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INHIBITION OF CATECHOLAMINE OXIDATION BY INDOLES

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Abstract—Various 5-substituted indoles are shown to inhibit effectively both the enzymatic and autoxidation of dopamine. The inhibition of the enzymatic oxidation is suggested by kinetic studies to be non-competitive. 5-Hydroxyindoles are also shown to inhibit the enzymatic oxidation of 3,4-dihydroxyphenylalanine.

RECENT reports by Vander-Wende and Johnson[1,2] indicated that 5-hydroxytryptamine interacts with both dopamine and noradrenaline forming a complex which inhibits the enzymatic and autoxidation of these catecholamines. It was further suggested that such an interaction might assist in modulating the activities of the brain catecholamines. Thus excessive amounts of 5-hydroxytryptamine in a particular brain area could lead to a depletion in melanin content such as is observed in the *substantia nigra* in human Parkinsonism.

A further report by Greiner[3] demonstrates that melanin is excessively produced in schizophrenic patients, a disorder that is associated with high dopamine levels.

Our studies of the relationship between catecholamines and indoles in the pineal gland prompted a wider investigation into the inhibition of catecholamine oxidation by indole metabolites. Our experiments show that both the enzymatic and autoxidation of dopamine is effectively inhibited not only by 5-hydroxyindoles but also by 5-methoxyindoles and to a lesser extent by 5-fluoro substituted indoles. The enzymatic inhibition of dopamine by 5-hydroxy and 5-methoxy indoles is apparently non-competitive.

The report also includes studies of the inhibition of the enzymatic oxidation of 3,4 dihydroxyphenylalanine (DOPA).

MATERIALS AND METHODS

Mushroom tyrosinase, dopamine HCl, D-L-tryptophan and 5-hydroxytryptamine creatinine sulphate were purchased from Koch–Light Lab., Ltd. L-DOPA, D-L-5-hydroxytryptophan and *N*-acetyl 5-hydroxytryptamine were obtained from Sigma Chemical Co., D-L-5-fluorotryptophan, melatonin and 5-methoxytryptamine HCl were supplied by Ralph N. Emanuel Ltd., 5-Fluorotryptamine HCl was purchased from Fluka A.G. Chemische Fabrik.

Both the enzymatic and autoxidation of dopamine and DOPA were assayed spectrophotometrically by measuring the increase in absorbance of the dopachrome

type intermediate of the Raper–Mason[4,5] pathway at 310 nm. The Unicam S.P. 800 spectrophotometer was adapted with an S.P. 820 attachment to measure absorbance at the fixed wavelength with respect to time, 1 cm Silica cells were used. When inhibitor was present a solution of buffer, tyrosinase and the appropriate indole was placed in the reference beam. Otherwise, a solution of buffer and tyrosinase; or in the autoxidations, buffer alone was used as reference. Measurements were made at a constant temperature at 25° after each sample had been shaken vigorously for 25 sec. Percentage inhibitions were calculated after 1 min 50 sec.

Tyrosinase activity. One unit is equivalent to the amount of enzyme required to increase the absorbance at 280 nm by 0·001 per min at 25° and pH 6·5 using 10^{-3} M L-tyrosine solution as substrate. The present sample possessed an activity of 160 units/mg.

Enzymatic oxidation of dopamine. Approximately 25 units of mushroom tyrosinase (0·15 ml of a 1 mg/ml solution) was used per assay to facilitate the oxidation of a $1{\cdot}67 \times 10^{-3}$ M solution of dopamine in 0·1 M phosphate buffer pH 6·8. The total assay volume was 4 ml.

Inhibition of the enzymatic oxidation of dopamine by indoles

To determine the percentage inhibition, a solution of dopamine was mixed with an equimolar solution of indole together with tyrosinase and buffer as before, to produce a final concentration of $1{\cdot}67 \times 10^{-3}$ M.

Kinetic studies were carried out using varying dopamine concentrations between $0{\cdot}83 \times 10^{-3}$ and $3{\cdot}34 \times 10^{-3}$ M. Each substrate level was examined using 5-hydroxytryptamine concentrations in the range of $0{\cdot}415 \times 10^{-3}$ to $1{\cdot}67 \times 10^{-3}$ M. This was repeated using 5-methoxytryptamine as inhibitor. Solutions of tyrosinase and buffer were as described before.

Autoxidation. Three methods were evaluated. The methods employed were modifications of those used by Vander-Wende and Johnson[1] using 0·01 N NaOH solutions and Sassetti and Fudenberg[6] using 0·05 M borate buffer pH 9·8 and finally 0·2 M Tris buffer pH 9·8 was used. Dopamine and inhibitor concentration was adjusted to $0{\cdot}78 \times 10^{-3}$ M with appropriate buffer pH 9·8 or in the first method by adjustment of pH to 9·8, with 0·01 N NaOH. The total volume was 7 ml.

Inhibition of the enzymatic oxidation of DOPA by 5-hydroxyindoles

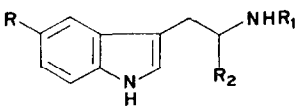
To determine percentage inhibition, solutions of DOPA ($1{\cdot}67 \times 10^{-3}$ M) were mixed with an equimolar solution of hydroxyindole together with tyrosinase and buffer solutions as described for dopamine.

