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Tetrahydroisoquinolines derived from noradrenaline–aldehyde condensations—Pyrogallol-sensitive *O*-methylation in rat homogenates*

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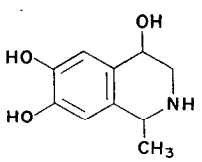
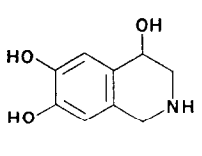
THE CATECHOL *O*-methyltransferase (COMT) pathway is a well established metabolic route for endogenous catecholamines (CAs).[1] With rat brain and liver homogenates, we have examined *O*-methylation in the presence and absence of the COMT inhibitor, pyrogallol, of tetrahydroisoquinoline (TIQ) alkaloids (Fig. 1) which are cyclized derivatives of noradrenaline (NA) and acetaldehyde (AcH) or formaldehyde (HCHO). It has been suggested that TIQ alkaloids may form in neuronal and chromaffin cells during alcohol metabolism and assume a physiological role in the development of alcoholism.[2–4] *O*-methylation of some TIQs structurally related to dopamine (DA) has been recently observed.[5–7] The substrate specificity of COMT has now been extended to include the TIQ alkaloids having a β-hydroxylated CA skeleton.



TIQ I



TIQ II

FIG. 1. 4,6,7-Trihydroxy-tetrahydroisoquinoline derivatives of noradrenaline.

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TABLE 1. EFFECT OF PYROGALLOL ON THE *O*-METHYLATION OF NORADRENALINE AND TWO TETRAHYDROISOQUINOLINE DERIVATIVES IN RAT BRAIN AND LIVER HOMOGENATES

	Brain			Liver		
^{3}H-compound	% *O*-methylation* Saline	Pyrogallol	% Inhibition due to pyrogallol	% *O*-methylation* Saline	Pyrogallol	% Inhibition due to pyrogallol
NA (5)†	33·6 ± 4·6‡	15·5 ± 3·0	53·9	35·3 ± 4·6§·‖	17·6 ± 3·4	50·2
TIQ I (8)	21·5 ± 6·1‡	2·0 ± 1·0	90·7	78·2 ± 8·3§·¶	31·0 ± 4·6	60·4
TIQ II (8)	29·3 ± 4·5	7·9 ± 3·2	73·1	58·1 ± 4·5‖·¶	6·2 ± 5·1	89·4

* Per cent of added substrate that was *O*-methylated, out of total radioactivity, corrected for recovery (recovery = 70 ± 5 per cent).
† Number of determinations for each saline and each pyrogallol experiment.
‡ Statistical significance by Student's unpaired *t*-test—$P < 0.005$.
§ Statistical significance by Student's unpaired *t*-test—$P < 0.001$.
‖ Statistical significance by Student's unpaired *t*-test—$P < 0.001$.
¶ Statistical significance by Student's unpaired *t*-test—$P < 0.001$.

Male Holtzman Sprague–Dawley rats, weighing 300–500 g each, were injected with pyrogallol (250 mg/kg, i.p.) in 1 ml of 0·9% saline or with 0·9% saline only. One-half hr after pyrogallol or saline injection, rats were decapitated and livers and brains were removed and washed in cold Krebs–Ringer phosphate buffer, pH 7·4. Homogenization was done with a chilled Teflon homogenizer in 2 vol. of ice-cold buffer which contained L-ascorbic acid, 0·5 mg/ml. To each homogenate containing 500 mg of tissue was added 100 μmoles of $MgCl_2$ (0·1 ml), 2 nmole (1 μCi) of the catechol substrate to be metabolized (1 μl), and a self-regenerating COMT cofactor system[8] consisting of 5 μmoles each of DL-methionine (0·25 ml), adenosine triphosphate (0·1 ml) and *S*-adenosyl-L-methionine chloride (SAM) (0·1 ml). The total volume of each incubation mixture was 2·05 ml.

