

MULTIPLE AROMATIC AMINE N-ACETYLTRANSFERASES
IN THE PINEAL GLAND

M. A. A. Namboodiri

Department of Biology
Georgetown University
Washington, D. C.

P. Voisin

Zoologie Biologie Cellulaire Faculte des Sciences
Poitiers, France

D. C. Klein

Laboratory of Developmental Neurobiology
National Institute of Child Health and Human Development
Bethesda, Maryland

Acetylation of aromatic amines has been an area of active investigation during the last 30 years. Initial studies were focused on drug acetylation because it was observed that drugs such as isoniazid (INH) and dapsone (DDS) are excreted in urine in the N-acetylated form (1). These studies led to the discovery of acetyl CoA as the endogenous acetyl donor for acetylation reactions, and also resulted in a detailed description of the N-acetylating system in the liver, including its purification and characterization and the finding of genetic polymorphism (2-4). Acetylation of aromatic amines in the liver was initially considered to be primarily a detoxification mechanism. However, recent experimental evidence indicates that it is also an essential step in the metabolic activation process which converts aromatic amines to potent carcinogens (5).

Acetylation of aromatic amines has also been studied in some detail in blood, brain, retina and the pineal gland. The best understood is the acetylation of serotonin in the pineal gland because of the role N-acetylation plays in the synthesis and regulation of melatonin, a putative hormone (Fig. 1).

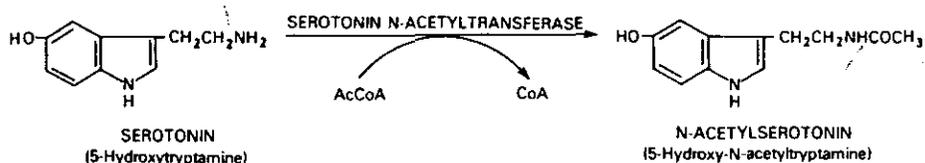


Fig. 1. N-Acetylation of serotonin.

I. SEROTONIN \longrightarrow MELATONIN CONVERSION

Interest in the N-acetylation of serotonin in the pineal gland started when Aaron Lerner discovered that melatonin, the skin lightening compound found in the pineal gland, is an N-acetylated derivative of 5-methoxytryptamine (6). Subsequently, Weissbach and co-workers demonstrated that serotonin was converted to melatonin in the pineal gland (7-8). The pathway involves the N-acetylation of serotonin by serotonin N-acetyltransferase¹ (serotonin NAT) to form N-acetylserotonin, which is O-methylated by hydroxyindole-O-methyltransferase (HIOMT) to form melatonin. HIOMT received most attention at that time because it was discovered that HIOMT activity and melatonin content of the pineal gland are regulated by a photo-neural mechanism (9-10). This influenced early investigators to suspect that HIOMT was responsible for the regulation of melatonin synthesis, and the concentration of melatonin in the pineal gland and in the circulation.

Investigations of the serotonin N-acetylation in the pineal gland received a major stimulus when Axelrod and his colleagues discovered that the synthesis of melatonin from tryptophan was increased by adrenergic drugs (11). This observation prompted Klein and co-workers to study HIOMT under this condition to understand the mechanism involved in the

¹ The term, arylalkylamine N-acetyltransferase, has also been used in the text to denote the same enzyme.

regulation of melatonin biosynthesis and the role of HIOMT. It was soon discovered that HIOMT showed only a marginal increase when melatonin synthesis from tryptophan was increased several fold by adrenergic drugs (12). This was interpreted by Klein and co-workers that perhaps another mechanism was responsible for the increase in melatonin synthesis. They analyzed the intermediates in the conversion of tryptophan to melatonin and discovered that the increase in melatonin synthesis caused by treatment with adrenergic drugs was associated with a large increase in the melatonin precursor, *N*-acetylserotonin (12). This led to interest in serotonin NAT and the discovery that adrenergic agonists increase the activity of serotonin NAT 10- to 100-fold by a direct action on pineal cells (13-15). Subsequently, they found that a large increase in the activity of serotonin NAT occurred at night, and this appeared to be responsible for the concomitant decrease in serotonin as well as the increase in melatonin in the pineal gland (16-17, Fig. 2). These observations brought pineal serotonin NAT to the forefront of pineal research and led to extensive investigations of the neural regulation of this enzyme and the role it plays in the circadian regulation of indole metabolism in the pineal gland.

II. NEURAL REGULATION OF SEROTONIN NAT IN THE PINEAL GLAND

It is now well established that pineal NAT in the rat exhibits a circadian rhythm with 50 to 70-fold increase in activity at night (18-19). This increase is caused by the stimulation of the pineal gland by a circadian clock in the suprachiasmatic nucleus (SCN); a neural circuit involving both central and peripheral structures, including superior cervical ganglia (SCG), links the SCN to the pineal gland (20-22). The neurotransmitter which stimulates pineal cells physiologically is norepinephrine (23-24). It is released from sympathetic nerve terminals whose cell bodies are located in the SCG (23).

