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ON THE ROLE OF *O*-METHYLATION IN THE METABOLISM OF *S*-ADENOSYLMETHIONINE IN RAT BRAIN

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Abstract—The effects of tropolone and pyrogallol in areas of the rat brain with a high and low density of catecholaminergic innervation, i.e. the striatum and cortex, on *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) concentrations were studied and related to the extent of catechol-*O*-methyltransferase (COMT) inhibition. Moreover, the effects of drugs enhancing dopamine (DA) or noradrenaline (NA) utilization in these areas were also investigated. Pyrogallol reduced the concentrations of SAM in a similar manner in both areas and increased SAH much more in the cortex than in the striatum; these effects corresponded to that on *O*-methylation in terms of dose–effect relationships, indicating that there is no compartmentation of SAM with respect to the methylation process in which it is used. Tropolone increased SAM and decreased SAH in the striatum only, and these effects occurred at somewhat higher doses than the inhibition of COMT. Together with the data showing that DA antagonists decrease SAM in the striatum, this suggests that a significant proportion of SAM metabolism in this area results from *O*-methylation of DA (or its deaminated metabolite). A number of antidepressants did not alter the levels of SAM in either area, but some of the drugs increased SAH in the cortex. However, this was not correlated with their effects on the noradrenergic system. Inhibition of the synthesis and decarboxylation of SAM by cycloleucine and methylglyoxal bis(guanylhydrazone) (MGBG), respectively, did not cause the expected pattern of changes, i.e. decreases of both SAM and SAH in the former case and either increase or no change in both parameters in the latter. Instead, both cycloleucine and MGBG increased SAH while decreasing SAM, suggesting an involvement of other properties of these drugs.

S-Adenosine-L-methionine (SAM) is the principal methyl donor in a large number of biological methylation reactions, one of them being the *O*-methylation of catecholamines and their catechol-containing metabolites by catechol-*O*-methyltransferase (COMT). Administration of substrates of COMT, such as L-DOPA or pyrogallol (which as a competing substrate can be used as a COMT inhibitor) leads to a decrease in SAM concentration in the brains of rats and mice [1, 2], and, as demonstrated more recently, to a marked increase in those of *S*-adenosyl-L-homocysteine (SAH), formed from SAM in the transmethylation process [3, 4]. Moreover, it has been reported that various drugs interfering with catecholaminergic neurotransmission, such as the monoamine oxidase (MAO) inhibitor pargyline (but not other MAO inhibitors, i.e. pheniprazine, iproniazid or tranylcypromine) and the inhibitors of monoamine uptake, imipramine and desipramine, considerably reduced the levels of SAM in rat or mouse whole brain [1, 5]. Neuroleptic agents and reserpine showed no or small effects. Discrepant results were reported with D-amphetamine [1, 5]. In all these instances, the alterations of the levels of SAM were associated with the ability of these drugs to enhance synaptic availability of noradrenaline and/or its metabolite 3,4-dihydroxyphenylglycol for *O*-methylation [1, 5].

Tropolone inhibits COMT *in vivo* without undergoing *O*-methylation, unlike pyrogallol [1, 6]. Consequently, it does not decrease rat hypothalamic SAM concentration and prevents the depletion induced by L-DOPA [4]. It seemed of interest to study whether tropolone is able to increase SAM levels in a brain area with a high catecholamine concentration, where a significant proportion of this cofactor might be consumed by *O*-methylation. The area of choice for this purpose is, of course, the corpus striatum. On the other hand, drugs which enhance DA turnover might specifically or preferentially deplete the levels of SAM in this area. Moreover, it seemed of interest to investigate whether the reported SAM decreases by desipramine and imipramine are restricted to areas with a predominant noradrenergic innervation, e.g. the cortex. In the course of the study, it was also attempted to relate the extent of inhibition of COMT *in vivo* to the changes in tissue concentrations of SAM, and to see whether the latter were reflected in the concentration of SAH.

Pyrogallol was studied in comparison with tropolone. It was chosen from the class of competitive substrates despite its relatively low therapeutic margin, because it is known to become extensively methylated and thus represents an extreme opposite of tropolone in this respect. Both compounds enter the brain readily [6].

MATERIALS AND METHODS

Female Tif:RAIf (SPF) rats weighing 160–200 g and female Tif:MAGf (SPF) mice weighing 20–25 g were obtained from Tierfarm Sisseln (Switzerland).

