Biochemical Pharmacology, Vol. 22, pp. 1905–1913. Pergamon Press, 1973. Printed in Great Britain.

INHIBITION OF BINDING OF ALDEHYDES OF BIOGENIC AMINES IN TISSUES*

FRIEDA UNGAR,† BORIS TABAKOFF and SPYRIDON G. A. ALIVISATOS
Department of Biochemistry, The Chicago Medical School, Chicago, Ill., U.S.A.

(*Received* 5 *June* 1972; *accepted* 2 *February* 1973)

Abstract—The binding of aldehydes derived from the biogenic amines (i.e. serotonin, dopamine, tryptamine, etc.) is dependent on the substituents attached to the carbonyl residue, as well as the aldehydic group *per se*. The hydroxyl group on the aromatic nucleus of "biogenic" aldehydes contributes significantly to the attachment of the moieties to brain tissue. Thus, 5-hydroxyindole and catechol were found to compete selectively with the "biogenic" aldehydes for tissue binding sites. The attachment of the "biogenic" aldehydes to tissue may also be prevented *in vitro* by various reducing or trapping agents, such as ascorbate, cysteine or glutathione. The possible physiologic significance of aldehyde binding to cellular components is discussed.

IN THE PRESENCE of monoamine oxidase (MAO) activity, biogenic amines, such as serotonin, are converted to their aldehyde derivatives. Alivisatos and Ungar[1] have demonstrated that such aldehydes are incorporated into various subcellular components and certain characteristics of this MAO-dependent incorporation of indoleacetaldehydes have been described[2, 3]. Since previous work on the attachment of aldehydes to macromolecules has centered on the derivatives of the indoleamines, it was of interest to determine whether aldehydes produced from other physiologically active amines would also bind to cell components.

The various chemical substituents of indoleamines have been shown to contribute to their orientation and association with synaptic receptor sites in the central nervous system (CNS)[4, 5]. If the aldehyde derivatives of several structurally different amines bind to tissues, one may question whether the binding is dependent solely on the presence of the carbonyl residue, or whether the other molecular constituents contribute to the choice of binding site. Accordingly, we utilized several structural analogues of the compounds in question, which did not contain a carbonyl residue and aldehydes containing various substituents in an attempt to ascertain whether these analogues would compete for orientation of the "biogenic" aldehyde at the binding site(s).

In addition, we had recently demonstrated[6, 7] that certain agents (i.e. ascorbate, glutathione and cysteine) interact with aldehydes derived from dopamine and prevent their participation in condensation reactions, such as those leading to the formation of tetrahydroisoquinolines. Consequently, it was of interest to investigate whether ascorbate, glutathione or cysteine would also be effective in preventing the binding of aldehyde derivatives of the biogenic amines to cell components.

* This work was partly supported by grants from the National Institute of Mental Health (MH15410 and MH20758), The National Science Foundation (GB-30295), The State of Illinois Department of Mental Health (101-13-RD), Licensed Beverage Industries, Inc., William S. Deree, and Marg and Clem Caditz.

† Present address: Department of Biochemistry, The Chicago Medical School, Chicago, Ill. 60612.

MATERIALS AND METHODS

All chemicals were of the highest purity obtainable through commercial sources. Dihydroxyphenylethylamine-1-^{14}C hydrobromide (spec. act., 6·28 Ci/mole), 1-^{14}C-β-phenylethylamine hydrochloride (spec. act., 3·61 Ci/mole) and 3^{1}-^{14}C-tryptamine bisuccinate (spec. act., 60·0 Ci/mole) were obtained from New England Nuclear Corp., while 3^{1}-^{14}C-5-hydroxytryptamine creatinine sulfate (spec. act., 57·0 Ci/mole) was obtained from Amersham/Searle Corp. When necessary, nonradioactive serotonin creatinine sulfate (Calbiochem) or tryptamine hydrochloride and dopamine hydrochloride (Sigma Chemical Company) were added to obtain the specific activities as stated in the tables. Indoleacetaldehyde (3^{1}-^{14}C) was prepared from labeled tryptophan (New England Nuclear) by the method of Gray[8] and stored as the bisulfite complex.[7] Indoleacetaldehyde free of the bisulfite was prepared immediately prior to use by extracting a solution of the indoleacetaldehyde bisulfite complex with 3 vol. of ether, after adjusting the pH of the aqueous solution to pH 12. The ether containing the indoleacetaldehyde was evaporated over water. 5-Hydroxyindoleacetaldehyde was prepared enzymatically from serotonin-3^{1}-^{14}C utilizing rat liver MAO as described by Tabakoff *et al.*[9] The solutions containing the aldehydes were assayed and used immediately.

