

Arylamine *N*-Acetyltransferase and Arylalkylamine *N*-Acetyltransferase in the Mammalian Pineal Gland*

(Received for publication, February 1, 1984)

Pierre Voisin, M. A. Aryan Namboodiri, and David C. Klein

From the Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205

Amine *N*-acetylation in the pineal gland is of special importance because it is the first step in the synthesis of melatonin from serotonin. In the present study the *N*-acetylation of arylamines and arylalkylamines by homogenates of rat and sheep pineal glands was investigated. The arylamines studied were *p*-phenetidine and aniline; the arylalkylamines studied were tryptamine, serotonin, 5-methoxytryptamine, 6-fluorotryptamine, and phenylethylamine. These amines were acetylated by pineal homogenates of both species, although marked interspecies differences in apparent K_m and V_{max} values were found. A series of observations in both species indicate that aromatic amine *N*-acetylation is catalyzed by two distinct enzymes; one preferentially acetylates arylamines and the other preferentially acetylates arylalkylamines. First, isoproterenol treatment of the rat increased arylalkylamine *N*-acetylation 100-fold without increasing arylamine *N*-acetylation. Second, cycloheximide treatment in sheep reduced arylalkylamine *N*-acetylation at night to one-tenth control values, without altering arylamine *N*-acetylation. Third, arylamine *N*-acetyltransferase and arylalkylamine *N*-acetyltransferase inactivated at different rates at 4 °C. Fourth, the two enzymes were resolved by size exclusion chromatography. These results clearly establish that the pineal gland contains an arylamine *N*-acetyltransferase and a second, independently regulated arylalkylamine *N*-acetyltransferase which appears to be primarily responsible for the physiological conversion of serotonin to melatonin via the intermediate *N*-acetylserotonin.

Amine *N*-acetylation in the pineal gland is of special importance because it is the first step in the serotonin (5-hydroxytryptamine) → *N*-acetylserotonin → melatonin (*N*-acetyl-5-methoxytryptamine) pathway (1). Melatonin is the pineal hormone which controls photic-induced seasonal changes in the physiology, including reproduction (2, 3). Large day/night differences in the production and release of melatonin are caused by changes in the enzymatic *N*-acetylation of serotonin, which is under photic-neural control (4).

Aromatic amine *N*-acetylation has been reported in many tissues and studied in detail in the liver where it appears that a single enzyme, arylamine:acetyl-CoA *N*-acetyltransferase (EC 2.3.1.5), acts on both arylamines and arylalkylamines with broad specificity (5). The same identification has been used for the pineal enzyme. However, limited studies in the

rat indicate that pineal and liver *N*-acetyltransferase differ in specificity (6, 7), stability (8), inhibition by melatonin (9), and apparent size (10). Also, whereas the enzymatic *N*-acetylation of serotonin increases 30-70-fold in the rat pineal gland at night, neither the liver nor any other tissue exhibit such a large change in activity (11). In addition to these tissue-related differences, interspecies differences in the pineal enzyme have also been reported. These include differences in the day/night ratio of activity (12), inhibition by melatonin, and stability (9). Most recently, comparative studies on rat and sheep in our laboratory have revealed not only that there are marked differences in the day/night activity ratios, but also that the mechanisms regulating serotonin *N*-acetylation are different in these two species (13).¹ In addition, these studies revealed that pineal serotonin concentration is 50-fold greater in rat than in sheep, suggesting that the K_m of *N*-acetyltransferase for serotonin may also be different.

The apparent diversity among tissues and species in the acetylation of aromatic amines and the special importance of serotonin acetylation in the pineal gland have prompted us to study the *N*-acetylation of arylamines and arylalkylamines by rat and sheep pineal homogenates. The results indicate that in both species the pineal gland contains an arylamine *N*-acetyltransferase and a distinct, independently controlled, arylalkylamine *N*-acetyltransferase which is involved in melatonin synthesis. The latter is identified as acetylCoA: arylalkylamine *N*-acetyltransferase. Distinct interspecies differences in the kinetic characteristics of both enzymes also exist.