Tropolone and pyrogallol were purchased from Fluka, cycloleucine from Calbiochem, scopolamine HBr from Merck (Darmstadt, F.R.G.), haloperidol from Cilag AG (Schaffhausen, Switzerland) and

methylglyoxal-bis-(guanylhydrazone) (MGBG) from Sigma Chemical Co. (St Louis, MO). Idazoxan and clozapine were generously donated by Reckitt & Colman Ltd. (Hull, U.K.) and Sandoz AG (Basel, Switzerland). Imipramine HCl and desipramine HCl are marketed compounds and (+)- and (−)-oxaprotiline HCl and brofaromine–HCl experimental compounds of CIBA-GEIGY.

Determination of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Striata were dissected from rat brain and stored frozen at −20° until analyzed. Pairs of striata were homogenized in 2 ml of the mobile phase for the HPLC separation described below, containing 1000 ng vanillic acid per extract as an internal standard. Cell debris was removed by centrifugation. Fifty to 200 μl of the supernatant were injected into a BAS liquid chromatography system (Bioanalytical Systems, W. Lafayette, IN) fitted with a C_{18}-μBondapak reversed phase column (Waters Ass., Milford, MA), a TL3 electrochemical detector cell, and a LC4 controller. The detector cell contained cp_w carbon paste and the potential was set to +0.85 V. The mobile phase contained 0.1 mol/l citric acid, 0.075 mol/l Na_2HPO_4, 2.5% tetrahydrofuran, and 0.05 mmol/l sodium octylsulphate and was brought to pH 3.0 with HCl. Column temperature was set between 28–40° and flow between 1–1.3 ml/min, as required to obtain optimal separation. Typical retention times were: DA: 3.9 min; 5-HT: 6.9 min; DOPAC: 8.45 min; tryptophan: 10.8 min; HVA: 16.7 min; vanillic acid: 19.6 min. Five animals were used per group.

Determination of 3-methoxytyramine (3-MT) accumulation after MAO inhibition by clorgyline. Rats received the test drug orally or intraperitoneally 5 min before the injection of 10 mg/kg s.c. clorgyline and were killed by microwave irradiation (10 kW operating power, 2450 MHz, exposure 1.7–1.8 sec, Pueschner Mikrowellen-Energietechnik, Schwanewede/Bremen, F.R.G.) 30 min later. After cooling of the animals, the striata were dissected out and homogenized in 2 ml 0.1 mol/l citric acid, 0.075 mol/l Na_2HPO_4, 2.5% tetrahydrofuran, and 0.05 mmol/l sodium octylsulphate, brought to pH 3.0 with HCl and containing 1000 ng vanillic acid as an internal standard. Cell debris were removed by centrifugation. Fifty to 200 μl of the supernatant were injected into a BAS liquid chromatography system (Bioanalytical Systems, W. Lafayette, IN) fitted with a C_{18}-μBondapak reversed phase column (Waters Ass., Milford, MA) and an ESA model 5100 A coulometric detector with a model 5010 detector cell (ESA Inc., Bedford, MA, potential of detector 2: +0.45 V, detector 1 switched off). The mobile phase was a citrate–phosphate buffer (made by mixing 0.1 M citric acid and 0.1 M Na_2HPO_4 at pH 3.0) containing 10% ethanol and 1.55 mmol/l sodium octylsulphate, pumped at a rate of 1.3 ml/min. Typical retention times for 3-MT and compounds which might potentially interfere with its determination are: DA: 6.0 min; DOPAC: 8.2 min; 3-MT: 10.8 min; 5-HT: 11.8 min; 5-HIAA: 14.6 min; tryptophan: 16.6 min; HVA: 18.4 min: vanillic acid: 19.4 min. Five animals were used per group.