Labeled substrate (amine or aldehyde) was incubated with rat brain homogenate (0·04 g original tissue) prepared in 0·25 M sucrose. The incubation mixture also contained 100 mM potassium phosphate, pH 7·4, in 1 ml total volume. When present, the various inhibitors of aldehyde incorporation or MAO activity were added prior to the addition of substrate. When catechol or indole derivatives were used as displacing agents, a 10-min preincubation was performed before the addition of substrate; incubation mixtures containing pargyline were preincubated for 20 min. Sucrose was utilized to maintain equal tonicity in all incubation mixtures, and the solutions of all inhibitors were adjusted to pH 7·4 before addition to incubation mixtures. Incubations were terminated by the addition of 0·25 ml of 50 per cent trichloroacetic acid (TCA) in ice and the precipitate was washed an additional six times with 10 per cent TCA, as described by Alivisatos and Ungar.[1] After washing, the precipitate was solubilized in 1 ml of Hydroxide of Hyamine (Packard) and transferred with 15 ml of scintillation fluid [2,5-diphenyloxazole, 0·5 per cent; 1,4-bis-2-(4-Methyl-5-phenyloxazolyl) benzene, 0·03 per cent, in toluene] to counting vials for the determination of the bound radioactive material using a Packard Tri-Carb scintillation spectrometer. Counting efficiency was determined to be approximately 80 per cent with the aid of internal standard. It had been previously demonstrated[1] that the labeled material bound to subcellular components under these conditions is the aldehyde derivative of the biogenic amines.

MAO activity in rat brain homogenates in the presence of ascorbate, glutathione and cysteine was determined spectrophotometrically by the method of Tabakoff and Alivisatos.[10] A modification of the radioisotopic method of McCaman *et al.*[11] was used to determine MAO activity with the biogenic amines.

RESULTS

The addition of ascorbate or cysteine (Table 1) to incubation mixtures containing labeled serotonin, tryptamine or indole-3-acetaldehyde greatly diminishes the amount of label incorporated into TCA-insoluble material from rat brain. When β-phenylethylamine or its substituted derivative, dopamine, was incubated with rat brain

TABLE 1. EFFECT OF ASCORBATE AND CYSTEINE ON THE INCORPORATION OF RADIOACTIVITY FROM LABELED SEROTONIN, TRYPTAMINE AND INDOLE-3-ACETALDEHYDE INTO RAT BRAIN HOMOGENATES *in vitro*

Labeled substrate*	Addition	Incorporation ($\times 10^{-6}$ moles/g brain)	Inhibition (%)
Serotonin	None	6·13	0
Serotonin	Ascorbate	1·35	78
Serotonin	Cysteine	0·06	99
Tryptamine	None	5·67	0
Tryptamine	Ascorbate	1·76	69
Tryptamine	Cysteine	0·02	99
Indole-3-acetaldehyde	None	0·09	0
Indole-3-acetaldehyde	Ascorbate	0·04	56
Indole-3-acetaldehyde	Cysteine	0·03	66

* 3¹-^{14}C-5-hydroxytryptamine ¹creatinine sulfate (1×10^{-3}M; 0·29 Ci/mole), 3¹-^{14}C-tryptamine bisuccinate (1×10^{-3}M; 0·16 Ci/mole) and 3¹-^{14}C-indole-3-acetaldehyde (2×10^{-5} M; 0·042 Ci/mole) were incubated in 100 mM potassium phosphate, pH 7·4, with rat brain homogenate (0·04 g brain, wet wt) for 120 min at 37°. Ascorbate, $1{\cdot}5 \times 10^{-1}$ M, or cysteine, 9×10^{-2} M, was added where indicated. Other conditions were as described in the text. Values are averages of two to three separate experiments, each done in duplicate.

