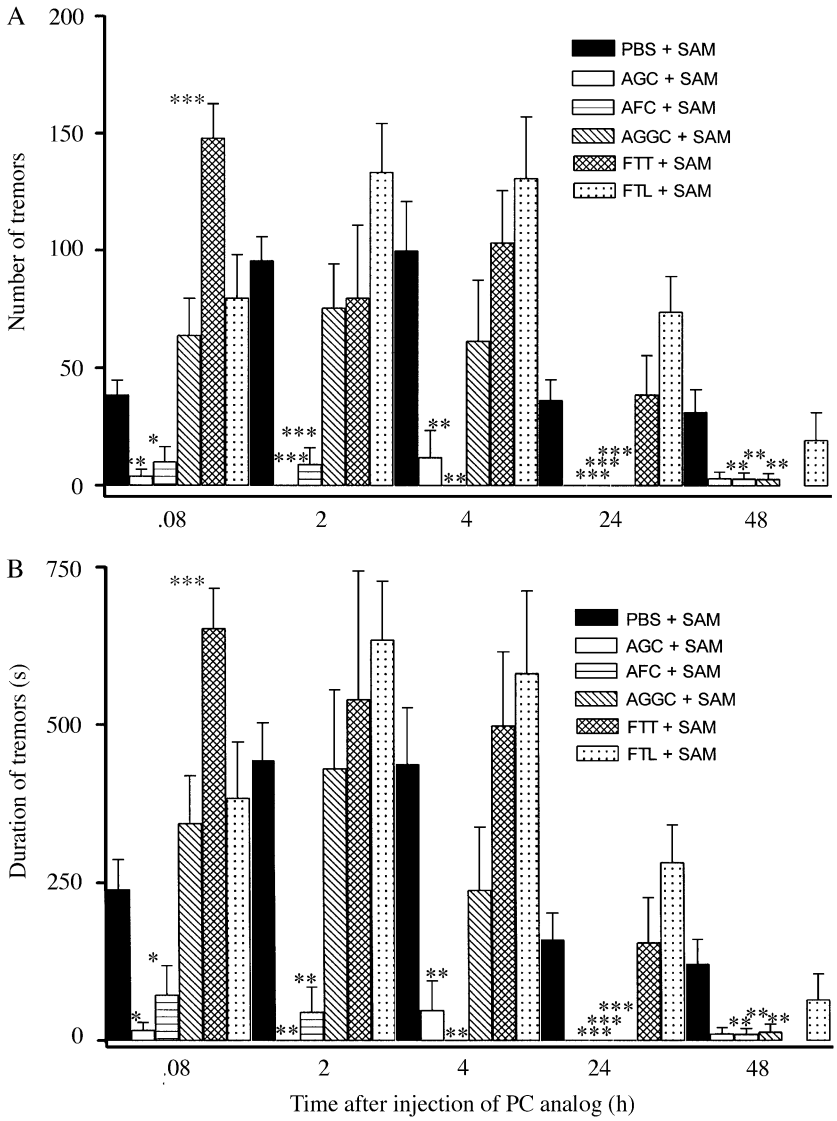

Fig. 6. Effect of repeated SAM injections following a single administration of PC analog. Cannulated rats received 1 μmol intracerebroventricular injections of the indicated PC analogs followed by repeated injections of 1 μmol of SAM at the indicated times. After each SAM injection, their movement activities were recorded on a Tremor Monitor over a 20-min period and the recorded data analyzed for the number (panel A) and duration (panel B) of tremors as before (Lamango et al., 2000). The results are the means ± S.E.M., $N=6$, * $P<.05$, * * $P<.01$, * * * $P<.001$ when compared to the SAM-treated animals by one-way ANOVA followed by Newman–Keuls multiple comparisons.

induced symptoms. Long-term studies were also conducted on the effects of AGC and AFC. As shown in Fig. 5, AGC and AFC were immediately effective soon after injection and remained so for at least 48 h as depicted by the number and duration of tremors following repeated injections of SAM. On the contrary, similar treatment of rats with FTL and FTT displayed a lack of effectiveness by both compounds. For the first 4 h after injection with 1 μmol of AGGC, no significant difference in the number and duration of tremors relative to the controls was observed. However, SAM treatment of the same animals 24 and 48 h posttreatment with AGGC revealed a significant reduction of tremors relative to control (Fig. 6).

3.4. Antagonistic effects of SAM and FTE on the activity of amphetamine-injected rats

When cannulated rats were injected intraperitoneally with amphetamine, a significant increase in activity over PBS controls was observed as depicted by the movement time (700%), total distance (660%), horizontal activity (550%) and the number of movements (425%, Fig. 7). Animals that received intracerebroventricular injections of SAM plus amphetamine (ip) displayed movement activity parameters that were respectively 13%, 80%, 35% and 30% less than those that received amphetamine alone. On the contrary, intracerebroventricular injections of FTE increased