EXPERIMENTAL PROCEDURES

Materials—The following compounds were purchased: [acetyl-1-¹⁴C]coenzyme A (53 Ci/mol), [methyl-¹⁴C]γ-globulins (15 μCi/mg), [methyl-¹⁴C]bovine serum albumin (10 μCi/mg), [methyl-¹⁴C]ovalbumin (10 μCi/mg), [methyl-¹⁴C]carbonic anhydrase (10 μCi/mg), [methyl-¹⁴C]cytochrome *c* (15 μCi/mg) (New England Nuclear); tryptamine (Schwartz/Mann); acetophenetidine, serotonin, 5-methoxytryptamine, cycloheximide, and bovine serum albumin (Sigma); paralyline, 6-fluorotryptamine, and phenylethylamine (Aldrich); *p*-phenetidine citrate and aniline (City Chemical Corp., New York, NY); DL-isoproterenol HCl (Regis Chemical Co., Morton Grove, IL); acetyl coenzyme A and dithiothreitol (Boehringer).

Animal Treatments—Control and superior cervical ganglionectomized Sprague-Dawley rats, 150-200 g (Zivic-Miller Labs) were housed in LD 14:10, with lights off at 1900 and killed at 1400. To stimulate rat pineal *N*-acetyltransferase activity, animals were injected with DL-isoproterenol (10 mg/kg) in 0.01 M sodium phosphate buffer, pH 7, containing 0.9% NaCl (phosphate-buffered saline) 4 h before killing (14). Sheep (12-14-month-old male Dorset × Rambouillet, weighing 50-60 kg) were obtained from a breeding herd under the supervision of the National Heart, Lung and Blood Institute. Animals were kept in a light-tight box stall (LD 12:12, lights off at 1900). Daytime

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ M. A. A. Namboodiri, D. Sugden, D. C. Klein, L. Tamarkin, and I. N. Mefford, unpublished results.

animals were killed at 1000; nighttime animals were killed in the dark at 2300. Cycloheximide (4 mg/kg in phosphate-buffered saline) or phosphate-buffered saline alone was administered to sheep intravenously 30 min before lights off and all animals were killed 30 min after lights off.¹

Tissue Treatments—All pineals were quickly dissected out and immediately frozen on solid CO₂ before storing at -30 °C. Rat pineals were sonicated in groups of 10–20. Individual sheep pineals were minced and homogenized in a glass homogenizer. Blood samples were collected and frozen on solid CO₂. Aliquots were homogenized by sonication.

Enzyme Assays—*N*-Acetyltransferase activity was routinely measured with 0.5 mM [¹⁴C]AcCoA² (specific activity = 2 Ci/mol) and 10 mM amine substrate as previously described (15). When assaying the enzyme activities in HPLC fractions, 0.1 mM [¹⁴C]AcCoA (specific activity = 10 Ci/mol) and either 10 mM tryptamine or 3 mM *p*-phenetidine were used. The reaction products were extracted into chloroform, and the organic phase was rinsed once with sodium phosphate buffer, 0.1 M, pH 6.8, and twice with 1 M NaOH. In a preliminary experiment the ¹⁴C-acetylated products formed from *p*-phenetidine, aniline, and phenylethylamine were identified using thin layer chromatography on precoated silica gel plates with chloroform/methanol/acetic acid (90:10:1) as solvent. Recovery of the acetylated products by chloroform extraction was >95%.

Kinetic Analyses—The enzyme was prepared and assayed as follows: individual sheep pineals or a pool of 20 rat pineals were homogenized in 0.1 M sodium phosphate buffer, pH 6.8 (1 mg, wet weight, per 10 μl). When the acetyl acceptor was used at varying concentrations, the 10,000 × *g* pineal supernatant was incubated with 1 mM pargyline at 37 °C for 10 min to inhibit specifically monoamine oxidase activity (16, 17); 1.5 mM [¹⁴C]AcCoA (specific activity = 3.3 Ci/mol) was also included to prevent thermal inactivation of the *N*-acetyltransferase during this incubation (18). After cooling on ice, this solution was used as the source of enzyme and AcCoA. When the AcCoA concentration was varied, or when studying the partially purified enzyme, monoamine oxidase activity was not inhibited. The concentration range of the varied substrate in the assay was adjusted from 0.2 to 5-fold *K_m*(app) and the fixed substrate was either 1 mM [¹⁴C]AcCoA (specific activity = 3.3 Ci/mol), 10 mM tryptamine, 1 mM tryptamine, or 3 mM *p*-phenetidine. The assay was done in 20 μl of 0.1 M sodium phosphate buffer, pH 6.8, at 37 °C for 10 min and stopped by the addition of 1 ml of chloroform. The reaction products were extracted into chloroform except for *N*-acetylserotonin. When serotonin was used as substrate, the reaction was stopped by placing the tubes on solid CO₂ and 20 μl of ethanol containing 10 μg of *N*-acetylserotonin was added. The reaction product was separated by TLC as previously described (19), and eluted with 1 ml of ethanol. To measure the degradation of [¹⁴C]AcCoA, the reaction was stopped on solid CO₂ and 20 μl of ethanol was added. The [¹⁴C]AcCoA was isolated by TLC using ethanol, 1 M ammonium acetate (5:2), eluted with 0.5 ml of water, and counted in aqueous fluor (20). Boiled enzyme was used as a blank. The kinetic constants were calculated using the "Ligand" program (21).

