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N-Acetyltransferase of Brain: Some Properties of the Enzyme and the Identification of β-Carboline Inhibitor Compounds

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SUMMARY

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Several β-carboline derivatives, such as harmane, harmol, 6-methoxyharman, and melatonin, inhibited the *N*-acetylation of tryptamine by brain *N*-acetyltransferase. The enzyme of brain was active toward several indole- and phenylethylamine substrates. In contrast to the enzyme of brain, the enzyme of pineal was not inhibited by harmaline and harman, and 3,4-dimethoxyphenylethylamine was a relatively poor substrate. β-carboline inhibitors may be useful aids for studying the various *N*-acetyltransferases and for evaluating the physiological role of the brain enzyme.

INTRODUCTION

N-Acetyltransferase (acetyl-CoA:arylamine *N*-acetyltransferase; EC 2.3.1.5) catalyzes acyl transfer from acetyl-CoA to a wide variety of aromatic and other amines. Serotonin *N*-acetyltransferase of pineal gland has been intensively investigated because of its role in the synthesis of melatonin (1, 2). Liver *N*-acetyltransferase has been investigated because it acetylates isoniazid, various sulfonamides, and other structurally related drugs. The activity of the liver enzyme is low in some individuals, which leads to susceptibility to isoniazid toxicity (3). Liver *N*-acetyltransferase also catalyzes the acetylation of endogenous amines, including serotonin, tryptamine, histamine, phenylethylamine, tyramine, octopamine, and normetanephrine (4). Acetylation of certain amines by liver may be important after treatment with monoamine oxidase inhibitor drugs (5). The existence of *N*-acetyltransferase activity in brain has been reported (4, 6, 7); however, the physiological role of the enzyme in the brain is not known. The possibility that brain *N*-acetyltransferase might be important for the metabolism of the endogenous amines and, perhaps, certain drugs prompted us to search for inhibitor drugs and to compare some of the characteristics of the *N*-acetyltransferase of brain with the enzyme of pineal. A preliminary report of this investigation has been published (8).

MATERIALS AND METHODS

Materials. [1-^{14}C]Acetyl-CoA, 57.6 mCi/mmole, was purchased from New England Nuclear Corporation. Sprague-Dawley male rats 150–200 g, were obtained from Zivic-Miller Laboratories. β-Carboline and indole derivatives were purchased from Regis Chemical Company.

Enzyme assay. *N*-Acetyltransferase activity was measured by the method of Deguchi and Axelrod (9) with some modification. The enzyme was incubated for 30 min at 37° with 1 mM amine substrate (tryptamine, 5-methoxytryptamine, 2-phenylethylamine, or 3,4-dimethoxyphenylethylamine) and 0.07 mM [1-^{14}C]acetyl-CoA in a

total volume of 100 μl of 0.05 M potassium phosphate buffer (pH 7.4). The reaction was terminated by adding 0.5 ml of 0.5 M borate buffer (pH 10) and 6 ml of a toluene–isoamyl alcohol mixture (97:3 by volume). After mixing and centrifugation, 5 ml of the organic phase were transferred to a counting vial and evaporated under a stream of air. Aquasol (New England Nuclear), 5 ml, was added to the residue, and radioactivity was measured. Amine substrates were omitted for determination of blank values. Protein was determined by the method of Lowry *et al.* (10), with bovine serum albumin as standard.

Preparation of N-acetyltransferase. Rat brain was homogenized with 7 volumes of 1 mM EDTA containing 0.2 mM dithiothreitol in a Waring Blendor. The homogenate was adjusted to pH 5 with 1 N HCl. After standing for 1 hr at 0°, a precipitate formed which was removed by centrifugation at 16,000 × *g* for 20 min. The supernatant was adjusted to pH 7.0, with 1 N NaOH and subjected to ammonium sulfate fractionation. Enzymatically active protein was collected between 45% and 65% saturation with ammonium sulfate. The precipitated protein was dissolved in a small volume of 0.05 M potassium phosphate buffer, pH 7.4, containing 0.1 mM dithiothreitol, and dialyzed against the buffer overnight. Significant enzyme activity was lost if dithiothreitol was not included in the buffer. Moreover, *p*-chloromercuribenzoate (0.1 mM) almost completely inhibited the enzyme, suggesting that a sulfhydryl moiety is essential for activity. The specific activity of the preparation was about 1.3 nmoles of acetyltryptamine per hour per milligram of protein, and it was free of monoamine oxidase and choline acetyltransferase activity.