Norepinephrine acts through a mechanism involving both α - and β -adrenergic receptors to increase cAMP, which in turn increases serotonin NAT activity 50 to 70-fold (25-27). cAMP acts to increase serotonin NAT activity at least in two ways. First, it increases the synthesis of new proteins by a mechanism involving both transcriptional and translational processes (28-30). Second, it stabilizes serotonin NAT, probably by a cAMP dependent phosphorylation mechanism (31). The stabilizing effect of cAMP is apparent when the cAMP concentration in cultured pineal cells is decreased. This leads to a rapid

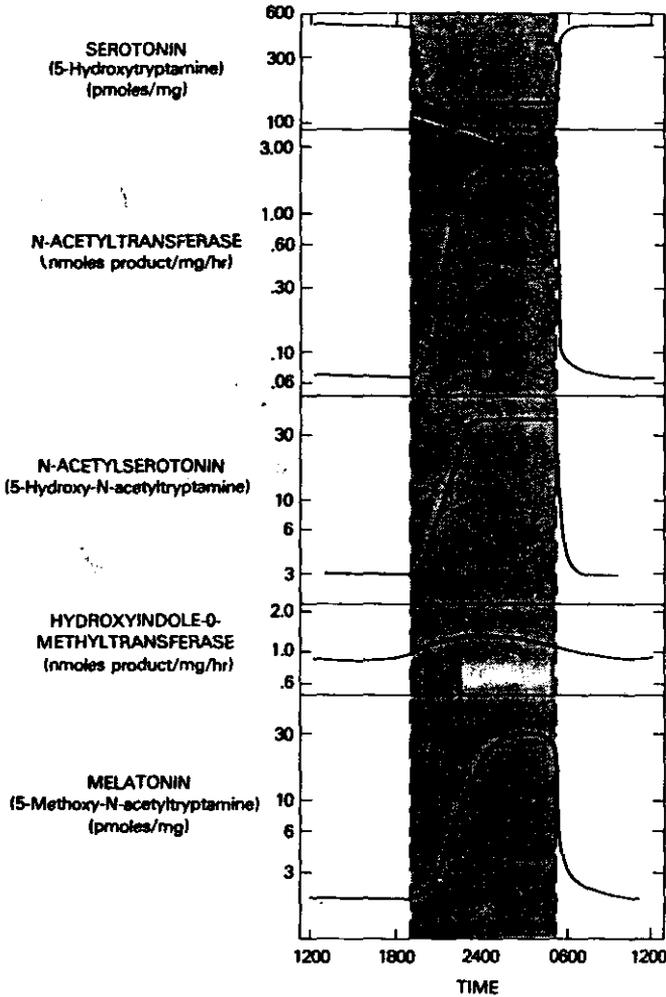


Fig. 2. Rhythms in indole metabolism in the rat pineal gland. The shaded portion indicates the dark period of the lighting cycle. The data have been abstracted from reports in the literature. 5-HT, 5-hydroxytryptamine, serotonin; Nact, N-acetyltransferase; Nac 5-HT, N-acetyl 5-hydroxytryptamine; HIOMT, hydroxyindole-O-methyltransferase; Nac 5-MT, N-acetyl 5-methoxytryptamine, melatonin.

decrease in NAT activity (31). This decrease also occurs in intact rats when they are exposed to light at night; light appears to act by a gating mechanism at the level of the SCN to inhibit the neural stimulation of the pineal gland (32). These large and rapid changes in serotonin NAT activity generate the rhythm in melatonin concentration in the pineal gland and in the circulation (33).

Regulation of pineal serotonin NAT has been studied in several species other than the rat, including chicken, sheep and hamster. These studies show that all exhibit a circadian rhythm in serotonin NAT activity. However, notable differences in the mechanism of its regulation exist among these species. Thus, in chicken the serotonin NAT rhythm is not controlled entirely by the SCN, but to a large degree by a biological clock located within the pineal gland (34). Also, the mechanism of the nocturnal increase in serotonin NAT does not always seem to involve norepinephrine (34). There appear to be two notable differences in sheep. They are the rapid nocturnal increase in serotonin NAT activity (maximum reached in about 30 min.) and the small magnitude (3-5-fold) of the rhythm (35). In addition, pineal serotonin NAT in sheep appears to be only partly responsible for the regulation of melatonin synthesis (36). The hamster pineal serotonin NAT rhythm has characteristics of that in both the rat and sheep; the apparent amplitude of the rhythm is small, as in sheep, while the nocturnal response exhibits a lag as in the rat (37).

The molecular mechanisms involved in the regulation of the serotonin NAT rhythm are only beginning to be understood. As noted above, the increase in activity at night appears to involve transcriptional and translational processes. However, it is not clear whether new molecules of serotonin NAT are produced or some regulatory proteins are synthesized under this condition. The rapid decrease in the enzyme activity, which occurs on exposure to light, still remains a mystery. While a protein thiol:disulfide exchange mechanism has been proposed to be involved in this process, direct experimental support for this hypothesis is lacking (38-39). Progress toward understanding the molecular mechanisms of serotonin NAT regulation has been rather slow because of the lack of availability of the enzyme in the purified form. Purification of serotonin NAT has been difficult because of the unstable nature of the enzyme and the scarcity of adequate amounts of tissue containing the active enzyme (40-41). Recently, however, we have succeeded in purifying the enzyme from rat and sheep pineal glands using disulfide exchange, size exclusion and affinity chromatography (42-43). Accordingly, more information on the molecular mechanisms of serotonin NAT regulation is expected to become available in the near future.