TABLE 2. EFFECT OF VARIOUS COMPOUNDS ON THE INCORPORATION OF RADIOACTIVITY FROM LABELED DOPAMINE OR β-PHENYLETHYLAMINE INTO RAT BRAIN HOMOGENATES *in vitro*

Labeled Substrate*	Addition	Incorporation ($\times 10^{-8}$ moles/g brain)	Inhibition (%)
β-Phenylethylamine	None	6·39	0
β-Phenylethylamine	Ascorbate (9×10^{-2} M)	1·21	81
β-Phenylethylamine	Cysteine (9×10^{-2} M)	0·06	99
β-Phenylethylamine	Glutathione (9×10^{-2} M)	0·45	93
β-Phenylethylamine	Pargyline (4×10^{-4} M)	1·15	82
Dopamine	None	13·15	0
Dopamine	Ascorbate (9×10^{-2} M)	5·39	59
Dopamine	Cysteine (9×10^{-2} M)	0·26	98
Dopamine	Glutathione (9×10^{-2} M)	3·42	74
Dopamine	Pargyline (4×10^{-4} M)	1·71	87

* 1-^{14}C-3,4-dihydroxyphenylethylamine hydrobromide (4×10^{-5} M; 6·28 Ci/mole) or 1-^{14}C-β-phenylethylamine hydrochloride (4×10^{-5} M; 3·61 Ci/mole) was incubated in 100 mM potassium phosphate, pH 7·4, with rat brain homogenate (0·04 g brain, wet wt). Ascorbate, cysteine or glutathione, when present, was added just prior to the addition of substrate, while mixtures containing pargyline were preincubated for 20 min. Incubations were carried on for 90 min at 37°. Values are averages of two to three experiments, each done in duplicate.

homogenates, a substantial amount of radioactivity was again incorporated into acid-insoluble material. Glutathione, as well as ascorbate and cysteine, was shown to reduce the incorporation of this labeled material (Table 2). The incorporation of radioactive material from the phenylethylamines was shown to be dependent on the presence of MAO activity. Thus, pargyline (4×10^{-4} M), which was shown to inhibit MAO activity 96 per cent, greatly decreased the amount of material bound to brain tissue.

On the other hand, cysteine (0·15 M) was shown to inhibit MAO only 48 per cent, using the spectrophotometric assay of enzyme activity, but was highly effective in preventing the binding of the aldehyde products (Table 2). Ascorbate and glutathione in concentrations used in this study had no significant effect on MAO activity; however, these agents diminished the amount of aldehyde incorporated into tissue. Obviously, the partial inhibition of MAO activity by cysteine cannot explain the near total inhibition of incorporation of aldehyde derivatives produced by MAO, nor the inhibition of the incorporation of indoleacetaldehyde *per se* into brain macromolecules (Table 1 and Discussion).

We were interested in whether the substituent on the acetaldehyde structure, common to the deaminated products of amines utilized herein, contributed to the association of these aldehydes with the binding sites. Therefore, various structural analogues to the "biogenic" aldehydes were utilized to ascertain whether they would interfere with the attachment of the aldehydes to tissue constituents. 5-Hydroxyindole seemed to be the most potent agent in all cases (Table 3). However, when the various agents (i.e. 5-hydroxyindole, 5-methoxyindole, indole and catechol) were assayed for their ability to inhibit MAO activity using labeled amines as substrates (Table 4), a reassessment of the potency of these compounds as displacing agents became necessary. Thus, it can be gleaned from Table 3 that the ability of indole and 5-methyoxyindole to reduce incorporation of label derived from serotonin (3^{1-14}C) into TCA-insoluble material may be totally accounted for by inhibition of MAO.*