Determination of the accumulation of O-methyl-DOPA formed from exogenously administered L-DOPA in rat brain. Rats received the test compound 15 min before 50 mg/kg i.p. DOPA methyl-ester hydrochloride and were killed by decapitation 1 hr later. Striata were dissected and frozen at −70° until analyzed. After thawing, they were homogenized in the mobile phase used for HPLC determination (see below), from which the detergent had been omitted and which contained 500 ng vanillic acid as an internal standard. Cell debris were removed by centrifugation. One hundred μl of the supernatant were injected into a BAS liquid chromatography system (Bioanalytical Systems, W. Lafayette, IN) fitted with a C_{18}-μBondapak reversed phase column (Waters Ass., Milford, MA) and an ESA model 5100 A coulometric detector with a model 5010 detector cell (ESA Inc., Bedford, MA). The potential of detector 1 was set to +0.15 V, at which *O*-methyl-DOPA is not yet oxidized, to oxidize compounds of no interest, thus "cleaning" the chromatograms; actual measurements were made at detector 2, the potential of which was set to +0.35 V. A guard cell (ESA model 5020) was put before the injection valve and its potential was set to +0.45 V. The mobile phase consisted of a 0.15 M chloroacetate buffer (adjusted to pH 2.9 with 5 M NaOH) containing 0.2 mM EDTA and 0.24 mmol/l sodium octylsulphate, pumped at a rate of 1.0 ml/min at 42°. The retention time of *O*-methyl-DOPA was 14.0 min. Five animals were used per group.

Determination of the levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in rat brain. SAM and SAH were isolated from brain regions of rats killed by decapitation on SEP-PAK C_{18} cartridges [7] and separated and quantitated by HPLC with photometric detection (absorbance at 254 nm), using the conditions given by Chabannes *et al.* [8]. The HPLC apparatus was a Waters (Waters Ass., Milford, MA) system consisting of a control station model 840, two pumps model 510, an autosampler with a cooling system model WISP 710B, a temperature control system and a UV-VIS detector model 490. The analytical column was a Waters C_{18}-μBondapak 30 cm × 3.9 mm. Retention times were 3.5 min for SAH and 5.1 min for SAM.

RESULTS

Dose–response relationships and time courses of tropolone and pyrogallol with respect to COMT inhibition in the rat striatum in vivo

Tropolone reduced striatal concentrations of HVA, the accumulation of 3-MT after inhibition of MAO by clorgyline and the accumulation of 3-*O*-methyldopa after treatment with DOPA with very similar dose–response curves. Approximately 50% inhibition was found at 3 mg/kg i.p. In contrast, pyrogallol inhibited the accumulation of 3-MT more than that of *O*-methyldopa, and it was even less potent to reduce the levels of HVA; they were, in fact, significantly increased at 30 mg/kg i.p. (Fig. 1). Tropolone at 10 and 30 mg/kg i.p. significantly increased striatal concentrations of DOPAC to 134 ± 5 and 143 ± 3% of controls. Pyrogallol caused a much more marked increase, to 165 ± 7% and 287 ± 8% of controls at 30 and 100 mg/kg i.p., respectively (absolute control values 837 ± 29 ng/g, N = 5). At

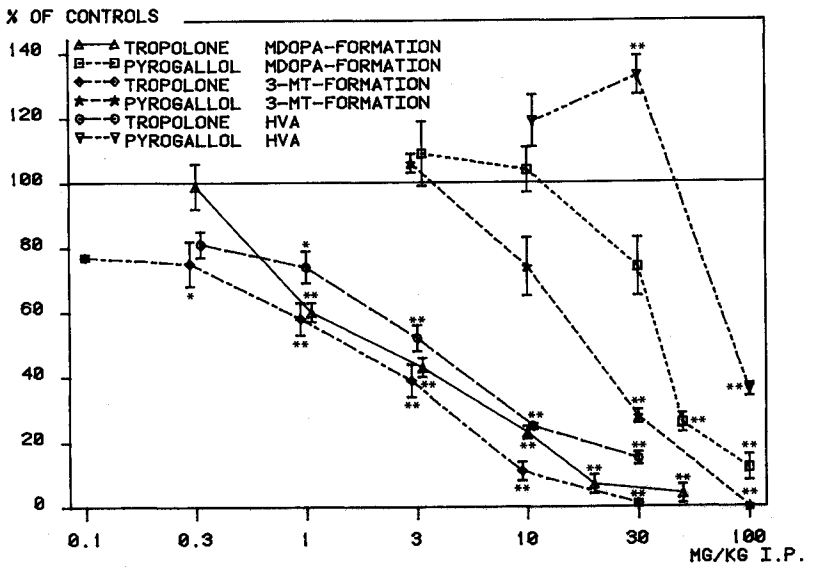

Fig. 1. Dose–response curves of effects of tropolone and pyrogallol in 3 experimental paradigms for the assessment of central COMT inhibition.

