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Rat liver *N*-acetyltransferase: inhibition by melatonin

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Mammalian liver *N*-acetyltransferase (EC 2.3.1.5.) has been studied extensively in rabbits, rats, monkeys and man [1, 2]. This enzyme (or family of enzymes) is responsible for the acetylation of various drugs, including isoniazid, sulfadiazine and other aromatic amines: it may also participate in the metabolism of endogenous serotonin [3–5]. Weber and Cohen [6] have reported that an acetylated end-product (*N*-4-acetylsulfamethazine) competitively inhibits *N*-acetyltransferase activity; others have observed inhibition by various psychotropic drugs, such as reserpine, iproniazid and harmine [5]. We now report that melatonin (*N*-acetyl-5-methytryptamine) and *N*-acetylserotonin are also potent inhibitors of rat liver *N*-acetyltransferase activity.

Rat liver *N*-acetyltransferase was prepared by the method of Saavedra *et al.* [7], which involves precipitation of the enzyme in a 40–65% saturated NH_4SO_4 fraction, and then dialysis. The enzyme assay was a modification of that used by Deguchi and Axelrod [8]: this method consisted of adding tryptamine or serotonin (final concentration 10^{-3}–10^{-6} M) and [^{14}C] or [^{3}H]AcCoA (1–10×10^{-5} M, at 60 mCi/m-mole and 0.74 mCi/m-mole, respectively; New England Nuclear Co., Boston, Mass.) to the enzyme (0.5–2.0 μl), and incubating for 10–30 min at 37° in 0.1 M phosphate buffer (pH 7.9, the enzyme's optimum), in a final incubation vol. of 50 μl. The reaction was stopped with 0.5 ml of 0.5 M borate buffer (pH 10.0) and the product extracted with 5 ml of isoamyl alcohol–toluene, 3:97 (for acetyltryptamine), or with 5 ml of pure isoamyl alcohol (for acetylserotonin). Four milliliters of the organic phase were removed and evaporated to dryness in a scintillation vial; 10 ml of toluene fluor were then added for counting.

Rat pineal *N*-acetyltransferase was similarly measured (incubation time, 10 min; pH 6.5, its optimum) in a homogenate of rat pineals, which had been collected at night in the dark and quick-frozen. We also studied a bovine pineal *N*-acetyltransferase, in a dialyzed 35–65% NH_4SO_4 fraction prepared according to Saavedra *et al.* [7] (incubation time, 10–60 min; pH 5.5–9.0, enzyme optimum about 6.5). Bovine pineals were 'mixed butcher run' from Pel-Freez (Rogers, Ark.). The activities of the rat liver and bovine pineal enzymes were linear for at least 30 min; that of the rat pineal enzyme was linear for only 10 min.

Rat liver *N*-acetyltransferase was found to have an apparent K_m for tryptamine of 6×10^{-5} M and a K_i for melatonin of 1×10^{-6} M at an AcCoA concentration of 3.5×10^{-5} M (Fig. 1a, 1b). When the tryptamine concentration was near saturation (10^{-4} M), [^{14}C]acetyltryptamine was formed at a rate of about 3 pmoles/min/mg of liver. The inhibition by melatonin of *N*-acetyltransferase activity was competitive with tryptamine and noncompetitive with AcCoA. Acetylserotonin was equipotent with melatonin in competitively inhibiting the enzyme. The liver enzyme acetylated serotonin at a lower rate than it did tryptamine, and melatonin was a correspondingly better inhibitor: 50 per cent inhibition of acetylation was obtained with 10 ng (43 pmole) of melatonin with tryptamine as substrate, compared to 5 ng (22 pmole) with serotonin as substrate (Fig. 2).

Rat pineal *N*-acetyltransferase, assayed at pH 6.5 with 3.5×10^{-5} M AcCoA, had an apparent K_m for tryptamine of 6×10^{-4} M. Concentrations of melatonin up to 10^{-4} M did not significantly inhibit this enzyme. With tryptamine as substrate, in a concentration near saturation (10^{-3} M), the rate of [^{14}C]acetyltryptamine formation was about 60 pmole/min/mg of pineal. The *N*-acetyltransferase found in the bovine pineal extract (assayed at pH 6.5, with 3.5×10^{-5} M AcCoA) exhibited a K_m for tryptamine of 10^{-5} M. Melatonin and acetylserotonin competitively inhibited this enzyme (K_i ca. 10^{-6} M). The activity of the bovine pineal enzyme with tryptamine concentration near saturation (10^{-4} M) was about 0.03 pmole/min/mg of pineal.