Comparison of brain and pineal N-acetyltransferase activities. Rats were treated with *l*-isoproterenol, 5 mg/kg intraperitoneally, and killed 3 hr later. This treatment increases pineal *N*-acetyltransferase activity (11). Pineal glands were removed immediately and homogenized with isotonic KCl, and enzyme activity was assayed as already described for brain and also by the method previously described by Deguchi and Axelrod (9).

RESULTS

Inhibition by β-carbolines of brain N-acetyltransferase. Marked enzyme inhibition was produced by β-carboline derivatives (Table 1). Harmane, harmol, and 6-methoxyharman at 10 μM inhibited enzyme activity by about 70% when 1 mM tryptamine was used as the substrate. Melatonin also produced considerable inhibition of the *N*-acetyltransferase of tryptamine. The inhibition of *N*-acetyltransferase by harmaline was competitive with tryptamine (Fig. 1). The drugs did not interfere with the extraction of acetyltryptamine, as their addition after the enzymatic reaction had no appreciable effect.

Comparison of brain and pineal N-acetyltransferase activities. Homogenates of pineal *N*-acetylated tryptamine, serotonin, and 5-methoxytryptamine, as well as 2-phenylethylamine and 3,4-dimethoxyphenylethylamine. Unlike the brain enzyme, however, the pineal enzyme was only slightly active toward 3,4-dimethoxyphenylethylamine (Table 2). Moreover, β-carboline derivatives inhibited the brain enzyme but not the pineal enzyme (Table 3).

DISCUSSION

One procedure for evaluating the physiological role of an enzyme in a biological system is to block the enzyme and look for functional changes. This report describes some of the properties of brain *N*-acetyltransferase and identifies some β-carboline derivatives that may be potentially useful aids for evaluating the physiological role of the brain enzyme.

We found that harmaline was apparently a competitive antagonist of the *N*-acetyltransferase of tryptamine. Harmane, harmol, and 6-methoxyharman, at $^1/_{100}$ the concentration of tryptamine, inhibited enzyme activity by about 70%. β-Carbolines are monoamine oxidase inhibitors; however, their ability to block this enzyme is probably unrelated to their ability to block *N*-acetyltransferase, as a structurally different monoamine oxidase inhibitor, pargyline (0.1 mM), was ineffective at the concentration tested.[1]

[1] Unpublished observations.

TABLE 1

Inhibition of N-acetyltransferase of rat brain by β-carboline and indole derivatives

Inhibitors (0.01 mM) were incubated at room temperature for 30 min with partially purified enzyme, after which tryptamine (1 mM) and acetyl-CoA (0.07 mM) were added and the incubation was continued at 37° for 30 min. The specific activity of the enzyme preparation was 1.3 nmoles/hr/mg protein.

Inhibitor	Activity
	% control
Harmaline	73
Harmine	50
Harmane	30
Harmol	28
6-Methoxyharmalan	51
Yohimbine	99
Melatonin	33
6-Hydroxymelatonin	62
6-Methoxyharman	32
Harminic acid	84

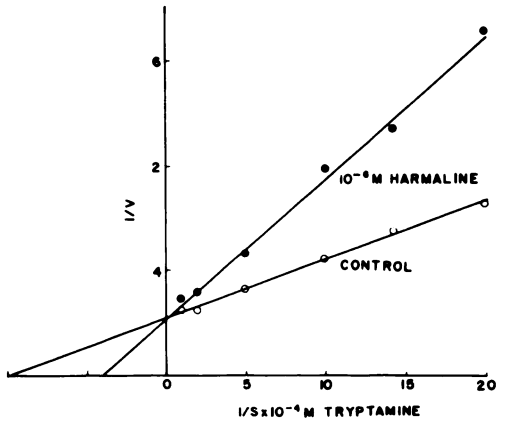

FIG. 1. *Lineweaver-Burk plot of acetylation of tryptamine in the presence and absence of harmaline*

Partially purified enzyme was incubated with 1 μM harmaline for 30 min at room temperature. [1-^{14}C]Acetyl-CoA (0.07 mM) and tryptamine were then added, and the incubation was continued for 30 min at 37°. The radioactive product was extracted as described in MATERIALS AND METHODS.