(a) Groups of 5 rats were treated with graded doses of the two compounds 1 hr before decapitation. HVA levels were determined in the striata. Data are means ± SEM in percent of controls (of which the absolute values were 557 ± 18 and 557 ± 31 ng/g in the two experiments).

(b) Groups of 5 rats received graded doses of the two compounds 5 min before 10 mg/kg s.c. clorgyline and were killed by microwave irradiation 30 min thereafter. 3-MT was determined in the striata. Data are means ± SEM in percent of the group receiving clorgyline alone (of which the means of the absolute values were between 227 and 264 ng/g).

(c) Groups of 5 rats were treated with graded doses of the two compounds 15 min before the injection of 50 mg/kg i.p. DOPA and were decapitated 1 hr later. *O*-methyl-DOPA (MDOPA) levels were measured in the striata. Data are means ± SEM in percent of the group receiving MDOPA alone (of which the means of the absolute levels were between 1496 and 1749 ng/g).

*$P < 0.05$ **$P < 0.01$ vs respective controls (Dunnett's test).

these doses of pyrogallol, the decreases of HVA were certainly not more marked than with 10 and 30 mg/kg tropolone, and the levels of DA were not affected (results not shown).

In one of the experiments in which the accumulation of 3-MT was studied after clorgyline, HVA and DOPAC were also measured. Pyrogallol at 10 mg/kg i.p. approximately doubled the levels of both metabolites of DA with respect to those measured after clorgyline alone. At higher doses, the levels of HVA gradually fell to reach 30% of the clorgyline controls at 100 mg/kg, whereas those of DOPAC rose to about 1200% at this dose (Table 1).

The duration of action of the two compounds was assessed by measuring striatal HVA (Fig. 2). Maximal decreases were found after 1 hr with both compounds. The disappearance of the effect of tropolone was slower than that of pyrogallol, being nearly complete after 6 hr. With the latter, a significant ($P < 0.05$, Dunnett's test) increase of HVA (126 ± 5% of controls) was seen after 2 hr, which subsided thereafter.

Time-courses of effects of tropolone and pyrogallol on SAM levels

Tropolone (30 mg/kg i.p.) gradually increased the

Table 1. Effect of pyrogallol on the striatal concentrations of 3-MT, DOPAC and HVA after treatment with clorgyline

Treatment	Dose (mg/kg)	3-MT (ng/g)	DOPAC (ng/g)	HVA (ng/g)
Controls		64 ± 27 (5)	646 ± 57 (5)	457 ± 38 (5)
Clorgyline	10 s.c.	244 ± 15 (5)	115 ± 7 (5)	177 ± 20 (5)
Pyrogallol	10 i.p.	148 ± 22 (5)	239 ± 31 (5)	352 ± 47 (5)*
+ clorgyl.	10 s.c.			
Pyrogallol	30 i.p.	42 ± 30 (4)*	687 ± 115 (4)*	244 ± 51 (5)*
+ clorgyl.	10 s.c.			
Pyrogallol	100 i.p.	0 (4)	1338 ± 88 (4)*	54 ± 33 (4)*
+ clorgyl.	10 s.c.			

Rats received graded doses of pyrogallol 5 min before clorgyline, and were killed by microwave irradiation 30 min after the latter. Data are means ± SEM of the concentrations measured in the striata.

*$P < 0.01$ (Dunnett's test) vs clorgyline.

