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5,6-Dihydroxytryptamine is a substrate for catechol *O*-methyltransferase

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THE ABILITY of 5,6-dihydroxytryptamine (5,6-DHT) to be taken up mainly by serotoninergic nerve endings and to cause degenerative changes in the neuron[1] has made it a useful tool in the study of serotoninergic pathways in the brain. More recently, 5,7-dihydroxytryptamine (5,7-DHT) has been shown to act in the same general way [2] Certain differences in the actions of these two drugs have been observed For instance, Baumgarten and Lachenmayer[2] reported that 5,7-DHT was less selective against serotonin neurons and also caused functional impairment of noradrenergic neuronal systems. Morgane *et al.*[3] also observed that 5,7-DHT was less specific in reducing serotonin levels than was 5,6-DHT; 5,7-DHT reduced norepinephrine as well as serotonin levels in various regions of rat brain. Since 5,6-DHT contains a catechol grouping, we postulated that it might be attacked by catechol *O*-methyltransferase (COMT), an enzyme whose major physiologic role is apparently to inactivate catecholamines. Metabolism of 5,6-DHT by COMT could then influence actions of that compound *in vivo*; for example, 5,6-DHT might exert less pharmacologic effect in brain regions rich in COMT or might have little effect on noradrenergic neurons because of their COMT content With this possibility in mind, we determined whether 5,6-DHT could act as a substrate for COMT from rat brain and liver *in vitro*.

COMT from rat liver or whole brain was prepared by the method of Axelrod and Tomchick[4] through the stage of ammonium sulfate fractionation. COMT activity was assayed in incubation mixtures containing 0·08 M sodium phosphate buffer pH 7·9, 12·5 mM $MgCl_2$, 2×10^{-6} M *S*-adenosylmethionine-methyl-^{14}C (sp. act., 60·4 mCi/m-mole), 5,6-DHT at various concentrations and enzyme. With the brain enzyme, each assay tube contained 42 μg protein; with the liver enzyme, each assay tube contained 14 μg protein. Protein determinations were by the biuret method The total incubation mixture had a volume of 15 μl. After incubation at 37° for 20 min, the unreacted *S*-adenosylmethionine-methyl-^{14}C was precipitated by the addition of Reinecke salt.[5] The methylated product in the supernatant fluid after centrifugation was determined by liquid scintillation spectrometry

Figure 1 shows a comparison of 5,6-DHT with catechol as COMT substrates. Both with the enzyme from brain and with the enzyme for liver, 5,6-DHT was a substrate. Its rate of methylation was only slightly below that of catechol itself at each of the three concentrations tested with the brain enzyme; the difference was somewhat greater with the liver enzyme.

An experiment designed to show that the methylation of 5,6-DHT was accomplished by COMT and not by some other methyl-transferring enzyme is reported in Table 1. Omitting magnesium ion, which is required by COMT but not by other *N*- or *O*-methyltransferases that attack monoamines, reduced the activity to only 7 per cent of the control Adding tropolone, a known inhibitor of COMT, inhibited the

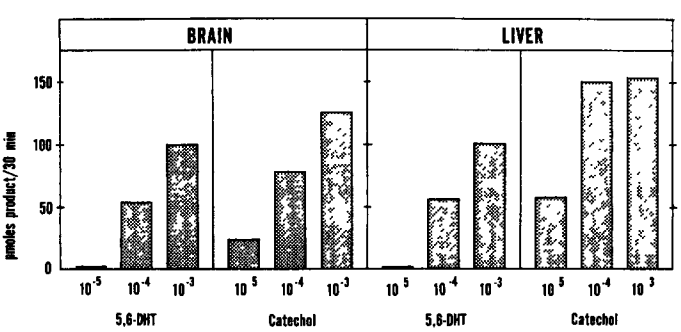

FIG. 1. Comparison of 5,6-DHT and catechol as substrates for COMT from rat brain and liver. Incubation mixtures as described in the text contained 5,6-DHT or catechol at the molar concentrations indicated

TABLE 1. EVIDENCE THAT COMT IS THE ENZYME THAT METHYLATES 5,6-DHT*

Incubation conditions	Methylated product formed (pmoles/30 min)
Complete	84·7
Without $MgCl_2$	5·6
With tropolone (10^{-3} M)	7 3
5-Hydroxytryptamine in place of 5,6-DHT	0·0

* Incubation mixtures containing brain enzyme were prepared as described in the text 5,6-DHT (or 5-hydroxytryptamine) was present at 10^{-3} M.

reaction by 91 per cent. Substituting 5-hydroxytryptamine for 5,6-DHT, so that a catechol group was no longer available, completely abolished the methylation.

Axelrod and Lerner[6] had previously shown that 5,6-dihydroxyindole was a substrate for rat liver COMT. It is not surprising then that 5,6-DHT can also serve as a COMT substrate, i.e. that the addition of the 2-aminoethyl substituent does not destroy substrate activity. Axelrod and Lerner[6] showed that rat liver COMT mostly methylated on the 6-hydroxyl of 5,6-dihydroxyindole, about twice as much 5-hydroxy-6-methoxyindole being formed as 5-methoxy-6-hydroxyindole. We have not attempted to identify the product(s) from the reaction with 5,6-DHT because we do not have reference standards of the two possible products—5-methoxy-6-hydroxy-tryptamine and 5-hydroxy-6-methoxy-tryptamine. It seems probable that either product would be inactive or less active than 5,6-DHT in affecting serotoninergic nerve terminals.

Axelrod and Lerner[6] found that pineal hydroxyindole *O*-methyltransferase could also *O*-methylate 5,6-dihydroxyindole, and that enzyme may likewise be capable of acting on 5,6-DHT. However, the localization of that enzyme in pineal tissue compared to the ubiquitous distribution of COMT suggests that the latter enzyme is more likely to have a role in the metabolism of 5,6-DHT *in vivo*.

If 5,6-DHT is attacked by COMT to a significant degree *in vivo*, then the action of 5,6-DHT in discrete regions of brain or in cells rich in COMT activity might be prevented, and the selectivity of 5,6-DHT's action relative to 5,7-DHT (which does not have the catechol grouping) might be influenced. The possibility that 5,6-DHT might be more active and exert relatively different effects in animals treated with a COMT inhibitor is raised

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