On the other hand, neither 5-hydroxyindole nor catechol inhibits MAO sufficiently for this phenomenon to explain the decreased incorporation into macromolecules in their presence. Therefore, a more thorough examination of the inhibition of the deamination of serotonin, tryptamine and dopamine by 5-hydroxyindole and catechol was performed, in addition to studying the effect of these agents on aldehyde binding. The K_m for the deamination of tryptamine was found to be $8{\cdot}7 \times 10^{-5}$ M with rat brain homogenates, while the K_m for serotonin was found to be approximately $2{\cdot}5 \times 10^{-4}$ M. No inhibition of tryptamine metabolism by MAO was obtained utilizing up to 1 mM 5-hydroxyindole or catechol, even when the tryptamine concentration in incubation mixtures was below the K_m value (1×10^{-5} M). However, 5-hydroxyindole was found to be a competitive inhibitor of the deamination of serotonin with a K_i of approximately $7{\cdot}5 \times 10^{-4}$ M, while catechol was a less potent inhibitor with a K_i of $2{\cdot}2 \times 10^{-3}$ M. The inhibition of the deamination of dopamine by 5-hydroxyindole was found to be of a noncompetitive nature with a K_i of $6{\cdot}2 \times 10^{-4}$ M. Catechol proved to be a better inhibitor of the deamination of dopamine ($K_m \cong 5{\cdot}5 \times 10^{-4}$ M) than of the deamination of serotonin. The inhibition was of a mixed type[13] and the K_i was approximately $6{\cdot}7 \times 10^{-4}$ M. Although 5-hydroxyindole and catechol did not interfere with the deamination of tryptamine, these agents did prevent the subsequent binding of this aldehyde to neuronal components (Table 3).

* Inhibition of MAO by indole has also been demonstrated by McEwen *et al.*[12]

TABLE 3. INHIBITION OF MAO AND INCOPORATION OF ALDEHYDES IN RAT BRAINHOMOGENATES BY INDOLE, 5-HYDROXYINDOLE, 5-METHOXYINDOLE AND CATECHOL*

Inhibitor (mM)	Substrate	MAO activity ($\times 10^{-8}$ moles /g brain/30 min)	Inhibition of MAO activity (%)	Incorporation $\times 10^{-8}$ moles /g brain)	Inhibition of incorporation (%)	Net inhibition of incorporation (%)
None	Serotonin	12·40 ± 0·1	0	6·25 ± 0·16	0	0
Indole						
0·05	Serotonin	10·90 ± 0·2	12	5·56 ± 0·03	11	< 0
0·25	Serotonin	6·93 ± 0·4	44	3·56 ± 0·05	43	< 0
1·00	Serotonin	2·73 ± 0·8	68	2·13 ± 0·04	66	< 0
5-Hydroxyindole						
0·05	Serotonin	12·61 ± 0·1	0	3·56 ± 0·20	43	43
0·25	Serotonin	9·89 ± 0·8	20	0·31 ± 0·02	95	75
1·00	Serotonin	4·70 ± 2·0	62	0·38 ± 0·02	94	32
5-Methoxyindole						
0·05	Serotonin	9·52 ± 0·2	23	5·32 ± 0·04	15	< 0
0·25	Serotonin	5·07 ± 0·2	59	3·19 ± 0·06	49	< 0
1·00	Serotonin	1·86 ± 0·5	85	0·88 ± 0·02	86	1
Catechol						
0·05	Serotonin	12·99 ± 0·3	0	5·25 ± 0·07	16	16
0·25	Serotonin	12·25 ± 0·2	0	4·13 ± 0·07	34	34
1·00	Serotonin	11·13 ± 0·5	10	2·38 ± 0·02	62	52
None	Tryptamine	16·30 ± 0·6	0	6·96 ± 0·06	0	0
Indole						
0·05	Tryptamine	16·45 ± 0·4	0	6·62 ± 0·06	5	5
0·25	Tryptamine	16·78 ± 0·7	0	6·83 ± 0·28	2	2
1·00	Tryptamine	16·60 ± 0·6	0	6·97 ± 0·06	0	0
5-Hydroxyindole						
0·05	Tryptamine	17·26 ± 0·7	0	2·72 ± 0·21	61	61
0·25	Tryptamine	18·57 ± 0·9	0	0·28 ± 0·10	96	96
1·00	Tryptamine	16·45 ± 2·2	0	0·07 ± 0·07	99	99
Catechol						
0·05	Tryptamine	16·15 ± 0·8	0	5·78 ± 0·04	17	17
0·25	Tryptamine	16·45 ± 0·5	0	2·79 ± 0·20	60	60
1·00	Tryptamine	18·76 ± 1·2	0	0·56 ± 0·04	92	92
None	Dopamine	21·37 ± 0·9	0	13·15 ± 0·06	0	0
Indole						
0·05	Dopamine	14·56 ± 0·5	32	11·58 ± 0·03	12	< 0
0·25	Dopamine	10·69 ± 0·3	50	8·16 ± 0·02	38	< 0
1·00	Dopamine	4·23 ± 0·9	80	3·68 ± 0·06	72	< 0
5-Hydroxyindole						
0·05	Dopamine	18·00 ± 0·6	16	6·35 ± 0·04	52	36
0·25	Dopamine	11·33 ± 0·5	47	0·85 ± 0·05	94	47
1·00	Dopamine	4·23 ± 1·0	80	0·66 ± 0·06	95	15
Catechol						
0·05	Dopamine	22·88 ± 1·2	0	10·65 ± 0·02	19	19
0·25	Dopamine	21·95 ± 0·8	0	4·99 ± 0·03	62	62
1·00	Dopamine	17·49 ± 0·9	18	1·84 ± 0·05	86	68