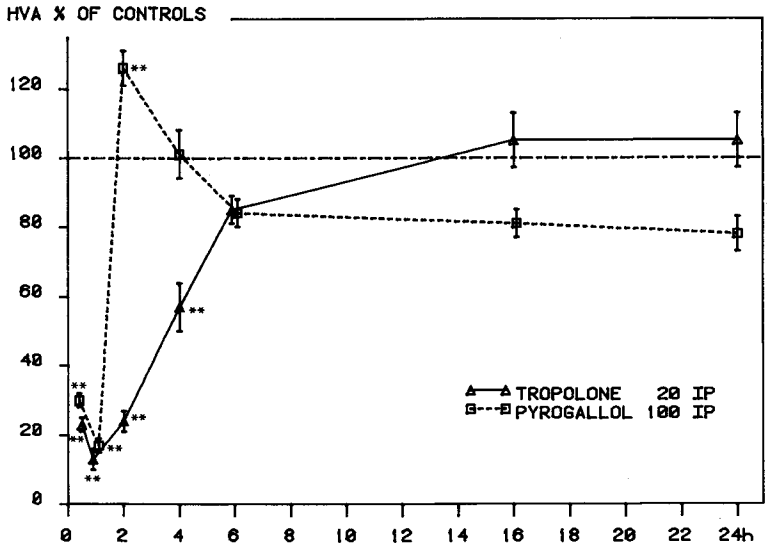

Fig. 2. Time-course of the effects of tropolone (20 mg/kg i.p.) and pyrogallol (100 mg/kg i.p.) on rat striatal HVA concentrations. Data are means ± SEM in percent of controls (N = 5). Absolute control levels were 646 ± 37 and 925 ± 66 ng/g in the two experiments. **P < vs controls (Dunnett's test).

concentration of SAM in the rat striatum with a maximum after 2 hr. The effect subsided after 6 hr. In the cortex, however, tropolone was without effect. Pyrogallol (300 mg/kg i.p.) caused marked and similar decreases of the levels of SAM in both striatum and cortex. The effect was maximal between 0.5 and 2 hr after administration and had disappeared at 6 hr. In the cortex, pyrogallol caused a marked rise in the levels of SAH after 30 min, which gradually subsided to reach control values after 6 hr. In the

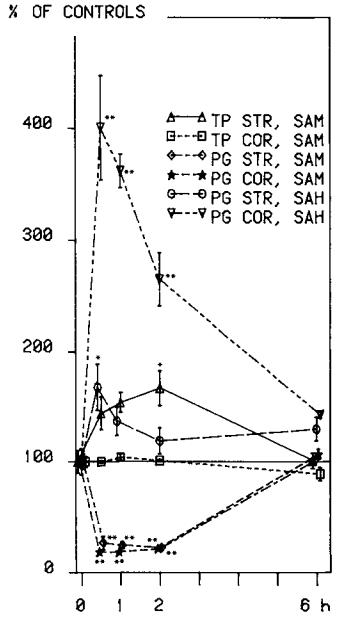

Fig. 3. Time-course of the effects of tropolone (30 mg/kg i.p.) and pyrogallol (300 mg/kg i.p.) on SAM and SAH concentrations. Groups of 5 rats were decapitated at various intervals after treatment with the two compounds. SAM was determined in both experiments in the striatum and cortex, SAH in the experiment with pyrogallol only. Data are means ± SEM in percent of controls. Absolute control values in the two experiments were: SAM striatum: 10,802 ± 784 and 8342 ± 877 ng/g; SAM cortex: 7864 ± 338 and 14,243 ± 539 ng/g; SAH striatum: 1512 ± 187 ng/g; SAH cortex: 760 ± 34 ng/g. *P < 0.05 **P < 0.01 vs controls (Dunnett's test).

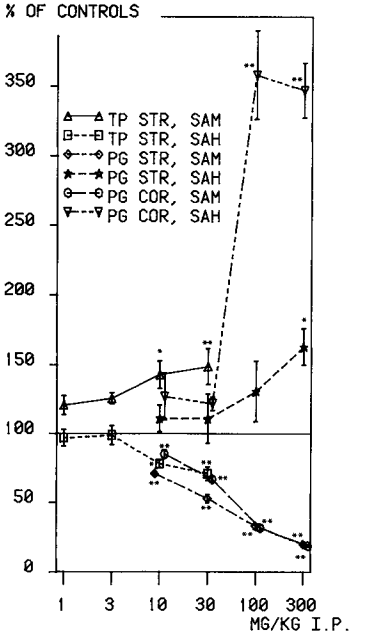

Fig. 4. Dose-response curves of the effects of tropolone (TP) and pyrogallol (PG) on SAM and SAH concentrations. Groups of 5 rats were decapitated 1 hr after treatment with graded doses of the two compounds. SAM and SAH were determined in the striatum only with TP and in the striatum and the cortex with PG. Data are means ± SEM in percent of controls. Absolute control values in the two experiments were: SAM striatum: 6667 ± 311 and 7847 ± 300 ng/g; SAM cortex: 7226 ± 266 ng/g; SAH striatum: 1432 ± 51 and 1481 ± 143 ng/g; SAH cortex: 650 ± 81 ng/g. *P < 0.05 **P < 0.01 vs controls (Dunnett's test).