III. REGULATION OF SEROTONIN NAT IN THE EYE

The retina is another tissue in which serotonin NAT exhibits a true circadian rhythm. This rhythm has been detected in a variety of species, although it has been studied in some detail only in chicken and frog (44-45). In both chicken and frog the rhythm is of small magnitude (5-10-fold), persists in constant darkness, and is suppressed by light (45-47). It has been possible to demonstrate the above characteristics of the rhythm in cultured frog eye cups, indicating that the rhythm is controlled by a biological clock located inside the eye (48). cAMP appears to be involved in the increase in the enzyme activity at night, although the mechanism generating the cAMP is not known (45). In addition, catecholamines appear to exert an inhibitory influence on the avian retinal serotonin NAT rhythm, opposite to their stimulatory effects in the rat pineal gland (46,49). The rhythm in retinal serotonin NAT seems to regulate a similar rhythm in melatonin, which is believed to be involved in the control of rhythmic retinal functions, including disk shedding (49).

IV. ARYLAMINE AND ARYLALKYLAMINE NATS IN THE PINEAL GLAND

In the early stages of the investigations of biosynthesis of melatonin in the pineal gland, it was assumed that pineal serotonin NAT was the same as the relatively nonspecific aromatic amine NAT in the liver. The discovery of the adrenergic regulation of pineal serotonin NAT, however, began to change this view. It seemed possible that adrenergic regulation of pineal NAT could involve a different molecular species of NAT. In pursuit of this, Klein and Weber found in unpublished studies that the increase in pineal NAT caused by isoproterenol, an adrenergic agonist, was detectable only if serotonin or tryptamine was used as the amine substrate, not when INH, a known substrate for the liver enzyme, was used.

This possibility was further pursued and it was discovered that both the rat and sheep pineal gland contain two distinct types of NATs which can act on aromatic amines (50). One type acetylates arylamines such as aniline and *p*-phenetidine preferentially, whereas the other acts on arylalkylamines such as serotonin, tryptamine and phenylethylamine with high specificity. Both enzymes are located in pineal cells. The K_m of acetyl CoA for arylamine NAT is unusually high (2 to 4 μ m), about 20-fold higher than that of arylalkylamine NAT; this suggests that perhaps the endogenous acetyl donor for this enzyme

may not be acetyl CoA. As described below, these two enzymes can be distinguished further by their (1) regulation, (2) stability, and (3) molecular size.

A. Regulation

Regulation of pineal NATs has been studied under three different conditions: 1) isoproterenol treatment, 2) exposure to darkness at night and 3) cycloheximide treatment (50). Treatment of rats with isoproterenol during the day increased arylalkylamine NAT about 100-fold, without affecting arylamine NAT to any significant extent (Table 1). This indicates that the nocturnal increase in NAT activity in the rat pineal gland is specific for arylalkylamine NAT. Another instance in which the differential regulation of the two enzymes is evident is in their rapid response to darkness at the onset of night in the sheep pineal gland. The arylalkylamine NAT increased about 5-fold 30 min. after the exposure to darkness at night; arylamine NAT remained unchanged under this condition (Table 2). Further, treatment of sheep with cycloheximide 30 min. before exposure to darkness caused a 90% reduction in arylalkylamine NAT measured 30 min. after exposure to darkness, but did not have any effect on arylamine NAT (Table 3). These observations show that pineal arylamine and arylalkylamine NATs are differentially regulated.

TABLE I. Effect of isoproterenol treatment on arylamine and arylalkylamine NAT activities in rat pineal gland

Substrate	NAT activity (nmol/min./mg protein)	
	control day time	isoproterenol
Tryptamine	0.011 \pm 0.007	1.5 \pm 0.25
Phenylethylamine	0.019 \pm 0.005	1.5 \pm 0.15
p-Phenetidine	0.20 \pm 0.036	0.13 \pm 0.006
Aniline	0.14 \pm 0.007	0.08 \pm 0.018

Rats received isoproterenol (10 mg/kg) or saline and were killed during the daytime. Enzyme activity was measured with 0.5 mM AcCoA and 10 mM amine substrate. Results are expressed as the mean \pm S.E. of triplicate determinations (from Ref. 50).

TABLE II. Ovine pineal arylalkylamine and arylamine NAT activities 30 min. before and after lights off at night

Substrate	NAT activity (nmol/min./mg protein)	
	Day	Night
Tryptamine	0.18 \pm 0.04	0.71 \pm 0.07
Phenylethylamine	0.13 \pm 0.05	0.66 \pm 0.07
p-Phenetidine	0.38 \pm 0.11	0.42 \pm 0.10
Aniline	0.12 \pm 0.04	0.12 \pm 0.05

Groups of five sheep were killed 30 minutes before lights off and 30 minutes after lights off (1800 hours). NAT activities were measured with 0.5 mM AcCoA and 10 mM amine substrate. Results are expressed as the mean \pm S.E. of triplicate determinations (from Ref. 36).