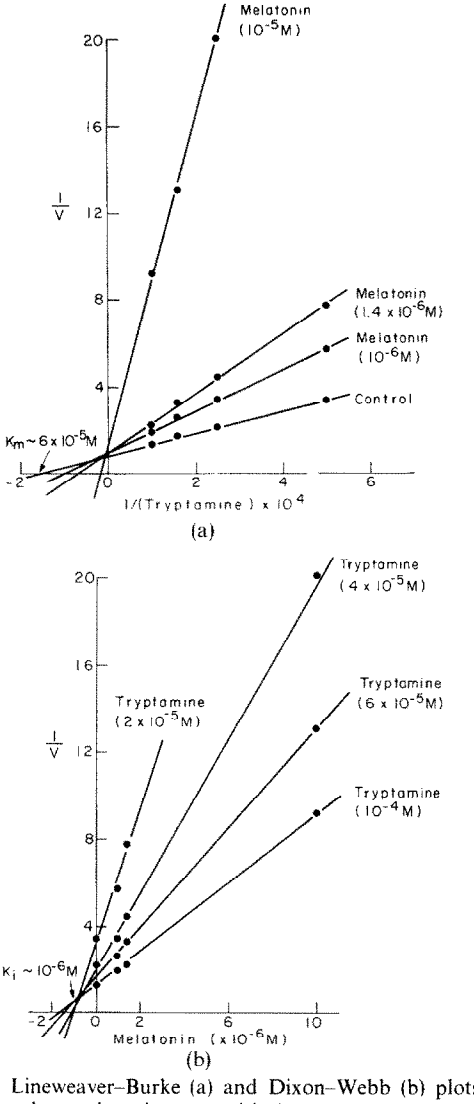

Fig. 1. Lineweaver–Burke (a) and Dixon–Webb (b) plots showing that melatonin competitively inhibits tryptamine acetylation by rat liver *N*-acetyltransferase. Samples containing [^{14}C]AcCoA in a concentration of 3.5×10^{-5} M were incubated for 10 min; $1/v$ represents 1/cpm $\times 10^4$.

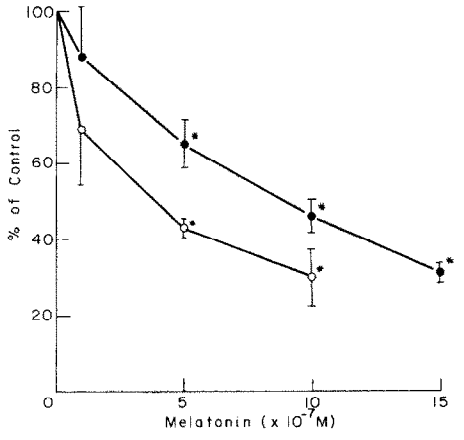

Fig. 2. Inhibition of rat liver *N*-acetyltransferase by various concentrations of melatonin, using tryptamine or serotonin as substrates. Incubation time was 30 min; [^{3}H]AcCoA concentration was 1.4×10^{-5} M: tryptamine (●) and serotonin (○) concentrations were 10^{-6} M. All samples were run in triplicate: tryptamine controls gave 1995 ± 120 (S.E.M.) cpm, with blanks of 280 ± 20 cpm; serotonin controls gave 1200 ± 95 cpm, with blanks of 690 ± 40 cpm. Results are plotted as per cent of control; bars show S.E.M.; asterisks indicate statistical significance ($P < 0.05$). 1×10^{-7} M melatonin = 1.15 ng in 50 μl.

These studies demonstrate that rat liver *N*-acetyltransferase and a bovine pineal *N*-acetyltransferase are quite sensitive to inhibition by melatonin and acetylserotonin; in contrast, the *N*-acetyltransferase activity in pineals of rats killed at night appears to be insensitive to these indoles. (We were unable to examine the effect of melatonin on homogenates of pineals obtained from animals killed during the day, because the basal enzyme activity was too low.) The fact that melatonin and *N*-acetylserotonin can inhibit the liver enzyme does not, of course, prove that these indoles normally do affect *N*-acetyltransferase activity *in vivo*. The concentrations of these compounds in blood are probably very low; their concentrations in the liver are unknown. Liver *N*-acetyltransferase activities vary widely in animal and human populations [1, 9]; it is thus possible that inhibition of this enzyme by melatonin or *N*-acetylserotonin might be significant in the low-acetylator phenotype, or in conditions associated with elevated levels of melatonin or acetylserotonin (e.g., after L-DOPA administration [10], or, possibly, in patients with pineal or carcinoid tumors).