* Rat brain homogenate (0·04 g brain, wet wt) was preincubated for 10 min in 100 mM potassium phosphate, pH 7·4 in the presence or absence of indole, 5-hydroxyindole, 5-methoxyindole or catechol. 3¹-^{14}C-5-hydroxytryptamine creatinine sulfate (1×10^{-5} M; 55 Ci/mole,) 3¹-^{14}C-tryptamine bisuccinate (1×10^{-5} M; 32 Ci/mole) or 1-^{14}C-3,4-dihydroxyphenylethylamine hydrobromide (4×10^{-5} M; 6·28 Ci/mole) was added after the preincubation period.

To determine MAO activity after 30 min of incubation at 37°, the reaction was stopped by the addition of 0·4 ml of 3 N HCl and the mixture was extracted with 7 ml ether. An aliquot of the ether extract was taken to dryness in a counting vial, 1 ml Hydroxide of Hyamine (Packard Instrument Company) and 15 ml scintillation fluid were added, and radioactivity was determined. To determine the amount of aldehyde incorporated into brain macromolecules, the samples were incubated for 90 min at 37° and incorporated radioactivity was determined after washing, as described in the text. Values are the means of three to four separate experiments, each done in duplicate ± the standard deviation. Values of net inhibition of incorporation which are less than zero demonstrate MAO inhibition that is not totally reflected by inhibition of incorporation.

Another feature of interest also became evident (Table 3). If one compensated* for the contribution of MAO inhibition to the reduction in aldehyde binding, it was found that 5-hydroxyindole was a more potent displacer of aldehydes derived from serotonin compared to dopamine (Table 3, Net inhibition of incorporation).

TABLE 4. INHIBITION OF THE INCORPORATION OF RADIOACTIVITY FROM LABELED 3^1-^{14}C-5-HYDROXY-3-INDOLEACETALDEHYDE INTO RAT BRAIN*

Inhibitor (mM)	Substrate 3^1-^{14}C-5-hydroxy-3-indoleacetaldehyde ($\times 10^{-6}$ M)	Incorporation ($\times 10^{-8}$ moles/g brain)	Inhibition of incorporation (%)
None	0·5	0·26 ± 0·05	0
None	4·0	1·96 ± 0·20	0
Indole			
1·00	0·5	0·29 ± 0·06	< 0
1·00	4·0	2·11 ± 0·08	< 0
5-Hydroxyindole			
1·00	0·5	0·12 ± 0·02	56
1·00	4·0	1·09 ± 0·05	44
5-Methoxyindole			
1·00	0·5	0·26 ± 0·02	0
1·00	4·0	1·97 ± 0·05	0
Catechol			
1·00	0·5	0·14 ± 0·03	46
1·00	4·0	1·18 ± 0·07	40
5-Methoxy-3-indole-carboxyaldehyde			
0·25	0·5	0·26 ± 0·05	0
0·25	4·0	2·03 ± 0·08	0
Acetaldehyde			
0·25	4·0	2·11 ± 0·15	0