TABLE III. Effect of cycloheximide treatment on arylamine and arylalkylamine NAT activities in the ovine pineal gland at night

Substrate	NAT activity (nmol/min./mg protein)	
	Control	Cycloheximide
Tryptamine	0.71 \pm 0.07	0.06 \pm 0.02
Phenylethylamine	0.66 \pm 0.07	0.075 \pm 0.08
p-Phenetidine	0.42 \pm 0.11	0.35 \pm 0.11
Aniline	0.12 \pm 0.05	0.10 \pm 0.03

Sheep received cycloheximide (4 mg/kg) or saline 30 minutes before lights off (1800 hours) and were killed in the dark 30 minutes after lights off. NAT activities were measured with 0.5 mM AcCoA and 10 mM amine substrate. Results are expressed as the mean \pm S.E. of triplicate determinations (from Ref. 50).

B. Stability

During the early stages of these investigations we found that arylamine NAT activity in the rat pineal homogenate was almost completely lost on storage overnight in the cold. This prompted us to compare the inactivation of arylamine and arylalkylamine NAT in the broken cell preparations in the cold. We found that the activity of arylamine NAT from both rat and sheep pineal glands decreased more quickly at 4°C than arylalkylamine NAT. Complete loss of arylamine NAT activity occurred after 24 hours in the rat and over 95% of the activity disappeared after 48 hours in sheep (Fig. 3). The corresponding inactivation of arylalkylamine NAT was 50% in the rat and 25% in sheep (Fig. 3).

Arylalkylamine NAT is more unstable at 37°C in broken cell preparations; about 90% of the activity is irreversibly lost in about 30 min. (40). It is not known if arylamine NAT also undergoes a similar inactivation at 37°C. Acetyl CoA and polyanions protect arylalkylamine NAT from this inactivation whereas disulfides such as cystamine and insulin, accelerate it (38-41). The rate of inactivation is reduced considerably in partially purified preparations of the enzyme suggesting

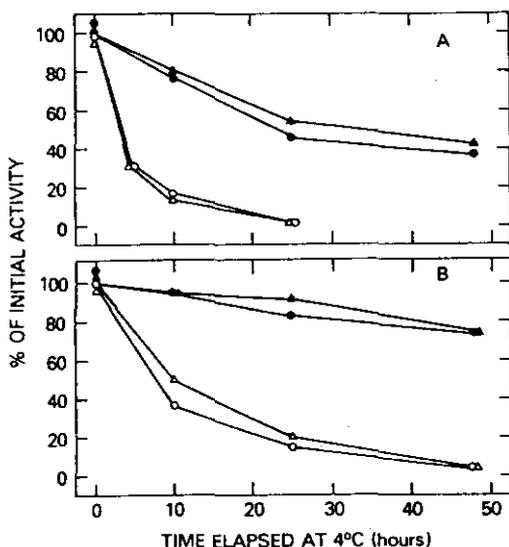


Fig. 3. Time course of inactivation (4°C) of pineal arylamine and arylalkylamine NATs. Samples of pineal supernatant preparations (10,000 xg) were stored at 4°C for the indicated time and assayed with 0.5 mM AcCoA and 10 mM amine substrate (from Ref. 50).

that some soluble factors, perhaps disulfides, present in the pineal supernatant are involved in the inactivation. However, our attempts to prevent the inactivation by treating the preparation with thiols has not been successful. Thus, the mechanism of this inactivation remains unclear.

C. Molecular size

Proof that the pineal gland contains separate arylamine and arylalkylamine NATs has come from the demonstration that these enzymes can be resolved using size exclusion HPLC. Chromatography of the 100,000 xg supernatant from isoproterenol treated rat pineal glands reveals that arylalkylamine NAT activity is present in two peaks (Fig. 4A). One contained molecules with an apparent molecular weight of about 100,000 and accounted for 75% of the recovered activity. The second peak contained molecules with an apparent molecular weight in the 10,000 to 20,000 range. Arylamine NAT activity was detected in a single peak of intermediate molecular size ($M_r = 35,000$). Recovery of activity was about 75% in both cases. As observed in the rat, size exclusion HPLC of the night sheep pineal gland supernatant also showed clear separation between arylamine and arylalkylamine NAT activities (Fig. 4B). However, the elution pattern of ovine arylalkylamine NAT activity was slightly different from that of the rat pineal preparation; the higher molecular weight peak contained 90% of the recovered activity; a distinct lower molecular weight peak was not apparent. As in the case of the rat pineal preparation, the arylamine NAT was detected in a single molecular form with an apparent molecular weight of about 35,000.

V. MULTIPLE FORMS OF ARYLALKYLAMINE NAT

An interesting characteristic of arylalkylamine NAT is that it can exist in different molecular forms depending on the ionic environment (42, 43, 50, 51). Three distinct forms of arylalkylamine NAT are detected during size exclusion chromatography using Sephacryl S-200 (low pressure) as well as using TSK 3000 columns (high pressure). In the presence of ammonium acetate (0.1 M, pH 6.5), DTT (10mM) and BSA (0.1 mg/ml), the enzyme is separated into two molecular forms as described above. However, in presence of sodium citrate (0.1 M, pH 6.5) containing DTT (10 mM) and BSA (0.1 mg/ml), it is detected in a single molecular form of intermediate molecular size ($M_r = 30,000$).