Partial Purification of Arylalkylamine *N*-Acetyltransferase—Rat pineal arylalkylamine *N*-acetyltransferase was partially purified by disulfide-exchange chromatography on a Sepharose-cystamine column (22). A Sepharose-cystamine column (0.5 ml) was equilibrated with 0.1 M sodium citrate, 10 mM sodium veronal buffer, pH 8.5. The 10,000 × *g* supernatant of 20 rat pineal glands in 25 mM ammonium acetate, pH 6.5, was mixed with 2 volumes of 0.3 M sodium citrate, 30 mM sodium veronal buffer, pH 8.5, and loaded on the column. The column was washed sequentially with (i) the equilibration buffer (10 ml) (ii) 0.1 M sodium citrate buffer, pH 6.5 (20 ml), and (iii) finally with 25 mM ammonium acetate buffer, pH 6.5, containing 10 mM dithiothreitol (20 ml). After standing overnight in the presence of this buffer, the column was washed with 25 mM ammonium acetate, pH 6.5 (20 ml), and the enzyme was eluted with 0.1 M sodium citrate buffer, pH 6.5. This method yields a 15-fold enrichment of activity.

HPLC Size Exclusion—Rat or sheep pineal glands were homogenized in 10 mM ammonium acetate, pH 6.8 (1 mg, wet weight, per 10 μl), and centrifuged at 100,000 × *g* for 30 min. The supernatant was injected into a system composed of two TSK columns (Kratos Analytical Instruments, Ramsey, NJ; total column volume = 27 ml, void

volume = 12 ml) pre-equilibrated with 100 mM ammonium acetate, pH 6.8, containing 10 mM dithiothreitol and 0.1 mg/ml of bovine serum albumin (22). The elution was done with the same buffer at a flow rate of 1 ml/min. Fractions of 0.4 ml were collected and assayed for *N*-acetyltransferase activity with tryptamine and *p*-phenetidine.

Protein Assay—Proteins were measured using a dye-binding method (23) with bovine serum albumin as standard.

RESULTS

Kinetic Studies (Table I)

The kinetic characteristics of amine-acetylation were studied using five arylalkylamines and two arylamines (Table I). As described under "Experimental Procedures," complicating effects of amine oxidation were minimized by inhibiting monoamine oxidase activity with pargyline. The degradation of 1 mM AcCoA was less than 10% in studies of amine *K_m*. Accordingly, it was unlikely that the apparent kinetic constants were significantly influenced by amine oxidation or acetyl-CoA degradation.

Acetylation of Arylalkylamines—The pineal supernatant from isoproterenol-treated rats appears to contain an enzyme which has a *K_m*(app) for tryptamine of 1.7–4.5 mM, in agreement with previous studies (7). A similar value (3.4 mM) was found after a 15-fold enrichment of this enzyme on a Sepharose-cystamine column. The *K_m*(app) and *V_{max}*(app) values obtained with 3 other indoleamines were generally similar to those obtained using tryptamine (Table I); the serotonin value agrees with a previous report (7). It should be noted that during the course of these experiments we observed an unexplained 2–3-fold variation in kinetic parameters using tryptamine. In view of this, all *K_m* values obtained using the crude homogenate must be viewed with some caution. A 10-fold higher *K_m*(app) was observed for phenylethylamine (Table I).

