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Tropolone antagonism of the L-dopa-induced elevation of *S*-adenosylhomocysteine: *S*-adenosylmethionine ratio but not depletion of adrenaline in rat hypothalamus

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Tropolone, an inhibitor of catechol *O*-methyl transferase, largely prevented the increase in SAH : SAMe ratio in rat hypothalamus following L-dopa injection. Tropolone did not prevent but instead enhanced the decrease produced by L-dopa of adrenaline concentration in rat hypothalamus. The results imply that the decrease in hypothalamic adrenaline concentration following L-dopa injection was not caused by the increase in SAH : SAMe ratio.

Administration of large doses of L-dopa to rats and mice decreases brain concentrations of *S*-adenosylmethionine (SAMe), the methyl donor for biological transmethylation reactions (Wurtman et al 1970; Chalmers et al 1971; Taylor & Randall 1975; Stramentinoli et al 1981). The decrease in SAMe concentration was postulated to result from its increased utilization caused by the *O*-methylation of L-dopa (Ordonez & Wurtman 1973), though initially there were no data to show that it was an increase in utilization rather than a decrease in formation that caused the SAMe depletion.

Recently we reported that the decrease in SAMe concentration following L-dopa administration to rats was accompanied by a marked increase in the brain concentration of *S*-adenosylhomocysteine (SAH) and in the ratio of SAH : SAMe concentrations (Fuller et al 1982). Since SAH is the product formed when the labile methyl group from SAMe is transferred to a methyl acceptor, this finding supports the earlier assumption that the decreased concentration of SAMe after L-dopa is associated with increased SAMe utilization. We postulated that the increase in SAH : SAMe ratio, by lending to a reduction in noradrenaline *N*-methyltransferase activity, might be responsible for the lowering of hypothalamic adrenaline concentration following L-dopa administration. To establish that the increase in SAMe utilization causes the lowering of its concentration and to examine the relationship between the increased SAH : SAMe ratio and the decreased adrenaline concentration, we considered inhibiting the *O*-methylation of L-dopa to see if the SAH : SAMe changes were prevented.

If increased SAMe conversion to SAH resulting from the *O*-methylation of L-dopa is the mechanism for the increased SAH : SAMe ratio, then an inhibitor of *O*-methylation should antagonize the elevation of the SAH : SAMe ratio following L-dopa administration. The enzyme responsible for this *O*-methylation, catechol *O*-methyl transferase (COMT: EC 2.1.1.6), acts on various catechols which themselves competitively inhibit the *O*-methylation of a particular substrate, e.g. L-dopa and therefore cannot be used in the present instance. Instead, tropolone, a COMT inhibitor that is not itself *O*-methylated, was used (Baldessarini 1966; Broch 1973). We describe here results showing that it antagonizes the L-dopa-induced conversion of SAMe to SAH in rat brain.

Method

Male Wistar rats 180–200 g from Harlan Industries, Cumberland, IN, were housed in groups of six in hanging wire cages. Tropolone (Aldrich Chemical Company, Milwaukee, WI) was injected at 100 mg kg^{-1} i.p. 2 h and L-dopa (Monsanto, St Louis, MO) at 200 mg kg^{-1} i.p. 1 h before rats were decapitated, and the hypothalamus was removed, frozen on dry ice, then stored at −15 °C before analysis. The concentrations of SAMe and SAH were measured by hplc with detection by ultraviolet absorbance (Perry & Fuller 1982).

Results and discussion

Table 1 shows the effect of L-dopa on SAMe and SAH concentrations in rat hypothalamus. At 1 h there was 49% depletion of SAMe, while SAH concentration was increased to 3·6 times the control value. Tropolone alone had no significant effect on SAMe or SAH concentration, but given before L-dopa attenuated the decrease in SAMe concentration by L-dopa, to only 27%. The concentration of SAH was only slightly increased by L-dopa in tropolone-pretreated rats so

Table 1. Hypothalamic concentrations of SAMe and SAH and ratio of their concentrations in rats treated with L-dopa, tropolone, or both drugs.