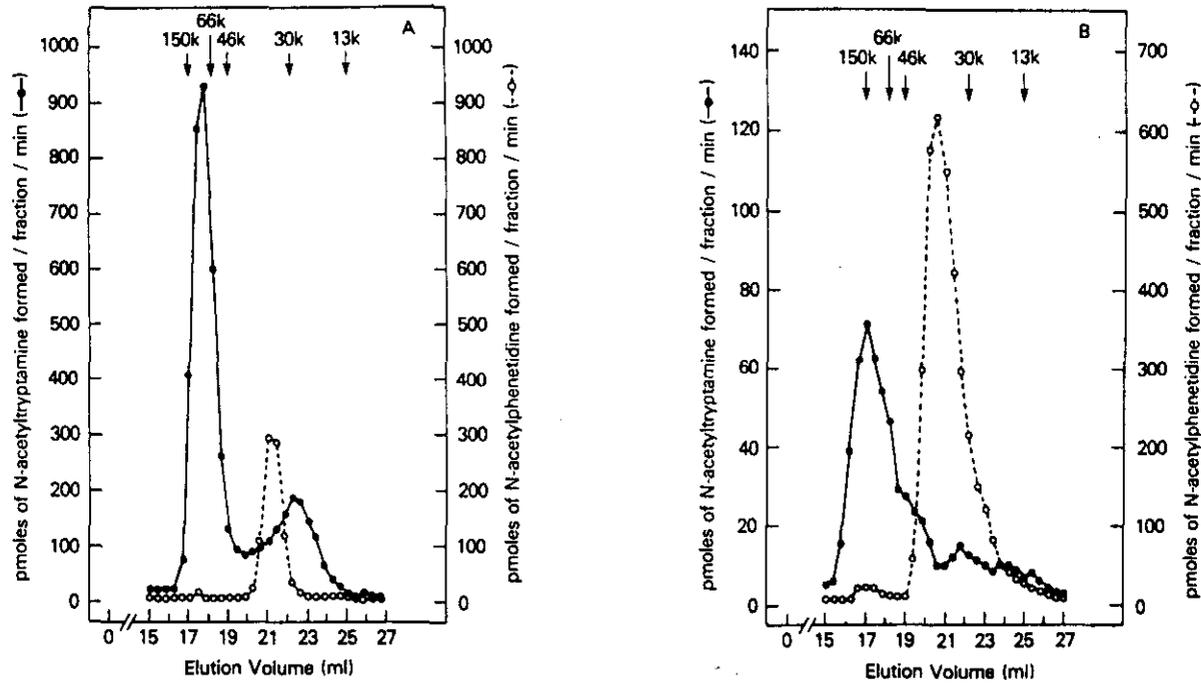


Fig. 4. Size exclusion HPLC profiles of arylamine and arylalkylamine NATs from rat and sheep pineal glands. The supernatants (100,000 \times g) from rat (A) and sheep (B) pineal homogenates were chromatographed. The fractions were assayed with 0.1 mM AcCoA and either 10 mM tryptamine or 3 mM p-phenetidine. Upper arrows indicate molecular weight markers: γ -globulins, 150,000; bovine serum albumin, 66,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; cytochrome c, 13,000 (from Ref. 50).

Another interesting observation is that the ratio of the two forms of the enzyme obtained on chromatography in the presence of ammonium acetate is changed after purification of the enzyme (42,50). The small molecular weight form becomes the predominant species after purification of the enzyme using chromatography based on disulfide exchange principles (42,51). In an earlier report Morrissey et al. have reported that rat pineal NAT in the 100,000 xg supernatant can be separated into two forms ($M_r = 39,000$ and $M_r = 10,000$) on Sephadex G100 chromatography in the presence of potassium phosphate (50 mM, pH 6.5) containing 4 mM mercaptoethylamine (52). Our results indicate that the multiple forms of the enzyme depend on the ionic environment whereas the results of Morrissey et al. seem to indicate that interaction between sulfhydryl groups is involved in this process.

The important variable in the ionic environment is not ionic strength. An increase in the concentration of ammonium acetate from 50 mM to 400 mM does not convert the $M_r = 100,000$ and $M_r = 10,000$ forms of the enzyme to the $M_r = 30,000$ form, whereas treatment with sodium citrate does (Fig. 5). Independent of these effects on molecular size are effects on activity. Increasing the ionic strength of buffers by adding sodium chloride, sodium citrate, sodium phosphate, ammonium acetate, potassium chloride or ATP causes as much as a 5-fold increase in enzyme activity (41). Thus, we believe there are two independent effects of ions on NAT. All salts appear to activate the enzyme in a dose-dependent manner, perhaps through a mechanism altering the physical characteristics of the active site environment. In contrast, it appears that certain salts which stabilize the enzyme, including sodium citrate, can convert the enzyme from the $M_r = 100,000$ and $M_r = 10,000$ forms to the $M_r = 30,000$ form; other salts including ammonium acetate can convert the $M_r = 30,000$ form to the $M_r = 100,000$ and $M_r = 10,000$ forms. One obvious difference between these salts is that one is monoionic and the other is polyionic. Perhaps this is the critical difference which determines the molecular form of arylalkylamine NAT. It is also possible that the apparent sensitivity of NAT to differences in salt composition may influence the intracellular form, activity and stability of this enzyme.