In the rat, the *K_m*(app) for AcCoA was 0.15 mM in the presence of tryptamine. This agrees with a previous report (7), and with the value (0.22 mM) found with the partially purified enzyme preparation. There was 35% and 10% degradation of 0.1 mM AcCoA, respectively, in these studies. The lower degradation of AcCoA in the partially purified preparation may reflect the absence of AcCoA hydrolase.

Individual sheep pineal supernatant preparations were used to study pineal arylalkylamine acetylation. The range of *K_m*(app) values for tryptamine in eight sheep pineal glands was 0.04–0.18 mM. A similar *K_m*(app) was found for the other indoleamines studied, although 5-methoxytryptamine tended to yield lower values (Table I). It is clear that the *K_m*(app) for indoleamines is at least 10-fold lower in sheep than in rat. With an individual sheep pineal, the *K_m*(app) for phenylethylamine (0.43 mM) was 10-fold higher than that for tryptamine (0.04 mM). Studies on sheep pineals obtained during the day or night indicated there were no day/night differences in the *K_m*(app) for tryptamine, serotonin, and AcCoA. The nocturnal increase of *V_{max}*(app) was typically 1.5–5-fold. However, a 3-fold variation in the absolute value of *V_{max}*(app) and its day/night ratio was observed between groups of pineals obtained over several weeks.

The *K_m*(app) for AcCoA was 0.078 mM in the presence of tryptamine. Degradation of 0.1 mM AcCoA at the end of the incubation was 35%.

Acetylation of Arylamines—Rat and sheep pineal supernatants prepared from glands obtained during the day or night acetylate *p*-phenetidine and aniline.

The rat gland appeared to contain an enzyme which had a *K_m*(app) for *p*-phenetidine (0.27 mM) similar to that for aniline (0.65 mM); the *V_{max}*(app) values for both substrates were also similar. In contrast, the sheep pineal gland appeared to

² The abbreviations used are: AcCoA, acetyl-CoA, HPLC, high pressure liquid chromatography, LD, a lighting schedule with the given hours of lighting and darkness.

TABLE I

Kinetics of arylalkylamine, and arylamine, acetylation with rat and sheep pineal supernatant preparations (10,000 × g)

All amine substrates were studied in the presence of 1 mM acetyl coenzyme A. Acetyl coenzyme A was studied in the presence of 10 mM tryptamine (a), 1 mM tryptamine (b), 3 mM *p*-phenetidine (c). Values are mean ± S.E. (number of determinations in parenthesis) or estimated from a single fit ± percentage of error on the fit.

Substrate	Rat		Sheep	
	$K_m(\text{app})$ mM	$V_{\text{max}}(\text{app})$ nmol/min/mg protein	$K_m(\text{app})$ mM	$V_{\text{max}}(\text{app})$ nmol/min/mg protein
Tryptamine	3.5 ± 0.5 (5)	2.75 ± 0.8 (5)	0.11 ± 0.02 (8)	0.44 ± 9% ^a
Serotonin	2.0 ± 13%	1.43 ± 7%	0.085 ± 0.013 (2)	0.67 ± 6.9% ^a
5-Methoxytryptamine	2.8 ± 8%	3.5 ± 4%	0.028 ± 0.001 (2)	0.36 ± 4.4% ^a
6-Fluorotryptamine	3.4 ± 9%	1.0 ± 5.7%	0.057 ± 0.027 (2)	0.49 ± 7.8% ^a
Phenylethylamine	22 ± 8.7%	7.13 ± 7.7%	0.43 ± 13%	4.01 ± 9%
<i>p</i> -Phenetidine	0.27 ± 0.07 (3)	0.85 ± 0.13 (3)	0.38 ± 0.02 (5)	8.52 ± 2.96 (5)
Aniline	0.65 ± 0.06 (2)	0.79 ± 0.12 (2)	3.8 ± 9%	3.75 ± 6.4%
Acetyl coenzyme A				
(a)	0.15 ± 11.6%	5.02 ± 4%		
(b)			0.078 ± 5.4%	1.53 ± 4%
(c)	5.9 ± 17%	3.5 ± 10%	1.59 ± 6%	29 ± 4.5%

^a $V_{\text{max}}(\text{app})$ values obtained on the same sheep pineal gland are presented to allow comparison between indoleamines.

contain an enzyme that had a $K_m(\text{app})$ for *p*-phenetidine (0.38 mM) which was one-tenth that for aniline (3.8 mM) and the $V_{\text{max}}(\text{app})$ with *p*-phenetidine was about 2-fold greater than with aniline. In sheep, a large individual variation in enzyme activity was observed using *p*-phenetidine. The same individual variation was found in aniline but not in tryptamine acetylation (data not shown).