Table 2. Effects of antidepressants and other drugs on the concentrations of SAM and SAH in rat striatum and cortex

Treatment	Dose (mg/gk)	Striatum SAM (% of controls)	Striatum SAH (% of controls)	Cortex SAM (% of controls)	Cortex SAH (% of controls)
Imipramine	20 i.p.	92 ± 6	104 ± 9	92 ± 3	99 ± 2
Desipramine	10 i.p.	88 ± 4	127 ± 7*	90 ± 4	123 ± 6†
(+)-Oxaprotiline	10 p.o.	105 ± 9	—	99 ± 1	131 ± 2*
	10 i.p.	87 ± 7	122 ± 5	90 ± 4	147 ± 7†
(−)-Oxaprotiline	10 p.o.	87 ± 3	—	96 ± 5	111 ± 6
	10 i.p.	83 ± 2	115 ± 4	94 ± 4	134 ± 3†
Idazoxan	30 p.o.	73 ± 6*	100 ± 1	101 ± 4	113 ± 7
Brofaromine	10 i.p.	81 ± 1	108 ± 7	126 ± 9*	122 ± 4
Scopolamine	10 i.p.	79 ± 6	113 ± 6	119 ± 8	139 ± 6†
MGBG	300 i.p.	65 ± 3†	99 ± 7	102 ± 6	134 ± 2†
Haloperidol	1 p.o.	67 ± 8*	—	88 ± 3	149 ± 3†
Clozapine	30 p.o.	63 ± 6†	—	82 ± 4†	144 ± 7†
Pyrogallol	300 i.p.	33 ± 3†	167 ± 12†	20 ± 2†	569 ± 10†
Cycloleucin	600 i.p.	79 ± 4†	119 ± 9	66 ± 8†	138 ± 12
	1000 i.p.	64 ± 1†	110 ± 6	63 ± 1†	253 ± 13†

Groups of 5 rats were treated with the drugs to be tested 1 hr before decapitation. Data are means ± SEM in percent of controls. The means of the absolute control values were between 7829 and 9639 ng/g (SAM striatum), 1142 and 2388 ng/g (SAH striatum), 6966 and 8317 ng/g (SAM cortex), and 528 and 778 ng/g (SAH cortex) in the four experiments made to generate the data presented in this table.

*$P < 0.05$.

†$P < 0.01$ vs controls (Dunnett's test).

striatum, a corresponding, though smaller effect was observed (Fig. 3).

Dose-dependence of effects of tropolone and pyrogallol on the levels of SAM and SAH

Tropolone dose-dependently increased the striatal concentration of SAH 1 hr after treatment. The effect reached statistical significance at 10 mg/kg i.p. The concentration of SAH was simultaneously decreased (Fig. 4). Pyrogallol at 10 mg/kg i.p. significantly decreased the levels of SAM to a similar extent in cortex and striatum. The levels of SAH in cortex rose enormously after doses above 30 mg/kg i.p., whereas the increase in the striatum was much smaller (Fig. 4), confirming the observation made in the time-course experiment.

Effects of antidepressants and other drugs on the concentrations of SAM and SAH in the rat striatum and cortex

In rats pretreated for 1 hr, none of the antidepressant agents tested (imipramine, desipramine, (+)- and (−)-oxaprotiline), nor the inhibitor of MAO A, brofaromine, significantly changed the striatal concentration of SAM. Scopolamine was also inactive. The α_2-noradrenoceptor antagonist idazoxan, the neuroleptic agents haloperidol and clozapine, MGBG, an inhibitor of SAM decarboxylase, cycloleucine, which was reported to inhibit the synthesis of SAM (among a host of other effects) and pyrogallol caused significant and substantial decreases. Striatal concentrations of SAH remained essentially unaltered by these drugs; desipramine induced a small, pyrogallol a somewhat more marked, increase. Cortical levels of SAM were not affected by the antidepressants, idazoxan, scopolamine, haloperidol and MGBG. They were slightly increased and decreased, respectively, by brofaromine and clozapine, and markedly decreased by cycloleucine and pyrogallol. Imipramine, idazoxan and brofaromine did not change cortical SAH, but desipramine, both enantiomers of oxaprotiline, scopolamine, the two neuroleptics and MGBG caused small increases; marked increases were found with pyrogallol and the higher dose of cycloleucine (Table 2).