VI. MULTIPLE AROMATIC AMINE NATS IN OTHER TISSUES

There is now convincing evidence indicating that multiple NATs having narrow specificity exist in different tissues. These have been studied extensively in liver in relation to

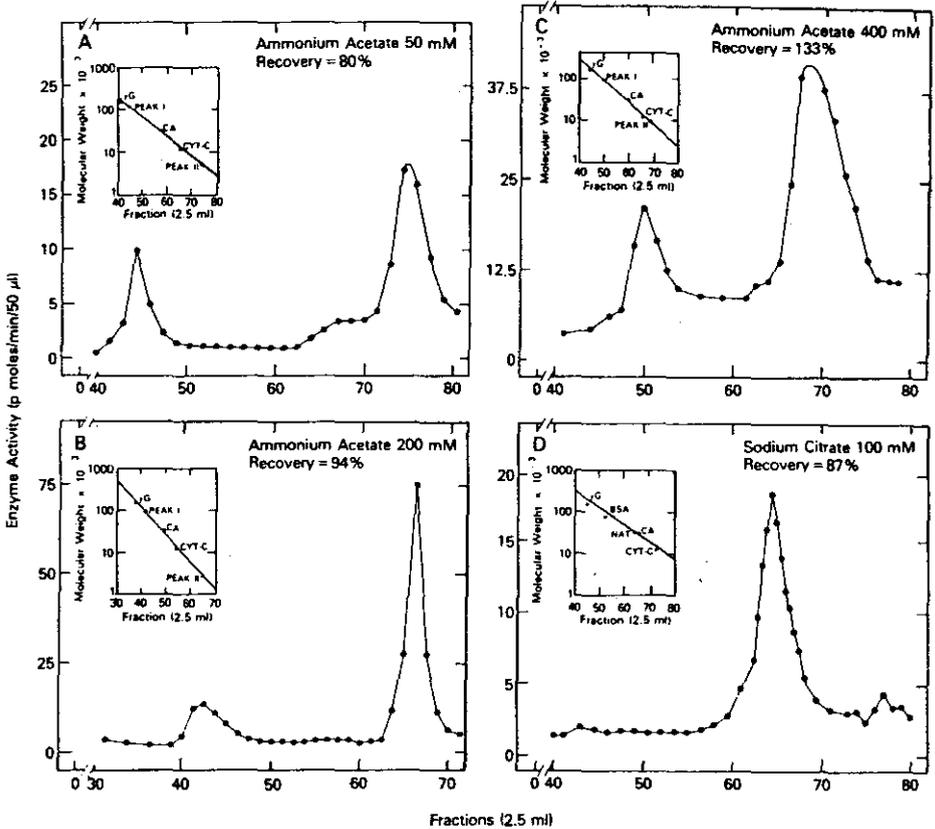


Fig. 5. Size exclusion chromatography of NATs using sephacryl S-200 under different ionic conditions. Four S-200 columns were equilibrated with four different buffers namely, ammonium acetate buffer, pH 6.5, 50 mM (1.5 x 100cm), 200 mM (1.5 x 95cm) and 400 mM (1.5 x 110cm), and sodium citrate buffer, pH 6.5, 100 mM (1.5 x 110cm), all containing DTT (10 mM) and BSA (0.1 mg/ml). A 2.5 ml enzyme sample containing BSA (1 mg/ml) was applied to each column, the fractions were collected (flow rate 5 ml/h), and assayed for enzyme activity. The columns were calibrated using three proteins, namely γ -globulin ($M_r = 150,000$, ^{14}C -methylated), carbonic anhydrase ($M_r = 30,000$, ^{14}C -methylated), and cytochrome C ($M_r = 12,400$). Samples containing the three proteins (2.5 ml, 5 mg/ml) were applied to the columns and the fractions were collected. Cytochrome C was detected by absorption (400 nm) and the other two by radioactivity (from Ref. 42).

genetic polymorphism in drug acetylation. In rabbit liver, the most extensively studied system to date, two types of NAT activity can be distinguished. One type acetylates drugs such as DDS and INH, whereas the other acts on p-aminobenzoic acid (PABA) with high specificity; only the former exhibits genetic polymorphism with the rapid and slow acetylator phenotypes (5). Recent experimental evidence indicates that both these activities are located on the same protein (53). A similar pattern exists in mouse liver (54). However, in hamster liver the pattern appears to be exactly opposite; the PABA NAT activity exhibits polymorphism while the INH NAT activity remains unchanged in the various individuals (55). Recently, it has been possible to separate these two activities by ion exchange chromatography, showing that they represent distinct molecular forms (56). Preliminary studies in the rat indicate that its NAT pattern in the liver may be similar to that in the hamster (5).

Blood and brain NAT have also been studied in some detail. The blood enzyme is unusual because it acetylates PABA with very high specificity (57). The blood enzyme exhibits genetic polymorphism in mouse and hamster but fails to do so in rabbit, although the general properties of the enzyme are comparable in all the three species (5). The brain enzyme has been studied in some detail only in the rat. Substrate specificity and inhibition studies suggest that it is similar to the liver enzyme rather than to the pineal enzyme (58-59). It is not clear if it exhibits genetic polymorphism.