* Rat brain homogenate (0·4 g brain, wet wt) was preincubated for 10 min in 100 mM potassium phosphate, pH 7·4, in the presence or absence of the inhibitor. 3^1-^{14}C-5-hydroxy-3-indoleacetaldehyde (3·333 Ci/mole) was added after this period, and incubation continued at 37° for 30 min. Values are the means of three experiments ± standard deviation.

To assess further the specificity of the attachment of the aldehyde derivatives of the indoleamines, various agents including certain aldehydes (i.e. 5-methoxy-3-indole-carboxaldehyde and acetaldehyde) were preincubated with brain tissue before the addition of preformed 5-hydroxyindoleacetaldehyde (Table 4). Only catechol and 5-hydroxyindole were found to prevent the attachment of 5-hydroxyindoleacetaldehyde (5-HIAAld). 5-Hydroxyindole was found to be the more potent of the two agents when incubated with low concentrations of 5-HIAAld. When 5-hydroxyindole and

* As an approximation, one can subtract the per cent MAO inhibition from the per cent inhibition of incorporation. This is not totally satisfactory, since the rate of incorporation in relation to aldehyde production should also be considered. In addition, at high inhibitor concentrations, the inhibition of MAO obscures the displacement potential of the various agents that were employed. However, a justification for this approach may be provided by results in Table 4, since a similar rank order of displacement potential for catechol and indoles is obtained using the preformed aldehyde.

catechol were incubated with indoleacetaldehyde (Table 5), 5-hydroxyindole was again more potent than catechol in preventing the incorporation of even high concentrations of indoleacetaldehyde. Neither acetaldehyde nor 5-methoxyindolecarboxaldehyde interfered with the binding of 5-HIAAld (Table 4). In addition, when acetaldehyde-^{14}C (Schwarz-Mann; spec. act., 0·001 μCi/μmole) was incubated with rat brain tissue in concentrations up to 2×10^{-5} M, no significant binding of this aldehyde could be detected under our conditions.

TABLE 5. INHIBITION OF THE INCORPORATION OF RADIOACTIVITY FROM LABELED ^{14}C-3-INDOLEACETALDEHYDE IN RAT BRAIN HOMOGENATE BY INDOLE, 5-HYDROXYINDOLE AND CATECHOL*

Inhibitor (mM)	Incorporation ($\times 10^{-8}$ moles/g brain)	Inhibition of incorporation (%)
None	15·11	0
Indole		
0·05	15·13	0
0·25	15·16	0
1·00	15·11	0
5-Hydroxyindole		
0·05	12·73	16
0·25	11·93	21
1·00	10·00	34
Cathechol		
0·05	15·11	0
0·25	12·50	17
1·00	11·25	26

* Rat brain homogenate (0·04 g brain, wet wt) was preincubated for 10 min in 100 mM potassium phosphate, pH 7·4, in the presence or absence of the inhibitors. ^{14}C-3-indoleacetaldehyde (5×10^{-5} M; 0·04 Ci/mole) was added after this period, and samples were further incubated at 37° for 60 min. Values are the average of two experiments, each performed in duplicate.

DISCUSSION

Aldehyde derivatives of indoleamines were previously shown to be incorporated into particulate cell components.[3] In this current work, we have extended these observations with the demonstration that the aldehyde derivatives of other biologically active amines (β-phenylethylamine and dopamine) also bind to tissue.

Since the binding of metabolites of the biogenic amines depends on MAO activity, the amount of bound material, in turn, depends on the concentration of the amine available to MAO. Thus, a greater amount of binding would occur at concentrations of the amines which are above their respective K_m values with MAO (Table 1). However, even at low substrate concentrations (Table 3), a large portion of the deaminated "biogenic" amine is bound to tissue.

