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INHIBITION OF CATECHOL-O-METHYL TRANSFERASE BY SOME ACID DEGRADATION PRODUCTS OF ADRENALINE AND NORADRENALINE

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Abstract—The inhibitory activities of some acid degradation products of sympathomimetic catecholamines have been compared with those of catechol and pyrogallol in an *in vitro* test against catechol-O-methyl transferase. Unexpectedly the potency of the compounds used in the test appeared not to be related to the number of pairs of adjacent hydroxyl groups present, and possible explanations are presented. Two of the acid degradation products, adnamine and noradnamine, have been implicated in an hypothesis postulating that malfunction of catechol-O-methyl transferase might be responsible for endogenous depressive reactions. The finding that they are potent inhibitors of this enzyme is in accord with the hypothetical expectations.

PURE sympathomimetic catecholamines form multispots when chromatographed on paper from solution in hydrochloric acid.[1–3] The substances responsible for two of the extra spots have been identified as diamine ethers of the type described by Oppinger and Vetter[4] and dibenzocycloheptatrienes of the type described by Kawazu.[5] One of these latter compounds, 5-aminomethyl-2,3,7,8-tetrahydroxydibenzo-(*a*,*e*)-cyclohepta triene (noradnamine), obtained from noradrenaline, has been implicated as a depressive metabolite in an hypothesis concerning the biochemical cause of endogenous depression.[6] It was suggested that in the absence of functional catechol-O-methyl transferase (COMT) brain noradrenaline would be predominantly metabolised by monoamine oxidase (MAO), and that reduction of the deaminated oxidation product followed by dehydrative condensation with another molecule of noradrenaline could yield noradnamine. A report (subsequently unverified) that the adrenaline dibenzocycloheptatriene (adnamine) was itself an inhibitor of COMT led to the further speculation that only small amounts of noradnamine might be required to initiate depression, the condition then increasing in severity as the noradnamine first formed caused more noradrenaline to be exposed to MAO instead of COMT.

Also, it has been recently shown (to be published) that the cardiovascular effects of injected noradrenaline are potentiated following the administration of noradnamine; a phenomenon which could be explained in terms of interference with the normal catabolism of noradrenaline to normetanephrine.

We were interested, for these reasons, in testing noradnamine and some related compounds for inhibitory activity against catechol-O-methyl transferase.

METHODS

A crude enzyme preparation containing COMT and a methionine activating enzyme was prepared and used as follows (adapted from Axelrod and Tomchick[7]). Adult male rats were stunned and bled from the throat. The livers were immediately removed, chilled, weighed, and homogenized with ice-cold isosmotic KCl (4 ml/g tissue) for 45 sec in an Ato-Mix homogenizer. The homogenate was subjected to refrigerated centrifugation at 25,000 *g* for 30 min, and the supernatant fluid obtained was removed and stored at −20° until required.

COMT activity was determined by measuring the amount of metanephrine formed from adrenaline (1·2 μmoles) following incubation for 1 hr at 37° with 1 ml of enzyme solution, phosphate buffer (pH 8·2; 100 μmoles), magnesium chloride (20 μmoles), ATP (20 μmoles), L-methionine (40 μmoles) and distilled water to make a final volume of 2 ml. The inhibitors under test were incubated with the above enzyme mixture for 10 min before the adrenaline substrate was added.

The reactions were stopped by the addition of 2 ml 0·5 M borate buffer (pH 10) and the metanephrine was extracted in three stages (25 ml, 25 ml, 30 ml) into 80 ml of ethylene chloride containing 2% isoamyl alcohol, which was then itself extracted with 2 × 5 ml 0·1 N HCl. At all stages of the extraction shaking was for 2 min. The combined HCl fractions were clarified by centrifugation (10 min, 3000 rev/min) and metanephrine was determined fluorimetrically using an Aminco–Bowman spectrophotofluorimeter at an activating wavelength of 280 mμ and a fluorescence wavelength of 335 mμ (uncorrected instrumental values).

Recovery experiments using 100 μg metanephrine were carried out in the absence of and in the presence of the inhibitors under test. The inhibitory activities quoted in the text are corrected for recovery and any interference with recovery by the inhibitors.

Drugs used

(—) Adrenaline acid tartrate, metanephrine hydrochloride (L. Light & Co.), catechol, pyrogallol, ATP disodium dihydrogen salt, L-methionine (B.D.H. Ltd.) were obtained commercially. Diadrenaline ether hydrochloride,[4] adnamine hydrochloride and noradnamine hydrochloride (to be published) were synthesized in our laboratory. Quantities quoted in the text refer to free base where appropriate.