Incubations were carried out in open 25-ml Erlenmeyer beakers in a 37° shaking water bath. Control incubations were either heat-denatured homogenates (90°, 5 min) or those without the added COMT cofactor system. After 2 hr the reaction was deproteinated with 1 ml of 2 N HCl and centrifuged at 10,000 *g* for 15 min at 0°. Catechol compounds were separated from the non-catechol products using an aluminum hydroxide [$Al(OH)_3$] slurry method.[3] The radioactivity of a 1-ml aliquot of each fraction was measured in a solution of toluene–PPO–BBS-3 (Beckman, 100 ml:5 g:200 ml) by liquid scintillation spectrometry.† Thin-layer chromatography (TLC) with zonal scraping was carried out on aliquots of the fractions using conditions described below for the ^{3}H-TIQ substrates.

The substrate TIQs (Fig. 1) were produced in 1 M NaOAc pH 6 buffer by condensation of 7-^{3}H-*d,l*-NA (International Chemical & Nuclear, 500 mCi/mM) with excess aldehyde.[2] Reaction with predistilled AcH gave as the major product 1-methyl-4,6,7-trihydroxy-1,2,3,4-TIQ (I), while HCHO (Mallinckrodt Anal. Grade, 37% aqueous) yielded 4,6,7-trihydroxy-1,2,3,4-TIQ (II). The completeness of the reaction was determined by iodochrome monitoring[2] and by TLC. The TIQs were then separated from unreacted aldehyde by adsorption on aluminum oxide (Al_2O_3) columns at pH 8·3, washing twice with distilled H_2O, and elution with 1 N HCl. The eluates were readjusted to pH 7·4 and again were subjected to TLC and zonal scraping for proof of purity, prior to addition to the homogenates. ^{3}H-NA was prepurified on Al_2O_3 in the same manner. TLC glass plates (20 × 20 cm) were coated with 250 μ Adsorbosil-1 (Applied Science Labs), air-dried for 20 min, and heat-activated for 30 min at 110°. Development was in *sec*-butanol–formic acid–H_2O (15:3:2) under N_2 atmosphere in a tank lined with solvent-saturated filter paper. A second TLC system was also employed, using Brinkman cellulose MN 300 plates, 0·5 mm thick, developed in methanol–*n*-butanol–benzene–H_2O (40:30:20:10). Visualizing spray reagents were $K_3Fe(CN)_6$ (4·4 mg/ml in 0·2 M phosphate buffer, pH 8·3) followed by $FeCl_3$ (2·5 per cent $FeCl_3$ in H_2O freshly mixed with 1·5 vol. of acetone).[2]

A decrease in $Al(OH)_3$ adsorption of ^{3}H-NA or ^{3}H-TIQs after a 2-hr incubation indicated con-

† PPO = 2,5-diphenylopazole.

version to non-catechol metabolites. When examined by TLC, the non-catechol portions from ^{3}H-NA incubations, in agreement with a report[9] on NA metabolism in homogenates, contained radioactive normetanephrine and radioactive *O*-methylated products migrating with standards of 3-methoxy-4-hydroxy-phenylglycol and vanillylmandelic acid; these deaminated non-catechol products are included in the total percentages for NA in Table 1.

Homogenate metabolism of the two ^{3}H-TIQs was qualitatively different than the ^{3}H-NA "precursor." Non-catechol portions from incubations of brain or liver tissue with TIQ II consistently showed only one radioactive TLC zone which co-chromatographed with a synthesized standard[10] of the expected *O*-methylated metabolite, 4,7-dihydroxy-6-methoxy-TIQ. R_f values for this radioactive band (and cold carrier) were 0·28, Adsorbosil-1, or 0·27, Cellulose. Similarly, non-catechol effluent from incubations of TIQ I with either tissue demonstrated a single radioactive zone co-chromatographing with a standard (available as a synthetic mixture component)[10] of the expected 1-methyl-6-*O*-methylated product (R_f values: 0·43, Adsorbosil-1; 0·26, Cellulose).

For either ^{3}H-TIQ, the catechol portions contained radioactivity apparently associated only with unreacted ^{3}H-substrate (R_f values: TIQ I = 0·33, Adsorbosil-1, and 0·29, Cellulose; TIQ II, 0·18, Adsorbosil-1-, and 0·31, Cellulose). Thus monoamine oxidase action on the TIQs was insignificant in these homogenates.