The possibility that the binding we were investigating was nonspecific and dependent only on the presence of an aldehydic function, was investigated by the use of chemical

analogues lacking aldehydic residues and aldehydes containing substituents which differ from those of the "biogenic" aldehydes. Certain of these analogues, e.g. 5-hydroxyindole or catechol, prevent the incorporation of the aldehydes derived from the amines, and this phenomenon cannot be accounted for by their inhibition of MAO. This observation indicates that substituent groups on the carbonyl function participate in the orientation of the aldehyde prior to its attachment. Similarly, Alivisatos *et al.*[5] demonstrated that the indole and hydroxyl groups of serotonin participate in the reversible binding of the amine to CNS "receptors."

The presence of a free (unsubstituted) hydroxyl group on a constituent aromatic nucleus seems to be particularly important in the orientation and firm attachment of the "biogenic" aldehydes to tissue (Tables 3 and 4). Aliphatic aldehydes having free hydroxyl substituents have also been shown to be better protein cross-linking agents compared to those lacking the second functional group.[14] Both 5-hydroxyindole and catechol diminish the binding of aldehydes produced from dopamine, tryptamine and serotonin by MAO and also of the preformed indoleacetaldehyde and 5-HIAAld (Tables 3–5). These agents seemed to be more efficacious in preventing the binding of the nonhydroxylated aldehyde derived from tryptamine. This was also evident even when indoleacetaldehyde was utilized in incubation mixtures, since binding was diminished even at the high concentrations of indoleacetaldehyde used in these experiments.

5-Hydroxyindole was more potent in preventing the incorporation of 5-HIAAld than catechol (Table 4). Similarly, 5-hydroxyindole was significantly more potent as an inhibitor of MAO compared to catechol when serotonin was the substrate (Table 3). However, the differences in inhibition of MAO by 5-hydroxyindole and catechol were less pronounced when dopamine was the substrate (Results and Table 3). These results, together with the inability of 5-hydroxyindole and catechol to inhibit the deamination of tryptamine and the differences in the type of inhibition depending on the substrate, are consistent with the findings of others,[15] which demonstrate the presence of multiple forms of MAO in rat brain.

Our studies demonstrate an important pitfall in the use of radioactive "biogenic" amines for the determination of MAO activity with impure enzyme preparations. A substantial amount of the products formed by MAO is bound to tissue. This phenomenon would lead to underestimation of enzyme activity and errors in determination of kinetic constants. Although data derived by means of the radioisotopic assay may be used for comparative purposes with similar enzyme preparations and the same substrate, the bound material would have to be considered for the determination of absolute values of MAO activity.

The demonstration that cysteine, glutathione and ascorbate inhibit the incorporation of the "biogenic" aldehydes into rat brain tissue stems from our recent findings related to the inhibition of formation of tetrahydroisoquinoline alkaloids by various reducing or trapping agents.[6] The mechanism of that inhibition[7] depends on the nature of the micromolecule used as the inhibitor. Cysteine, for example, acts primarily as a trapping agent, forming thiazolidine derivatives[16] with the various aldehydes, while ascorbate probably acts as a reducing and complexing agent.

The same general mechanisms must be applicable to the inhibition of the incorporation into macromolecules reported here. This observation is in agreement with previously postulated mechanism(s) of attachment.[1, 2]

Attachment of indoleacetaldehydes may have important biological implications. This has been discussed previously[3, 17, 18] and supporting experimental evidence from studies *in vivo*, related to the possible role of indoleacetaldehydes in sleep, has been published.[19, 20] In our recent work, we found that barbiturates and acetaldehyde which are inhibitors of aldehyde metabolism in the brain,[21] enhance attachment of radioactivity derived from serotonin in midbrain homogenates.[22] More recent work in our laboratory indicates that such increased incorporation of radioactivity may also be observed *in vivo* after prolonged ethanol exposure.[23]

We have also demonstrated that "biogenic" aldehyde binding occurs in tissues, such as the heart and liver, that possess MAO activity. It is therefore pertinent to speculate that incorporation of aldehyde derivatives of indoleamines into proteolipids of endothelial membranes, with subsequent polymerization, may play a triggering role in the fibrous proliferations observed in carcinoid tumors and, as such, it may have a bearing on the molecular pathogenesis of certain symptoms occurring in these tumors.[24, 25] We are currently investigating the ameliorative effects *in vivo* of certain agents used in this study (i.e. ascorbate, cysteine) on tissue alterations caused by chronic administration of serotonin.