The $K_m(\text{app})$ for acetyl-CoA with *p*-phenetidine was 5.9 mM in the rat and 1.6 mM in the sheep preparations. These values are 20–40-fold greater than the $K_m(\text{app})$ for AcCoA measured with tryptamine. Comparison of the $V_{\text{max}}(\text{app})$ values for the rat and sheep arylamine *N*-acetylation indicates the sheep value is 10-fold greater than the rat value.

Differential Regulation of Pineal Arylamine *N*-Acetyltransferase and Arylalkylamine *N*-Acetyltransferase (Table II)

The presence of two forms of *N*-acetyltransferase was investigated by determining whether arylamine acetylation and arylalkylamine acetylation were controlled differently.³ In the rat, isoproterenol treatment, which increased daytime *N*-acetylation of tryptamine and phenylethylamine about 100-fold, did not significantly alter the acetylation of the two arylamines tested (Table II). In the sheep, cycloheximide treatment caused a 90% reduction in arylalkylamine acetylation but did not significantly alter arylamine acetylation (Table II). These observations provide evidence that arylamine and arylalkylamine acetylations are differentially regulated in the mammalian pineal gland.

Different Stability of Arylamine *N*-Acetyltransferase and Arylalkylamine *N*-Acetyltransferase (Fig. 1)

During the course of these studies, we found that the enzyme which acetylates *p*-phenetidine is labile upon storage in the cold. In both species arylamine acetylation decreased more quickly at 4 °C than arylalkylamine acetylation (Fig. 1). Complete loss of *p*-phenetidine and aniline acetylation occurred after 24 h in the rat and over 95% of the activity

disappeared after 48 h in the sheep. The corresponding inactivation of arylalkylamine acetylation was 50% in the rat and 25% in the sheep.

Chromatographic Separation of Arylamine *N*-Acetyltransferase and Arylalkylamine *N*-Acetyltransferase Activity (Fig. 2)

Size exclusion HPLC was used to attempt to separate arylamine *N*-acetyltransferase and arylalkylamine *N*-acetyltransferase; it has been previously established that tryptamine *N*-acetylation can be chromatographically resolved into two peaks (22, 24). Size exclusion chromatography of the 100,000 × g supernatant from isoproterenol-treated rat pineal glands revealed that tryptamine acetylation was present in two peaks (Fig. 2A). One contained molecules with an apparent molecular mass of about 100,000 daltons and accounted for 75% of the recovered activity; the $K_m(\text{app})$ for tryptamine was 0.86 mM (± 6%). The second peak contained molecules with an apparent molecular mass of 20,000–25,000 daltons; it had a $K_m(\text{app})$ for tryptamine of 1.8 mM (± 20%). The total recovery of applied activity in two runs was 50 and 80%. Arylamine *N*-acetylation was detected in a single intermediate peak (M_r = 35,000), with 80% recovery (Fig. 2A).

A significant level of tryptamine *N*-acetylation activity was detected in the peak fractions for the *p*-phenetidine acetylation activity, making it unclear whether one or two enzymes were present. To examine this, these fractions were pooled and tryptamine and *p*-phenetidine were added in saturating concentrations to determine if acetylation was additive in the pool. A positive result was obtained indicating that two enzymes were probably present in these fractions (Table III).

As observed in the rat, size exclusion HPLC of the night sheep pineal supernatant showed a clear separation between the peak of tryptamine *N*-acetylation activity and the peak of *p*-phenetidine *N*-acetylation activity (Fig. 2B). However, the elution profile of tryptamine *N*-acetylation was slightly different from that described above in the rat, since the higher M_r peak contained 90% of the recovered activity and a distinct lower M_r peak of activity was not apparent. The recovery for tryptamine *N*-acetylation was 50% in two runs. In addition, we observed a low but clear activity towards *p*-phenetidine in the peak fractions for tryptamine *N*-acetylation (Fig. 2B). *p*-Phenetidine *N*-acetylation was 2.5–4-fold lower than tryptamine

³ This agrees with the results of an unpublished experiment which found that isoproterenol-treatment of the rat results in an increase in tryptamine *N*-acetylation, but not in the *N*-acetylation of isoniazid or *p*-aminobenzoic acid by the pineal gland.