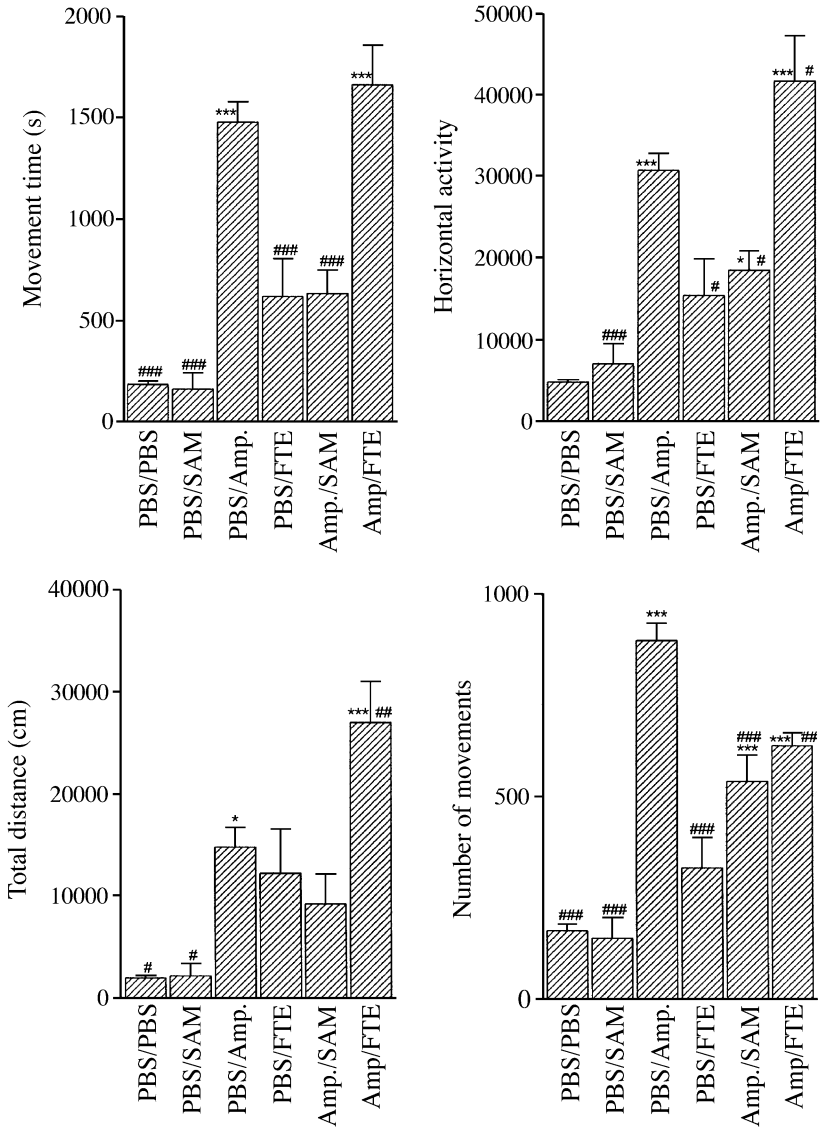

Fig. 7. Opposing effects of SAM and FTE on amphetamine-induced hyperactivity. Cannulated rats received intraperitoneal injections of either PBS or amphetamine (5 mg/ml, 5 mg/kg) followed by intracerebroventricular injections of PBS (5 μl), SAM (1 μmol) or FTE (1 μmol). They were then put in an Animal Activity Monitor and the corresponding movement parameters recorded over a 60-min period. The results are the means ± S.E.M., $N=6$, $^{*\#}P<.05$, $^{\#\#}P<.01$, $^{***\#\#\#}P<.001$ when compared to the PBS-only- and amphetamine-only-treated animals by one-way ANOVA followed by Newman–Keuls multiple comparisons.

the movement of rats by at least twofold over PBS controls. When injected in conjunction with an intraperitoneal administration of amphetamine, significant increases in the horizontal activity and the total distance over amphetamine-only-treated rats were recorded while at the same time significantly reducing the number of movements. This may suggest an increased tendency towards incessant movement (Fig. 7). It may be noted that SAM (PBS/SAM) caused no significant effect on the movement of the animals when compared to the PBS (PBS/PBS) controls (Fig. 7). This is due mainly to the fact that the SAM-treated animals suffer from tremor-related restlessness that is inevitably recorded as movement. This movement that is usually associated with the animals searching for a position to rest their heads at the onset of each tremor episode is absent from PBS-control animals once they finish investigating the cage at the beginning of the test period. Unfortunately, most of the SAM-induced tremors peak within the first 10 min (Lamango et al., 2000), thus making it difficult to include a conditioning period immediately following the injections. To summarize, SAM significantly decreased amphetamine-induced hyperactivity while FTE significantly increased this activity.