RESULTS AND DISCUSSION

Of the five substances tested for inhibitory activity against COMT, adnamine and noradnamine were the only ones that interfered with recovery of metanephrine. The corrected inhibitory activities of each compound are shown in Table 1. That all of the compounds are producing inhibition by the same mechanism is suggested by the parallelism of the log dose/percentage inhibition lines. This is shown in Table 1 by the similar figures for inhibitory activity. For example, 29 per cent inhibition of COMT is found with 100×10^{-5} M diadrenaline ether, 50×10^{-5} M catechol, 25×10^{-5} M noradnamine or adnamine and $12{\cdot}5 \times 10^{-5}$ M pyrogallol. It is apparent that the potency ratios of these compounds in the above order are 1:2:4:8 and this is confirmed by calculations of the slopes of the arithmetic dose/percentage inhibition regression lines. Since the common mechanism of action is presumably the presentation of catechol hydroxyl groups as substrate, however, these results are surprising.

TABLE 1. INHIBITION OF CATECHOL-O-METHYLTRANSFERASE BY VARIOUS COMPOUNDS

Inhibitor	Percentage inhibition produced by various concentrations (molar $\times 10^{-5}$) of inhibitors					
	6·25	12·5	25	50	100	200
Noradnamine	6·5 ± 1·2 (4)	14·0 ± 1·8 (4)	29·6 ± 1·5 (4)			
Adnamine	3·2 ± 2·3 (4)	11·9 ± 6·4 (3)	29·3 ± 1·2 (4)			
Diadrenaline ether			6·0 ± 2·8 (3)	13·5 ± 0·9 (3)	29·8 ± 0 (3)	41·8 ± 3·8 (2)
Catechol			9·5 ± 2·0 (3)	29·0 ± 2·7 (3)	55·3 ± 1·6 (3)	
Pyrogallol	29·2 ± 0·3 (2)	46·8 ± 0 (2)	70·3 ± 3·2 (2)	89·9 ± 0 (2)		

Substrate concentration was 6×10^{-4} M in each case.
Figures for inhibitory activity are means ± S.E. with the number of observations in parentheses.

If the inhibitory activity of catechol, with one pair of adjacent hydroxyl groups, is taken as the standard, the observation that noradnamine and adnamine, with two pairs of adjacent hydroxyl groups are twice as potent is in agreement with theoretical expectations. Diadrenaline ether, however, also has two catechol moieties and yet exhibits only one half of the inhibitory activity of catechol. Conversely, pyrogallol which has but three adjacent hydroxyl groups is four times more potent than catechol.

Assuming that inhibition of COMT in an *in vitro* test should be related to the number of catechol moeties in the molecule of a compound, the low activity of diadrenaline ether suggests that neither of its catechol groups are being properly bound to the enzyme. The flexibility of the ether linkage may well be such as to endow the molecule with sufficient mobility to prevent the required planar alignment of the aromatic rings with the enzyme surface; this situation does not arise in the inflexible molecules of noradnamine and adnamine. An explanation of the high activity of pyrogallol in terms of catechol groups presented to the enzyme is also difficult. The attack on the hydroxyl groups by the methyl group from S-adenosyl methionine is an electrophillic one, however, and compared with catechol, pyrogallol contains an additional electron-donating hydroxyl group. For an initial mono-O-methylation, therefore, pyrogallol might be expected to be a better substrate than catechol. Furthermore, when the hydroxyl group on position 1 is O-methylated, the resulting compound will still have two adjacent hydroxyl groups and again compared with catechol will contain an additional electron donating methoxyl group. This 1-methoxy-2,3 dihydroxybenzene might therefore also have much greater COMT inhibitory activity than catechol itself.

Irrespective of mechanism, however, adnamine and noradnamine are shown to be potent inhibitors of COMT. Whether the observation that they cause depressive symptoms in experimental animals (to be published) is related to this action remains to be investigated; the fact that other COMT inhibitors cause similar effects in animals suggests that it is.[8,9]

REFERENCES

1. D. J. ROBERTS, *J. Pharm. Pharmac.* **16**, 549 (1964).
2. D. J. ROBERTS, *Biochem. Pharmac.* **15**, 63 (1966).
3. K. J. BROADLEY and D. J. ROBERTS, *J. Pharm. Pharmac.* **18**, 182 (1966).
4. H. OPPINGER and H. VETTER *Med. und Chem.* **4**, 343 (1942).
5. M. KAWAZU, *J. pharm. Soc. Japan*, **78**, 399 (1958).
6. D. J. ROBERTS and K. J. BROADLEY, *Lancet*, **i**, 1219 (1965).
7. J. AXELROD and R. TOMCHICK, *J. biol. Chem.* **233**, 702 (1958).
8. A. B. MERLO and I. IZQUIERDO, *J. Pharm. Pharmac.* **15**, 629 (1963).
9. A. CARLSSON, *Progress in Brain Research*, vol. 8, p. 9. Elsevier, London (1964).