In an experiment with 200 mg/kg i.p. cycloleucine (administered 10–45 min before decapitation), no

Table 3. Effects of desipramine and the enantiomers of oxaprotiline on striatal and cortical levels of SAM and SAH in mice

Treatment	Dose (mg/kg)	Striatum SAM (% of controls)	Striatum SAH (% of controls)	Cortex SAM (% of controls)	Cortex SAH (% of controls)
Desipramine	10 i.p.	91 ± 6	112 ± 4	102 ± 2	98 ± 7
(+)-Oxaprotiline	10 i.p.	78 ± 4*	105 ± 7	97 ± 5	125 ± 3*
(−)-Oxaprotiline	10 i.p.	87 ± 8	121 ± 10	108 ± 1	118 ± 3

Groups of 4–5 mice were treated with the drugs to be tested 1 hr before decapitation. Data are means ± SEM in percent of controls. The absolute control values were 7582 ± 211 ng/g (SAM striatum), 8920 ± 522 ng/g (SAM cortex), 1466 ± 105 ng/g (SAH striatum) and 455 ± 11 ng/g (SAH cortex).

*$P < 0.05$ vs controls (Dunnett's test).

changes in the concentrations of SAM or SAH in the striatum and of SAM in the cortex were found (data not shown). On the other hand, the levels of cortical SAH gradually rose to reach 177 ± 9% ($P < 0.01$, Dunnett's test) of controls at 45 min.

Effects of desipramine and the enantiomers of oxaprotiline in mice

The compounds were given i.p. 1 hr before decapitation to mice, since desipramine has been reported to decrease the levels of SAM in the whole brain of this species [5]. Desipramine and (−)-oxaprotiline at 10 mg/kg had no significant effect on the striatal or cortical concentrations of SAM or SAH. (+)-Oxaprotiline significantly decreased the concentration of SAM in the striatum and increased that of SAH in the cortex (Table 3).

DISCUSSION

Tropolone was approximately equipotent in all three models used to assess its inhibitory effects on COMT activity in the striatum of the rat *in vivo*. In contrast, the potency of pyrogallol to reduce the accumulation of 3-MT or *O*-methyl-DOPA or the endogenous levels of HVA decreased in this order. In part, this difference might be explained by a short duration of action: the interval between its administration and death was 35 min in the 3-MT model and 75 min in the *O*-methyl-DOPA model. This does not explain the relatively poor potency to lower HVA with respect to that in the *O*-methyl-DOPA model, since the interval was 60 min in the case of the former. It is likely that pyrogallol interfered with the elimination of HVA and DOPAC from the tissue, since (a) at low doses, it increased HVA (Fig. 1); (b) a rebound effect was observed in the time-course experiment (Fig. 2); (c) it increased the concentration of DOPAC more markedly than tropolone; (d) it increased the concentrations of both metabolites of DA after clorgyline treatment. Thus, the data from the *O*-methyl-DOPA model might be more appropriate to compare the effects of pyrogallol on the activity of COMT and the concentration of SAM.

The time-courses of the effects of tropolone on the concentrations of SAM and HVA in the striatum were very similar, but higher doses were required to increase SAM than to inhibit *O*-methylation. A significant, approximately 140% increase of SAM (and a concomitant 20% decrease of SAH) was only found with 10 mg/kg i.p., at which *O*-methylation seemed to be inhibited by 80%. This suggests that a significant proportion, but not the majority, of SAM is used for *O*-methylation in this area. In the cortex, this proportion seems to be negligible, since tropolone had no effect. The finding that haloperidol and clozapine only weakly reduced the levels of SAM in the cortex, but markedly in the striatum, points in the same direction. This conclusion is also compatible with the fact that the amount of catechols per tissue weight metabolized in the striatum is considerably greater than in the cortex.

Pyrogallol reduced the concentrations of SAM to a similar extent and with a similar time-course in both striatum and cortex. These effects lasted longer than that on striatal HVA, which may be due partly to the use of a 3 times lower dose in the measurements of HVA and partly to the suspected interference with the elimination of this acid. Depletion of striatal SAM and inhibition of *O*-methylation occurred in similar doses. This suggests that there is no compartmentation of SAM, i.e. it is fully available for *O*-methylation, and that COMT can act as a sink for this methyldonor in the presence of enough substrate.