As shown in Table 1, *O*-methylation in brain homogenates proceeded to a similar extent for all three catechol compounds. Metabolism of TIQ I, however, was slightly but significantly less than that of NA. Liver, on the other hand, was found to metabolize thse substrates in a somewhat reverse order, TIQ I > TIQ II > NA.

Comparing the two TIQs, the AcH-derived TIQ I was a better substrate for liver COMT than was the HCHO-derived TIQ II, while the two were not significantly different in brain tissue. Pyrogallol inhibited the *O*-methylation in liver of TIQ II more than that of TIQ I, but the degree of inhibition was reversed in brain.

Pyrogallol inhibition never reached 100 per cent, possibly because: (a) pyrogallol is itself a substrate for COMT[11] and was partially consumed during the experiment, (b) different COMT isoenzymes[12] act in both tissues and respond differently to the inhibitor, or (c) other *O*-methylating enzymes which are pyrogallol-insensitive are active under the incubation conditions.[13] Also, inhibition of *O*-methylation was consistently 10–40 per cent greater for TIQ substrates than for NA. Differences in the relative affinities of the various substrates and their respective products for the COMT–SAM complex may be responsible for this latter effect.

In our experiments with liver tissue, *O*-methylated products derived from NA were always formed to a lesser extent than were *O*-methylated products from either TIQ. These findings were in accord with the report of the metabolism of the DA–AcH-derived TIQ (salsolinol) by rat liver COMT.[5] A further observation was that the 1-methyl group of TIQ I seemed to increase *O*-methylation in liver compared to TIQ II, but made little difference in the brain metabolism of the two alkaloids.

Although 6-*O*-methylation is expected because of the analogy to predominant meta-*O*-methylation among parent CAs, *O*-methylation of the TIQs on the 7- rather than on the 6-hydroxyl group is possible. Creveling *et al.*[6] have reported 7-*O*-methylation of (DA-related) 6,7-dihydroxy-TIQs by highly purified COMT preparations. In order for a 7-methoxy-4,6-dihydroxy-TIQ product to be present in our homogenates, its TLC migrations would have to be identical to its 6-*O*-methyl isomer. The possibiilty of 7-*O*-methylation of the 4-hydroxylated TIQs, as well as other enzymatic conversions which may have given products not separated by our TLC systems, will be examined further by gas chromatography in our laboratory.[14]

In the studies reported here, the formation of an *O*-quinone from a 4,6,7-trihydroxy-TIQ by non-enzymatic oxidation was probably eliminated by the addition of L-ascorbic acid to the incubation mixtures. Moreover, simple quinone standards were found to migrate close to the origin in the TLC systems used, and little radioactivity was seen in that area. Conjugation by glucuronyl transferase occurs *in vivo*, but systems *in vitro* require cofactor addition, as reported by Axelrod and Inscoe.[15] In support of this, the incubation of our homogenate products with limpet β-glucuronidase (Sigma) for 4 hr produced no clear changes in the TLC and column radioactivity patterns.

In regard to exploratory studies *in vivo*, the facile *O*-methylation of CA-derived TIQs should be taken into account by investigators concerned with TIQ detection and isolation. Difficulties experienced in detecting TIQs *in vivo*[16] could be due to their active conversion to *O*-methylated (and perhaps conjugated) metabolites. Indeed, our initial gas chromatographic results obtained from acute ethanol intoxication experiments with rats show that TIQ-like derivatives are detectable in brain and adrenal tissue when COMT is first inhibited.[17] Physiologically, such CA–aldehyde condensation products may enhance NA and DA activity by acting as competitive COMT inhibitors or by reducing available COMT cofactor, SAM. Single doses of L-dopa have been reported to act in the latter manner.[18] Hence, a metabolic relationship might exist between the formation of TIQs from aldehydes and CAs, and certain neurological aspects of chronic alcohol toxicity or of the alcohol withdrawal syndrome.

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