Acknowledgements—Sincere gratitude is extended to Mr. J. Soco and Mrs. Irma Roque for their fine technical assistance.

REFERENCES

1. S. G. A. Alivisatos and F. Ungar, *Biochemistry, N.Y.* **7**, 285 (1968).
2. S. G. A. Alivisatos, F. Ungar and S. S. Parmar, *Biochem. biophys. Res. Commun.* **25**, 495 (1966).
3. S. G. A. Alivisatos, F. Ungar, P. K. Seth and L. P. Levitt, *Biochem. Pharmac.* **19**, 401 (1970).
4. S. G. A. Alivisatos, A. D. Papaphilis, F. Ungar and P. K. Seth, *Nature, Lond.* **226**, 455 (1970).
5. S. G. A. Alivisatos, F. Ungar, P. K. Seth, L. P. Levitt, A. J. Geroulis and T. S. Myer, *Science, N.Y.* **171**, 809 (1971).
6. S. G. A. Alivisatos, F. Ungar, O. H. Callaghan, L. P. Levitt and B. Tabakoff, *Can. J. Biochem.* **51**, 28 (1973).
7. S. G. A. Alivisatos and P. K. Seth, in *Methods in Neurochemistry* (Ed. R. Fried), Vol. 2, p. 205. Dekker, New York (1972).
8. R. A. Gray, *Archs Biochem. Biophys.* **81**, 480 (1959).
9. B. Tabakoff, R. Anderson, F. Ungar and S. G. A. Alivisatos, *Trans. Am. Soc. Neurochem.* **3**, 126 (1972).
10. B. Tabakoff and S. G. A. Alivisatos, *Analyt. Chem.* **44**, 427 (1972).
11. R. E. McCaman, M. W. McCaman, J. M. Hunt and M. S. Smith, *J. Neurochem.* **12**, 15 (1965).
12. C. M. McEwen, Jr., G. Sasaki and D. C. Jones, *Biochemistry, N.Y.* **8**, 3952 (1969).
13. M. Dixon and E. C. Webb, *Enzymes*, p. 178. Academic Press, New York (1958).
14. R. A. Milch, *Gerontologia* **10**, 177 (1964–65).
15. M. B. H. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223**, 626 (1969).
16. D. French and J. Edsall, *Adv. Protein Chem.* **2**, 277 (1945).
17. S. G. A. Alivisatos and B. Tabakoff, in *Chemical Modulation of Brain Function* (Ed. H. Sabelli), p. 184. Raven Press, New York (1973).
18. B. Tabakoff, F. Ungar and S. G. A. Alivisatos, in *Alcohol Intoxication and Withdrawal: Experimental Studies* (Ed). M. Gross), *Adv. in Exp. Med. and Biol.*, Vol. V, p. 35. Plenum Press, New York, in press (1973).
19. H. C. Sabelli, W. J. Giardina, S. G. A. Alivisatos, P. K. Seth and F. Ungar, *Nature, Lond.* **223**, 73 (1969).
20. H. C. Sabelli, W. J. Giardina and S. G. A. Alivisatos, *Arnzeimittel-Forsch.* **20**, 68 (1970).
21. V. G. Erwin, B. Tabakoff and R. Bronaugh, *Molec. Pharmac.* **7**, 169 (1971).
22. B. Tabakoff, F. Ungar and S. G. A. Alivisatos, *Nature New Biol.* **238**, 126 (1972).
23. B. Tabakoff, W. O. Boggan, F. Ungar and S. G. A. Alivisatos, *Second Annual Meeting of the Society for Neuroscience*, p. 246. Program and Abstracts, Houston (1972).
24. M. Sandler, *Adv. Pharmac.* **6B**, 127 (1968).
25. A. R. Adamson, D. G. Grahame-Smith, W. S. Peart and M. Starr, *Lancet* **2**, 293 (1969).