Genetic regulation of aromatic amine NATs has been studied primarily in mice and hamster using inbred strains. The results of genetic analysis of liver and blood NAT activities are consistent with simple autosomal Mendelian inheritance of two co-dominant alleles at a single locus (60-61). These studies have also demonstrated the existence of minor modifying genes that segregate independently of the major NAT gene (60). Based on these results, it is tempting to speculate that the multiple NATs in different tissues are expressed from a single gene. It is possible that post transcriptional and/or post translational modifications play important roles in the formation of the multiple NATs. Thus, post translational modification, perhaps controlled by the modifying genes, may be involved in the slow and rapid acetylator phenotypes in the rabbit and mouse liver. The qualitative differences between the NAT activities of slow and rapid acetylator phenotypic rabbits combined with the lack of quantitative changes between them in the amount of the enzyme protein, determined using immunological methods, support this view (62-63). Post transcriptional modifications, perhaps involving differential splicing, may explain the different molecular forms of NAT in the pineal gland and hamster liver. Finally, it is also

possible that the multiple separable NATs in the pineal gland and hamster liver are altogether different gene products. These possibilities can be tested when appropriate monoclonal antibodies and cDNA probes become available.

VII. CONCLUSION

The pineal gland contains at least two types of NATs which can act on aromatic amines. One type acetylates arylalkylamines, such as serotonin, tryptamine and phenylethylamine, while the other acts on arylamines, such as aniline and phenitidine. The presence of these two enzyme activities in the pineal gland can be considered an evolutionary adaptation to generate the circadian rhythm in melatonin, without affecting the "house keeping" metabolism of arylamines. Therefore, one would predict that a similar enzyme system is present in other tissues exhibiting melatonin rhythm, such as the retina. Understanding the molecular mechanisms involved in the tissue specific expression of NATs in general and pineal NATs in particular is the next important step in this line of investigation.

REFERENCES

1. Weber, W. W. (1973). In "Metabolic Conjugation and Metabolic Hydrolysis" (W. H. Fishman, ed.) Vol. 3, p. 249, Academic Press, New York.
2. Weber, W. W. and Hein, D. W. (1979). Clin. Pharmacokinetics. 4, 401.
3. Weber, W. W. and Glowinski, I. B. (1980). In "Enzymatic Mechanism of Detoxification" (W. B. Jakoby, ed.) Vol. 2, p. 169, Academic Press, New York.
4. Reece, P. A. (1981). Med. Res. Rev. 1, 73.
5. Weber, W. W. and Hein, D. W. (1985). Pharmacol. Rev. 37, 25.
6. Lerner, A. B., Case, J. D., and Heinzelman, R. V. (1959). J. Am. Chem. Soc., 81, 6084.
7. Weissbach, H., Redfield, B. G. and Axelrod, J. (1960). Biochim. Biophys. Acta. 43, 352.
8. Axelrod, J. and Weissbach, H. (1960). Science, 131, 1312.
9. Axelrod, J., Wurtman, R. J. and Snyder, S. H. (1965). J. Biol. Chem. 240, 949.

10. Axelrod, J. and Wurtman, R. J. (1968). *Adv. Pharmacol.*, 6A, 157.
11. Axelrod, J., Shein, H. M. and Wurtman, R. J. (1969). *Proc. Natl. Acad. Sci. USA*, 62, 544.
12. Berg, G. R. and Klein, D. C. (1971). *Endocrinol.* 89, 453.
13. Klein, D. C. and Berg, G. R. (1970). *Advances in Biochem. Psychopharmacol.* 3, 241.
14. Klein, D. C., Berg, G. R. and Weller, J. L. (1970). *Science* 168, 979.
15. Klein, D. C. and Weller, J. L. (1973). *J. Pharmacol. Exp. Ther.* 186, 516.
16. Klein, D. C. and Weller, J. L. (1970). *Science*, 169, 1093.
17. Klein, D. C. (1974). In "The Neuroscience Third Study Programme" (P. O. Schmidt, ed.) p. 509, MIT Press, Massachusetts.
18. Axelrod, J. and Zatz, M. (1977). In "Biochemical Actions of Hormones" (G. Litwack, ed.) p. 249, Academic Press, New York.
19. Klein, D. C., Auerbach, D. A., Namboodiri, M. A. A. and Wheler, G. H. T. (1981). In "The Pineal Gland: Anatomy and Biochemistry" (R. J. Reiter, ed.) Vol. 1, p. 199, CRC Press, Florida.
20. Moore, R. Y. and Klein, D. C. (1974). *Brain Res.* 71, 17.
21. Klein, D. C. and Moore, R. Y. (1979). *Brain Res.* 174, 245.
22. Klein, D. C., Smoot, R., Weller, J. L., Higa, S., Markey, S. P., Creed, G. J. and Jacobowitz, D. M. (1983). *Brain Res. Bull.* 10, 647.
23. Axelrod, J. (1974). *Science*, 184, 1341.
24. Brownstein, M. and Axelrod, J. (1974). *Science* 184, 163.
25. Deguchi, T. and Axelrod, J. (1972). *Proc. Natl. Acad. Sci. USA* 69, 2547.
26. Deguchi, T. (1973). *Mol. Pharmacol.* 9, 184.
27. Vanecek, J., Sugden, D., Weller, J. L. and Klein, D. C. (1985). *Endocrinol.* 116, 2167.
28. Romero, J. A., Zatz, M. and Axelrod, J. (1975). *Proc. Natl. Acad. Sci. USA*, 72, 2107.
29. Morrissey, J. J. and Lovenberg, W. (1978). *Biochem. Pharmacol.* 27, 551.
30. Morrissey, J. J. and Lovenberg, W. (1978). *Biochem. Pharmacol.* 27, 557.
31. Klein, D. C., Buda, M., Kapoor, C. L. and Krishna, G. (1978). *Science*, 199, 309.
32. Klein, D. C. and Weller, J. L. (1972). *Science*, 177, 532.