In other studies, not reported in detail in this paper, we found that *o*-phenanthroline and α,α-dipyridyl inhibited *N*-acetyltransferase activity and that activity could be restored by adding a divalent metal ion such as Co^{2+} or Fe^{2+}. Although chelating agents can inhibit by many mechanisms, the studies suggest that a divalent metal is required for enzyme activity.

The β-carboline derivative 6-methoxy-1,2,3,4-tetrahydro-β-carboline increases the concentration of serotonin in rat brain (12, 13). This increase is more pronounced in cerebellum than in other regions. The effect is not due to inhibition of monoamine oxidase, as the compound is a poor inhibitor of this enzyme *in vitro* and it does not affect the level of 5-hydroxyindoleacetic acid when injected. In addition to increasing the endogenous serotonin concentration, the compound also retards the disappearance of intracisternally injected radioactive serotonin. In view of the inhibitory property of the β-carboline derivatives toward brain *N*-acetyltransferase, it appears that *N*-acetyltransferase of brain may play a role in the metabolism of endogenous amines, particularly indoleamines, in the cerebellum. This notion is supported by the recent identification of an *N*-acetylindolealkylamine in the cerebellum of the rat, which apparently did not originate in the pineal (14), and our finding that the rat cerebellum has the highest *N*-acetyltransferase activity of all the brain areas evaluated.[1] Furthermore, harmaline treatment produces tremors in animals which may be the consequences of a selective action on the olivo-cerebellar system (15).

5-Methoxytryptamine and melatonin are normally present in the hypothalamus of rat brain (16), and they are still present following pinealectomy (17). Brain *N*-acetyltransferase has a relatively low K_m (41 μM)[1] for 5-methoxytryptamine; thus it is possible that one of the functions of the enzyme is to catalyze the formation of melatonin in brain.

It is very likely that brain and pineal *N*-acetyltransferases are different. The enzyme of brain is more active toward 3,4-dimethoxyphenylethylamine than that of pineal. Harmaline and harman inhibited the brain enzyme but not the pineal enzyme. Moreover, the enzyme of brain did not exhibit a circadian rhythm.

In conclusion, we have presented some of the properties of *N*-acetyltransferase of brain and have demonstrated that β-carbolines are relatively potent inhibitors of this enzyme activity. These compounds may be useful aids in studying the various

TABLE 2

Comparison of pineal and brain N-acetyltransferase activities

Substrate	Concentration	pH	*N*-Acetyltransferase activity	
			Pineal[a]	Brain[b]
	mM		*nmoles/hr/mg protein*	
Tryptamine	1.5	6.5	4.2	
Tryptamine	1	7.4	2.0	1.8
5-Methoxytryptamine	1	7.4	2.0	2.4
3,4-Dimethoxyphenylethylamine	1	7.4	0.2	3.0
2-Phenylethylamine	1	7.4	1.0	1.3

[a] The pineal homogenate was prepared from rats treated with *l*-isoproterenol, 5 mg/kg intraperitoneally, 3 hr before death.

[b] Partially purified brain enzyme.

TABLE 3

Differential inhibition of N-acetyltransferase activities of brain and pineal by β-carbolines

Partially purified rat brain enzyme or homogenate of pineal was incubated with inhibitor (0.1 mM) at room temperature for 30 min. Tryptamine (1 mM) and acetyl-CoA (0.07 mM) were added, and the incubation was continued at 37° for 30 min.

Inhibitor	Inhibition
	%
Brain	
Harmane	88
Harmaline	75
Pineal	
Harmane	9
Harmaline	0

N-acetyltransferases and in evaluating the physiological role of the brain enzyme. There is now circumstantial evidence that the enzyme may serve a function in the cerebellum. Apparently the enzymes of brain and pineal are different.

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