TABLE II

Effect of selected treatments on arylamine and arylalkylamine *N*-acetyltransferase activities in rat and sheep pineal glands

Rats received isoproterenol (10 mg/kg) or saline and were killed during the daytime. Sheep received cycloheximide (4 mg/kg) or saline 30 min before lights off and were killed in the dark 30 min after lights off. *N*-Acetyltransferase 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.

Substrate	<i>N</i> -Acetyltransferase activity			
	Rat		Sheep	
	Control day	Isoproterenol	Control night	Cycloheximide
	<i>nmol/min/mg protein</i>			
Tryptamine	0.011 \pm 0.0007	1.5 \pm 0.25	0.71 \pm 0.07	0.06 \pm 0.015
Phenylethylamine	0.019 \pm 0.005	1.5 \pm 0.15	0.66 \pm 0.07	0.075 \pm 0.025
<i>p</i> -Phenetidine	0.20 \pm 0.036	0.13 \pm 0.006	0.42 \pm 0.1	0.35 \pm 0.1
Aniline	0.14 \pm 0.007	0.08 \pm 0.018	0.115 \pm 0.049	0.095 \pm 0.03

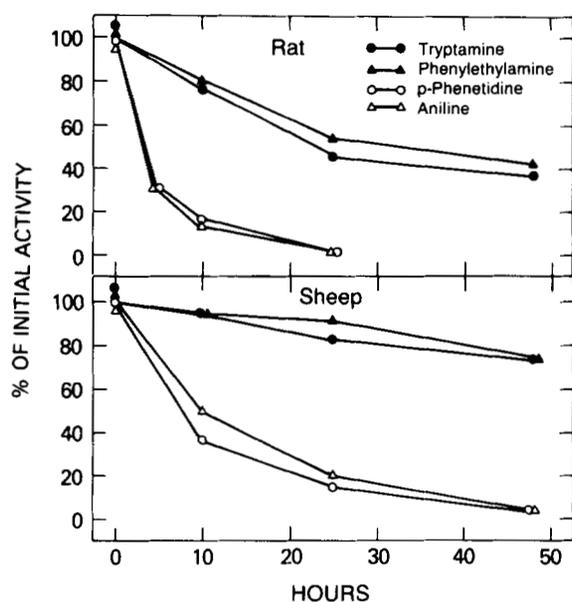


FIG. 1. Time course of inactivation (4 °C) of pineal arylamine and arylalkylamine *N*-acetyltransferases. Samples of pineal supernatant preparations (10,000 \times g) were stored at 4 °C for the indicated time and assayed with 0.5 mM AcCoA and 10 mM amine substrate.

tamine *N*-acetylation in these fractions, but accounted for only 2% of the total *p*-phenetidine *N*-acetylation activity. The presence of two enzymes in the peak fractions for tryptamine acetylation was investigated using the additivity test described above. The absence of additivity suggests that the tryptamine acetylating enzyme is also responsible for the low level of *p*-phenetidine acetylation in these fractions and that two enzymes are probably not present (Table III).

The peak in sheep *p*-phenetidine *N*-acetylation contained molecules with an apparent $M_r = 35,000$; it had 80% of the initial *p*-phenetidine *N*-acetylation activity.

Location of Arylamine *N*-Acetyltransferase (Table IV)

Possible cellular sites of arylamine *N*-acetylation in the pineal gland include blood cells and sympathetic nerve endings, in addition to the pineal cells. Although it has already been established that the enzyme which *N*-acetylates tryptamine in the rat is located in pineal cells (25), the location of arylamine *N*-acetylation activity is not known. To determine the relative contribution of blood arylamine *N*-acetyltransferase (26) to the *p*-phenetidine acetylation in the pineal,

the activity on *p*-phenetidine was measured in rat and sheep blood. The blood/pineal ratio of enzyme activity on a protein basis was about 1:2 in the rat and 1:20 in the sheep (Table IV). Given the low level of blood contamination in the pineal homogenates, it is unlikely that the blood enzyme contributes significantly to the activity measured in the pineal.

Arylamine *N*-acetyltransferase was also measured in denervated pineal glands, obtained from superior cervical ganglionectomized rats. Arylamine *N*-acetyltransferase activity in these glands did not decrease, as compared to intact glands (Table IV), suggesting arylamine *N*-acetyltransferase occurs primarily in pineal cells.