33. Reppert, S. and Klein, D. C. (1980). In "The Endocrine Functions of the Brain" (M. Motta, ed.) p. 327, Raven Press, New York.
34. Binkley, S. (1983). *Comp. Biochem. physiol.* 75, 123.
35. Namboodiri, M. A. A., Sugden, D., Klein, D. C., Tamarkin, L. and Mefford, I. (1985). *Comp. Biochem. Physiol.* 80B, 731.
36. Namboodiri, M. A. A., Sugden, D., Klein, D. C., Grady, Jr. R. and Mefford, I. (1985). *J. Neurochem.* 45, 832.
37. Vanecek, J. and Illnerova, H. (1982). *Experientia* 38, 513.
38. Namboodiri, M. A. A., Weller, J. L. and Klein, D. C. (1980). *J. Biol. Chem.* 255, 6032.
39. Namboodiri, M. A. A., Favilla, J. T. and Klein, D. C. (1981). *Science* 213, 571.
40. Binkley, S., Klein, D. C. and Weller, J. L. (1976). *J. Neurochem.* 26, 51.
41. Namboodiri, M. A. A., Nakai, C. and Klein, D. C. (1979). *J. Neurochem.* 33, 807.
42. Namboodiri, M. A. A. and Klein, D. C. (1981). In "Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects" (E. Usdin, N. Weiner, and M. B. H. Youdim, eds.) p. 701, MacMillan Publishers, London.
43. Namboodiri, M. A. A., Dubbels, R. and Klein, D. C. (1986). *Methods Enzymol.* (In press.)
44. Binkley, S., Hryshchyshyn, M. and Reilly, K. (1979). *Nature* 281, 479.
45. Iuvone, P. M. and Besharse, J. C. (1983). *Brain Res.* 273, 111.
46. Binkley, S., Reilly, K. B. and Hryshchyshyn, M. (1980). *J. Comp. Physiol.* 139, 103.
47. Ham, H. E. and Menaker, M. (1980). *Proc. Natl. Acad. Sci. USA*, 77, 4998.
48. Besharse, J. C. and Iuvone, P. M. (1983). *Nature* 305, 133.
49. Besharse, J. C., Dunis, D. A. and Iuvone, P. M. (1984). *Fed. Proc.* 43, 2704.
50. Voisin, P., Namboodiri, M. A. A. and Klein, D. C. (1984). *J. Biol. Chem.* 259, 10913.
51. Namboodiri, M. A. A., Brownstein, M. J., Weller, J. L. and Klein, D. C. (1982). *Fed. Proc.* 41, 2188.
52. Morrissey, J. J., Edwards, S. B. and Lovenberg, W. (1977). *Biochem. Biophys. Res. Commun.* 77, 118.
53. Hein, D. W., Hirata, M., Glowinski, I. B. and Weber, W. W. (1982). *J. Pharmacol Exp. Ther.* 220, 1.
54. Glowinski, I. B. and Weber, W. W. (1982). *J. Biol. Chem.* 257, 1431.

55. Hein, D. W., Omichinski, J. G., Brewer, J. A. and Weber, W. W. (1982). *J. Pharmacol. Exp. Ther.* 220, 8.
56. Hein, D. W., Kirilin, W. G., Ferguson, R. J. and Weber, W. W. (1985). *J. Pharmacol. Exp. Ther.* 234, 358.
57. Drummond, G. S., Kelker, H. C. and Weber, W. W. (1980). *Biochem. J.* 187, 157.
58. Paul, S. M., Hsu, L. L. and Mandel, A. J. (1975). *Life Sci.*, 15, 2135.
59. Yang, H. Y. T. and Neff, N. H. (1976). *Neuropharmacol.* 15, 561.
60. Glowinski, I. B. and Weber, W. W., (1982). *J. Biol. Chem.* 257, 1424.
61. Hein, D. W., Kirilin, W. G., Ferguson, R. J. and Weber, W. W., (1985). *J. Pharmacol. Exp. Ther.* 233, 584.
62. Patterson, E., Radtke, H. E. and Weber, W. W. (1980). *Mol. Pharmacol.* 17, 367.
63. Weber, W. W., Hein, D. W., Hirata, M. and Patterson, E. (1978). In "Conjugation Reactions in Drug Transformations" (A. Aito, ed.) p. 145, Elsevier-North Holland, Amsterdam.