4. Discussion

Previous work from this laboratory revealed that SAM-induced motor deficits similar to those observed in PD and are associated with the methylation of small molecular weight proteins (Lamango and Charlton, 2000). Prenylated proteins are substrates for methylation, so it was of interest to observe that the motor deficits were reversed by PC analogs. PC analogs mimic the C-terminal modifications of prenylated proteins and act as competitive inhibitors against their methylation. The inhibitory effects of PC analogs on SAM-induced symptoms strengthen the belief that the symptoms may be precipitated by an increased methylation of prenylated proteins. Although methylation may be responsible for the motoric changes caused by SAM, the lack of effect by FTT despite its inhibition of the methylation activity, implicates other cellular targets in the mechanism of action of PC analogs. FTT has been reported to stimulate superoxide release as well as inhibit methyltransferase activity with a K_i of 34 μM (Ding et al., 1994). Although somewhat opposite and depending on the structure of the particular analog, similar patterns have been observed whereby PC analogs that failed to act either as inhibitors or substrates of the methylating enzyme did however counteract platelet aggregation (Ma et al., 1994) and superoxide release (Ding et al., 1994). There is a significant body of evidence indicating that the portion of prenylated proteins that undergo methylation plays a major role in protein/protein interactions resulting in effector action (Hayes et al., 1999; Myung et al., 1999). The conformational changes that may arise as a result of methylation may render the methylated proteins unavailable for functional binding to certain cellular targets and thus precipitate the symptoms. Like kinase/phosphatase control mechanisms, methylation/demethylation reactions also involve the removal or addition of charges to the prenylated region of certain proteins that may result in allosteric changes. Methylation-induced depletion of the functional prenylated but unmethylated proteins might have been functionally replaced by the appropriate PC analog so as to avert the symptoms.

The carboxylic acid group of FTA is separated from the thioether link by one less methylene group when compared with AFC. As such, FTA was never expected to act as a competitive substrate. However, its ability to inhibit the enzyme activity and the symptoms was not surprising since it has been shown to have similar effects against platelets (Ding et al., 1994; Ma et al., 1994), bovine retina (Tan et al., 1991) PPMTase, fMet-Leu-Phe-stimulated superoxide release (Ma et al., 1995) and induction of cell apoptosis (Perez-Sala et al., 1998). A more interesting finding was the lack of inhibitory potency displayed by FTL against PPMTase and the symptoms. The only difference in structure between FTL and FTA is the additional methyl group on FTL. This might have resulted in steric hindrance and/or a nonfunctional enantiomer. Steric hindrance may be a more plausible reason since a racemic mixture would have resulted from a synthesis utilizing DL-thiolactic acid with one of the isomers being active.

Our data reveal a strong tendency of AGC, like AFC and AGGC, to act both as a substrate of the PPMTase and an inhibitor of the SAM-induced symptoms. Previous studies employing AGC, AFC and AGGC revealed a relatively insignificant effect of AGC on pancreatic exocrine secretion (Capdevila et al., 1997). The C10 prenyl chain length that is characteristic of AGC as opposed to AFC (C15) and AGGC (C20) has previously been shown to be relatively ineffective against EJ cell growth when the thiosalicylic acid derivatives were tested (Aharonson et al., 1998). In another study, only a small inhibitory effect on platelet aggregation was observed with AGC (Huzoor-Akbar et al., 1993). Our findings may indicate that only smaller concentrations of the PC analogs are required to reverse the SAM-induced physiological response and that a high local concentration of AGC might have been injected into the cerebrospinal fluid. Also, the lower potency of AGC observed in other studies may not be due to lack of specificity but a consequence of lower binding affinity of a shorter prenyl chain.

It is interesting to note the opposing effects of SAM and the PC analog, FTE, on the amphetamine-induced hyperactivity in rats. Amphetamine acts on dopaminergic neurons causing the release of and inhibits the reuptake of dopamine. Therefore, compounds that either inhibit or exacerbate the effects of amphetamine as well as induce or reverse SAM-induced changes are most likely interacting with dopamine signaling. Since the basal ganglia of the dopaminergic system is principally affected in PD, this may have significant implications to our understanding of PD; noting that

SAM and prenylated proteins are endogenous and the PC analogs mimic critical portions of the prenylated proteins. The association of some PC analogs with the superoxide free radicals (Ding et al., 1994), which are very destructive to cellular membranes with the induction of apoptosis in HL-60 cells (Perez-Sala et al., 1998) and the inhibition of cell growth (Marom et al., 1995), may hold strong implications for the neurodegenerative disorders such as PD, since SAM-dependent methylation induces PD-like symptoms in rats that are inhibited by PC analogs. PC analog injections induce a twofold increase in the net amphetamine-induced ipsiversive rotations of rats following a unilateral intrastriatal infusion of 6-hydroxydopamine (Lamango and Charlton, 2000). This suggests that the SAM-induced motor aberrations may be similar to those of PD since the 6-hydroxydopamine-lesioned rat is a widely accepted model for the study of PD. The methylation effects of SAM on various prenylated proteins involved in neurotransmitter signaling, cell proliferation and transforming activity indicate a possible mechanism by which aberrant SAM metabolism may cause the anatomical and symptomatic characteristics of neurodegenerative disorders such as PD. The biochemical and physiological effects of the PC analogs that mimic portions of these proteins are further testimony to the important role played by these proteins in normal and diseased states. The specificity of the PC analogs is revealed by the structural discrimination between those analogs that inhibited the symptoms and PPMTase interaction as well as the lack of activity by farnesol, the synthetic precursor of the analogs.

Acknowledgements

This work was supported by NIH Grants NS28432 and G12RR03020.

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