DISCUSSION

The findings in this report clearly establish that arylamine *N*-acetylation in the mammalian pineal gland is primarily catalyzed by one enzyme, and that arylalkylamine *N*-acetylation is catalyzed by another. There are three central observations supporting this conclusion. First, in both the rat and sheep, arylalkylamine *N*-acetylation markedly changes within hours as a result of drug treatment without a change in the activity of arylamine *N*-acetylation. Second, arylamine *N*-acetyltransferase activity is labile, whereas arylalkylamine *N*-acetyltransferase is relatively stable in the cold. Third, arylamine *N*-acetyltransferase can be chromatographically resolved from arylalkylamine *N*-acetyltransferase.

This report is the first body of evidence establishing the existence in one tissue of both an arylamine and a distinct arylalkylamine *N*-acetyltransferase. In contrast, it appears that the mammalian liver contains an enzyme activity which can acetylate arylamines, including *p*-phenetidine, and arylalkylamines, including serotonin (5). This enzyme activity varies on an individual basis in humans, monkeys, and rabbits, and is controlled genetically (5); individuals fall into either rapid or slow acetylator phenotype groups. Purified preparations of the rabbit liver enzyme have been shown to acetylate phenetidine, isoniazid, and serotonin (27, 28). Although it is possible that the two types of substrate specificity observed actually represent two enzymes in the rabbit liver, it has not yet been possible to separate them by chromatography. In contrast, recent studies have revealed that the rapid acetylator hamster liver contains two molecular forms of *N*-acetyltransferase, with different substrate specificity, which can be separated using ion-exchange chromatography (29, 30). However, the acetylation of arylalkylamines by these enzymes has not yet been described.

Pineal Arylalkylamine N-Acetyltransferase—These studies have revealed a number of interesting characteristics of the

FIG. 2. Size exclusion HPLC profiles of arylamine and arylalkylamine N-acetyltransferases from rat and sheep pineal glands. The supernatant ($100,000 \times g$) 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.

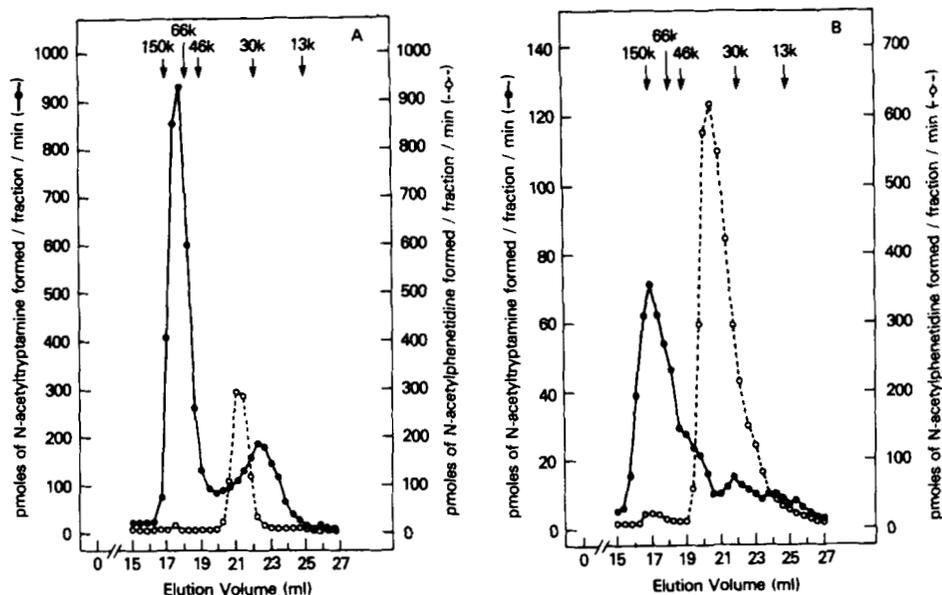


TABLE III

Test of the additivity of tryptamine and *p*-phenetidine N-acetylation in the HPLC fractions containing both enzyme activities

In the rat, the fractions from 20.2 ml to 22.2 ml were pooled (Fig. 2A). In the sheep, the fractions from 16.6 to 17.6 ml were pooled (Fig. 2B). The pools were assayed for N-acetyltransferase activity with 0.1 mM AcCoA and saturating concentrations of either tryptamine, *p*-phenetidine or both substrates together. Data is the mean \pm S.E. of the number of replicates in parenthesis.