The reported depleting effects of imipramine and desipramine on the concentrations of SAM in rat or mouse whole brain [1, 5] were not found in the areas investigated in our study, i.e. rat striatum and cortex, although similar doses and time schedules were used. Also, (+)-oxaprotiline, a potent inhibitor of NA uptake, was as inactive as its enantiomer, which does not possess this property. To exclude species differences, desipramine and the enantiomers of oxaprotiline were also tested in mice, but with the same negative result. Moreover, much higher doses of cycloleucine were needed to reduce the levels of SAM than those reported by Taylor and Randall [5]; our data are much more compatible with those of Lombardini and Talalay [9].

Brofaromine, a specific inhibitor of MAO A, also, did not lower the levels of SAM in either area. Baldessarini [1] found MAO inhibitors other than pargyline inactive, but ascribed this to incomplete inhibition of the enzyme. This can be excluded for the dose of brofaromine used in our study, suggesting that pargyline caused this effect for reasons unrelated to inhibition of MAO. The α_2-noradrenoceptor antagonist idazoxan decreased the levels of SAM somewhat in the striatum, but not in the cortex, contrary to what would be expected if its effects on the metabolism of NA were involved. Thus, our results suggest that an enhanced metabolism of NA via *O*-methylation does not lead to a decrease of the levels of SAM and this in turn indicates that this process consumes only a minor part of the SAM available in rat or mouse cortex.

The decrease of the concentration of SAM caused by MGBG, an inhibitor of SAM decarboxylase, is unexpected. However, the compound also inhibits copper-containing amine oxidases, and more weakly, polyamine-N^1-acetyltransferase. *In vivo,* on the other hand, it increases the activity and prolongs the half-life of this enzyme severalfold in many organs. Moreover, it accumulates in cells and affects mitochondrial function [10]. Although such effects may have obscured a small reduction of the concentration of SAM, decarboxylation seems to play a minor role in its metabolism in normal adult rat brain, which is in keeping with the low abundance of SAM decarboxylase in normal mammalian cells [10].

The endogenous concentration of SAH was lower and its increase after pyrogallol was much smaller in the striatum than in the cortex. Moreover, both enantiomers of oxaprotiline, haloperidol, clozapine, scopolamine, MGBG, and cycloleucine increased SAH only in the cortex. This suggests that cortical SAH more sensitively indicates changes in the turnover of SAM than that in the striatum, and that there is no causal involvement of noradrenergic activation.

The reasons for this higher sensitivity of cortical SAH are not clear. Removal of SAH proceeds via reversible hydrolysis by SAH hydrolase to adenosine

and homocysteine [11]. Although *in vitro* the equilibrium is on the SAH side, it seems that due to their further metabolism the net flow *in vivo* is towards adenosine and homocysteine [12]. There are no differences between the activities of SAH hydrolase [13] or the levels of adenosine in cortex and striatum [7, 14], but the concentration of homocysteine is twofold higher in the latter [15]. Wojcik and Neff [14] reported a rapid *post-mortem* increase of the levels of adenosine after decapitation, particularly in the striatum, which could be avoided by killing the animals with microwave irradiation; on the other hand, Gharib *et al.* [7] found the levels to be lowest after decapitation followed by immediate homogenization after dissection. Our procedure was similar to the latter, since the tissues were frozen immediately after dissection. Even if *post-mortem* increases had occurred under these conditions, this does not readily explain the more marked increases of SAH in the cortex in terms of an involvement of the reverse hydrolase reaction, for two reasons. First, it seems that the availability of homocysteine is the rate-limiting factor for the formation of SAH by SAH hydrolase [12], and second, if the rapid *post-mortem* accumulation of adenosine played a role, one would rather expect a more marked increase in the striatum. A lower rate of removal of homocysteine from cortex than from striatum seems unlikely, since the basal levels of SAH are lower in this area (SAM levels being similar) and homocysteine concentrations are the same in both.

In conclusion, our results suggest that *O*-methylation plays a quantitatively more important role in striatal than in cortical metabolism of SAM, which we tentatively ascribe to the higher absolute turnover of catechols in this area. Drugs which increase synaptic availability of NA and therefore its *O*-methylation (or that of its deaminated metabolite) do not appreciably affect the metabolism of SAM, at least in the rat cortex.

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