Inhibition of the tyrosinase catalysed oxidation of dopamine by indoles

Table 1 shows that 5-hydroxytryptophan (II) and *N*-acetyl 5-hydroxytryptamine (III) exhibit a similar degree of inhibition to that of 5-hydroxytryptamine (I) itself. All three molecules have the 5-hydroxy group which Vander-Wende and Johnson[1] reported was necessary for complex formation and therefore inhibition.

However, it is also demonstrated in Table 1 that 5-methoxytryptamine (IV) and melatonin (V) inhibited the oxidation process to almost as great an extent and that 5-fluorotryptamine (VI) and 5-fluorotryptophan (VII) gave considerable inhibition whilst tryptophan (VIII) itself had a slight effect.

TABLE 1. OXIDATION OF CATECHOLAMINES BY INDOLES



Indole substituent				Inhibition of enzymatic oxidation (%)		Inhibition of autoxidation (%)
No.	R	R_1	R_2	Dopamine	DOPA	Dopamine
I	HO	H	H	87·7 ± 0·6	70·3 ± 0·8	54·5 ± 4·0
II	HO	H	COOH	89·1 ± 0·9	81·0 ± 0·6	58·6 ± 1·0
III	HO	$COCH_3$	H	86·8 ± 2·5	71·6 ± 3·3	64·3 ± 2·5
IV	MeO	H	H	80·7 ± 1·0		40·2 ± 2·0
V	MeO	$COCH_3$	H	83·1 ± 0·6		35·7 ± 2·0
VI	F	H	H	25·5 ± 1·5		4·03 ± 1·3
VII	F	H	COOH	14·3 ± 0·3		7·04 ± 0·1
VIII	H	H	COOH	4·1 ± 1·3		4·00 ± 0·5

These molecules do not possess the necessary 5-hydroxy group for complex formation and indeed compound IV and V were reported by Vander-Wende and Johnson[1] not to form the fluorescent spot, a preliminary test for possible complex formation when the mixture of catecholamine and indole was sprayed with a diazotizing reagent.

It seems therefore, that in these examples at least an alternative mechanism must operate in the inhibition of the enzymatic oxidation of dopamine. Consequently, it was decided to re-examine the mechanism by which 5-hydroxytryptamine itself inhibited this oxidation process.

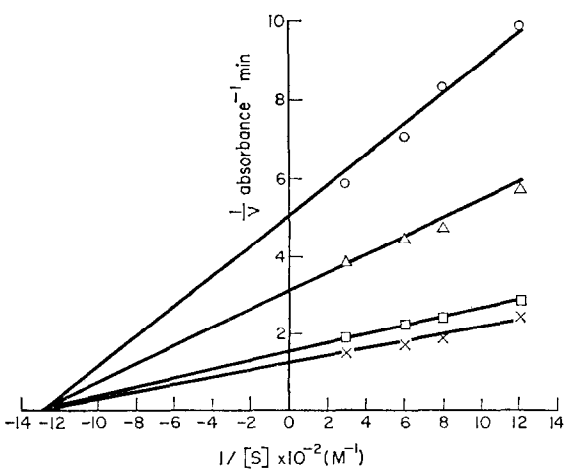


FIG. 1. The effect of 5-hydroxytryptamine on the enzymatic oxidation of dopamine. × Dopamine control, □ Dopamine and 5-hydroxytryptamine $0{\cdot}415 \times 10^{-3}$ M, △ Dopamine and 5-hydroxytryptamine $0{\cdot}83 \times 10^{-3}$ M, ○ Dopamine and 5-hydroxytryptamine $1{\cdot}67 \times 10^{-3}$ M.

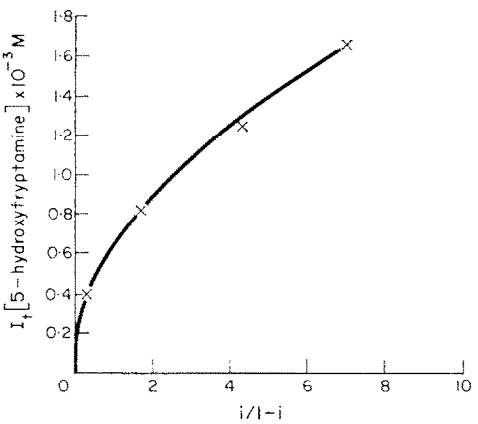


FIG. 2. The variation of $i/1\text{-}i$ with 5-hydroxytryptamine concentration. Dopamine concentration was $1{\cdot}67 \times 10^{-3}$ M.