Treatment group	SAMe nmol g^{-1}	SAH nmol g^{-1}	Ratio SAH : SAMe
Control	25·1 ± 0·5	0·69 ± 0·05	0·027 ± 0·002
L-dopa	12·7 ± 3·0*	2·51 ± 0·55*	0·298 ± 0·088*
Tropolone + L-dopa	18·4 ± 0·5*	0·86 ± 0·03*	0·047 ± 0·003*
Tropolone	22·6 ± 1·1	0·60 ± 0·04	0·027 ± 0·002

* $P < 0·05$, different from control group (Student's *t*-test).
Mean values ± standard errors for 6 rats per group are shown.

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Table 2. Hypothalamic concentrations of catecholamines in rats treated with L-dopa, tropolone, or the combination of drugs.

Treatment	Catecholamine concentration in hypothalamus, pmol g^{-1}		
	Adrenaline	Noradrenaline	Dopamine
Control	96 ± 6	7381 ± 160	1684 ± 61
L-dopa	53 ± 14*	7018 ± 304	8189 ± 1522*
Tropolone + L-dopa	16 ± 6*	4404 ± 349*	10755 ± 1157*
Tropolone	82 ± 4	5053 ± 229*	930 ± 39*

* $P < 0.05$, different from control group (Student's t-test).
Mean values ± standard errors for 6 rats per group are shown.

instead of a greater than 10-fold increase in ratio SAH : SAMe in rats treated with L-dopa alone, the ratio increased by less than 2-fold in rats treated with L-dopa after tropolone pretreatment. Thus tropolone markedly antagonized these effects of L-dopa. Since tropolone at the 100 mg kg^{-1} dose has been shown to inhibit COMT in rat brain (Broch 1973), the ability of tropolone to antagonize almost completely the elevation by L-dopa of the SAH : SAMe concentration ratio in brain supports the idea that the changes in SAH and SAMe occur as a consequence of the *O*-methylation of L-dopa by COMT.

Table 2 shows the effect of L-dopa and tropolone on hypothalamic catecholamine concentrations. The bottom line of the Table shows that tropolone itself significantly decreased dopamine and noradrenaline concentrations, probably by inhibition of tyrosine hydroxylase (Goldstein et al 1967; Ozawa & Suzuki 1971; Broch 1973). Decreases in mean catecholamine concentration after tropolone were 45, 32 and 15% for dopamine, noradrenaline and adrenaline respectively, the latter not being significant. Another tyrosine hydroxylase inhibitor, α-methyltyrosine, also produces little or no decline in adrenaline concentration at early times when dopamine and noradrenaline concentrations have already decreased in our experiments (unpublished data). The effect of tropolone itself on catecholamine concentrations complicates the interpretation of the remaining data, but some inferences can nonetheless be made. L-Dopa decreased adrenaline concentration, markedly increased dopamine concentration, and did not change noradrenaline concentration, as has been reported before (Fuller et al 1982). The injection of tropolone did not antagonize the lowering of adrenaline by L-dopa, as would be predicted if the lowering were due to an increased SAH : SAMe ratio, rather the lowering of adrenaline was enhanced in the group receiving both tropolone and L-dopa. The concentration of noradrenaline was slightly, but not significantly, lower in the group treated with tropolone plus L-dopa than in the group treated only with tropolone. Tropolone potentiated the rise in dopamine due to L-dopa.

The interpretation of the catecholamine data is thus complicated by the fact that tropolone itself altered catecholamine concentrations. However, the enhancement, rather than antagonism by L-dopa alone, of the lowering of adrenaline concentration relative to its antagonism of the rise in the SAH : SAMe ratio in rats pretreated with tropolone argues against the increase in the SAH : SAMe ratio being the mechanism by which L-dopa lowers adrenaline concentration as proposed by Fuller et al (1982). Tropolone should also be a useful tool for ascertaining if certain other changes reported after L-dopa, such as decreased *N*-methylation of tritiated histamine injected intracisternally into rats (Schwartz et al 1974), occur secondarily to the increase in SAH : SAMe ratio.

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