The stable, low-activity bovine pineal enzyme differs from the high-activity, highly labile rat pineal enzyme [11], and is not necessarily the same enzyme that catalyzes most of the acetylserotonin synthesis in bovine pineals. Its properties seem more like those of the *N*-acetyltransferase recently observed in rat brain tissue [12, 13]. Since melatonin may be present in the brain [14, 15], its effects on this latter enzyme would be worthy of investigation.

Rat pineal melatonin levels exhibit a daily rhythm, averaging less than 1 ng/pineal (ca. 5×10^{-6} M) during the day and about 6 ng/pineal (ca. 3×10^{-5} M) at night [16]. These concentrations failed, in the present study, to affect rat pineal *N*-acetyltransferase activity. Melatonin was similarly without effect on the conversion of [^{14}C]tryptophan to serotonin or melatonin by cultured rat pineal organs [17]. Melatonin could theoretically suppress its own synthesis by acting on hydroxyindole *O*-methyltransferase [18]: however, the concentration necessary to cause significant inhibition of this enzyme may be beyond that present in mammalian pineals.

The inhibition of rat liver *N*-acetyltransferase by melatonin makes possible an enzyme-inhibition assay for melatonin with a sensitivity of less than 2 ng. Although more sensitive assays are available [19, 20], these involve bioassay or immunochemical techniques. Hence, an assay based on enzyme inhibition might have use where facilities for bioassay or immunoassay are unavailable.

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REFERENCES

1. D. A. P. Evans and T. A. White, *J. Lab. clin. Med.* **63,** 394 (1964).
2. W. W. Weber, S. N. Cohen and M. S. Steinberg, *Ann. N.Y. Acad. Sci.* **151,** 734 (1968).
3. W. M. McIsaac and I. H. Page, *J. biol. Chem.* **234,** 858 (1959).
4. M. S. Steinberg, S. N. Cohen and W. W. Weber, *Biochim. biophys. Acta* **184,** 210 (1969).
5. W. Schloot, F.-J. Tiges, H. Blaesner and H. W. Goede, *Hoppe-Seyler's Z. physiol. Chem.* **350,** 1353 (1969).
6. W. W. Weber and S. N. Cohen, *Molec. Pharmac.* **3,** 266 (1967).
7. J. Saavedra, M. Brownstein and J. Axelrod, *J. Pharmac. exp. Ther.* **186,** 508 (1973).
8. T. Deguchi and J. Axelrod, *Analyt. Biochem.* **50,** 174 (1972).
9. J. W. Frymoyer and R. F. Jacox, *J. Lab. clin. Med.* **62,** 891 (1963).
10. H. J. Lynch, P. Wang and R. J. Wurtman, *Life Sci.* **12,** 145 (1973).
11. D. C. Klein and J. L. Weller, *Science, N.Y.* **169,** 1093 (1970).
12. H.-Y. T. Yang and N. H. Neff, *Pharmacologist* **16,** 199 (1974).
13. S. M. Paul, L. L. Hsu and A. J. Mandell, *Life Sci.* **15,** 2135 (1975).
14. S. H. Koslow, *Adv. biochem. Psychopharmac.* **11,** 95 (1974).
15. R. J. Wurtman, J. Axelrod and L. T. Potter, *J. Pharmac. exp. Ther.* **143,** 314 (1964).
16. H. J. Lynch, *Life Sci.* **10,** 791 (1971).
17. J. Axelrod, H. Shein and R. J. Wurtman, *Proc. natn. Acad. Sci. U.S.A.* **62,** 544 (1969).
18. B. Weiss, *Adv. Pharmac.* **6A,** 152 (1968).
19. C. L. Ralph and H. Lynch, *Gen. comp. Endocr.* **15,** 334 (1970).
20. L. Levine and L. Riceberg, *Res. Commun. chem. Path. Pharmac.* **10,** 693 (1975).