The extent of such inhibition was investigated with three levels of 5-hydroxytryptamine. Figure 1 demonstrated that the inhibition of the tyrosinase is non-competitive and that the observed K_m value is in good agreement with that reported by Vander-Wende.[7] In addition, it is shown in Fig. 2 that under the present conditions, the plot of total inhibitor concentration against $i/1\text{-}i$ gives a curve as opposed to the straight line reported by Vander-Wende and Johnson.[1]

The exercise was repeated using three concentrations of 5-methoxytryptamine. The results were very similar to those shown in Fig. 1 indicating once again non-competitive

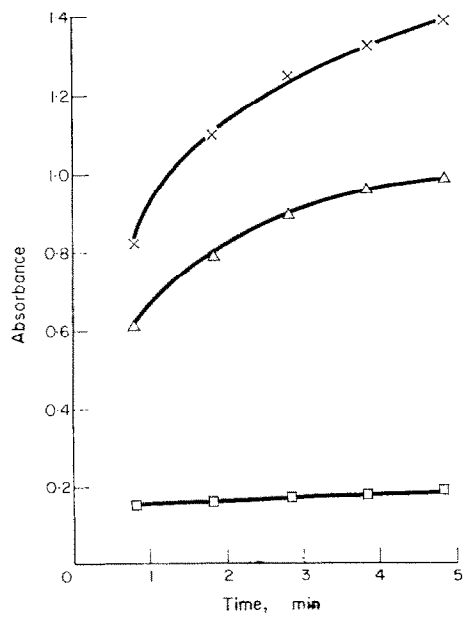


FIG. 3. The autoxidation of dopamine. Variation of absorbance with time.
× With 0·01 N NaOH, △ 0·2 M Tris buffer, □ 0·05 M Borate buffer.

inhibition. ($^{-1}/K_m = 14\ M^{-1} \times 10^{-2}$; $^{1}/V_{max} = 6{\cdot}25$ absorbance^{-1} min for inhibitor concentration $1{\cdot}67 \times 10^{-3}$ M). It seems, therefore, that a similar mechanism may operate in both the case of 5-hydroxytryptamine and its methyl homologue (IV) (Fig. 3).

Autoxidation of dopamine

Figure 3 shows that in the case of the borate buffer, the change in absorbance at 310 nm is linear with respect to time. However the absorbance was also found to be dependent on the borate concentration which at the levels investigated resulted in very low absorbance therefore, the technique was not used. Although adjustment of pH to 9·8 with 0·01 N sodium hydroxide gave reproducible results, the method using 0·2 M Tris buffer was employed to compensate for any small changes in pH due to the addition of the indole inhibitors.

Table 1 demonstrates that those indoles which inhibit the enzymatic oxidation by tyrosinase also inhibit the autoxidation of dopamine, although to differing extents. The hydroxy indoles—I–III produce only about 55–65 per cent reduction compared to the approximate 88 per cent in the enzymatic oxidation. The methoxy compounds IV and V exhibit a much smaller effect than in the enzymatic reaction whilst the fluorocompounds VI and VII only affect the autoxidation very slightly.

Since compounds IV and V did not produce a fluorescent spot,[1] indicating no complex formation as before, an alternative explanation must be found for the inhibition by these compounds at least. One possible mechanism could be that the indoles themselves are being oxidized, thus reducing the available oxygen in solution. However, this seems unlikely, since no indole oxidation products were observed in the u.v. or visible regions. Also, the u.v. spectrum of the indoles was not diminished during the autoxidation.

An alternative explanation might be that the indoles act as free radical quenching agents, thus slowing down the rate of autoxidation. Both these possibilities are at present under investigation.

Inhibition of tyrosinase catalysed oxidation of DOPA by 5-hydroxyindoles

It is shown in Table 1 that the 5-hydroxyindoles I–III also inhibit to a large extent the oxidation of DOPA by tyrosinase. Since DOPA and 5-hydroxytryptophan are well known to act as competitive substrates for aromatic amino acid decarboxylase,[8,9] the mechanism of the inhibition by 5-hydroxytryptophan of the oxidation of DOPA by tyrosinase is also being further examined.

DISCUSSION

It has been demonstrated that the inhibition of the tyrosinase catalysed oxidation of dopamine by 5-hydroxytryptamine and 5-methoxytryptamine respectively can be explained in terms of non-competitive inhibition of the enzyme.

In the case of 5-hydroxytryptamine, this offers an alternative mechanism to that proposed by Vander-Wende and Johnson.[1] Since 5-methoxytryptamine does not possess the necessary hydroxy group for complex formation,[1] substrate inhibitor complex inhibition could not in any case be applied to the methoxy indoles.

The fact that these workers obtained a straight line when I_t was plotted against $i/1\text{-}i$ can indicate such complex inhibition[10] but it is not conclusive proof of such a

mechanism. For example, in the decarboxylation of DOPA and 5-hydroxytryptophan, the results of Yuwiler *et al.*[9] and Rosengren,[8] may be treated in a similar way and a plot of I (assuming $I_t \simeq I$) against $i/1\text{-}i$ also produces a straight line. The molecules have the chemical group requirements for complex formation,[1] but the evidence points to substrate competition.

In the present work, however, under similar conditions, it is seen (Fig. 2) that such a plot gave not a straight line but a curve, thus indicating non-competitive inhibition.

It would appear, therefore, that any modulation of catecholamine activity by indoles would not be effected by catecholamine–indole complex formation.

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