Substrate	N-Acetyltransferase activity	
	Rat	Sheep
	<i>pmol/min/ml</i>	
Tryptamine (10 mM)	57 \pm 2 (2)	35 \pm 2 (3)
<i>p</i> -Phenetidine (3 mM)	58 \pm 2 (2)	15 \pm 1 (3)
Tryptamine (10 mM) + <i>p</i> -Phenetidine (3 mM)	110 \pm 18 (2)	30 \pm 2 (3)

TABLE IV

Location of pineal arylamine N-acetyltransferase

Experiment I, the $10,000 \times g$ supernatants from blood or pineal homogenates were assayed for arylamine N-acetyltransferase activity. Experiment II, pineal glands were obtained from control and superior cervical gangliorectomized rats. After sonication, the $10,000 \times g$ supernatants were assayed for arylamine N-acetyltransferase activity. Assays were carried out with 0.5 mM AcCoA and 10 mM *p*-phenetidine. Data is mean \pm S.E. of the number of determinations in parenthesis.

Sample	<i>p</i> -Phenetidine N-acetylation
	<i>nmol/min/mg protein</i>
Experiment I	
Rat	
Blood	0.110 \pm 0.007 (4)
Pineal	0.190 \pm 0.018 (3)
Sheep	
Blood	0.137 \pm 0.002 (4)
Pineal	3.2 \pm 0.07 (2)
Experiment II	
Rat	
Control pineal	0.250 \pm 0.017 (4)
Denervated pineal	0.410 \pm 0.09 (2)

enzyme which acetylates indoleamines in the pineal gland. First, this enzyme appears to have a greater affinity for the indole ring compared to the phenyl ring, as evidenced by the 10-fold lower K_m (app) values for four indoleamines as com-

pared to that of phenylethylamine in both species we studied. Substitutions on the indole ring do not significantly alter the kinetics of the reaction. Apparently the enzyme cannot differentiate between these analogs. The marked difference in affinity for the indoleamines as a group, as compared to phenylethylamine might be due specifically to the indole nitrogen or in a more diffuse effect to the larger ring structure of the indoleamines, a possibility which could be investigated using indane analogs.

The chromatographic resolution into two peaks of the pineal arylalkylamine N-acetyltransferase in the presence of ammonium acetate has been reported (22). Our finding that both forms had a similar K_m (app) for tryptamine in the rat suggests they are closely related; they might represent different quaternary structures of an identical gene product. This is also supported by the observation that pineal arylalkylamine N-acetyltransferase elutes in a single peak (M_r = around 30,000) in the presence of sodium citrate (22) or potassium phosphate (10). The K_m (app) for tryptamine in the first peak was lower than the values measured on the crude supernatant. This may be due to the presence of 10 mM dithiothreitol in the HPLC eluate, because the redox state of a thiol group carried by the arylalkylamine N-acetyltransferase is known to affect the catalytic activity (24). In the sheep, the presence of two peaks of tryptamine N-acetylation activity has been previously reported (24). However, the relative amplitude of these two peaks varies among individuals,⁴ which might account for the absence of a distinct lower M_r peak in the present study.

The enzymes from rat and sheep differ significantly in their kinetic properties and specificity. Comparison of the K_m (app) for all indoleamines indicate that the values for sheep are consistently lower. As mentioned in the Introduction, this was not unexpected because the concentration of serotonin in the sheep pineal gland is about 2% of that in the rat (13). Another difference is that, according to HPLC and additivity experiments, the tryptamine-acetyating enzyme from sheep pineal can act to a limited degree on *p*-phenetidine, whereas the rat enzyme cannot and shows a clear-cut specificity for arylalkylamines.

Pineal Arylamine N-Acetyltransferase—Multiple forms of arylamine N-acetyltransferase have been described in various

⁴ P. Voisin, M. A. A. Namboodiri, and D. C. Klein, unpublished results.

tissues including liver, gut mucosa, spleen, kidney, and blood where they play a role in the detoxification of aromatic amines (26, 31). This study has revealed the presence of an arylamine *N*-acetyltransferase in the pineal gland, and some interesting characteristics of this enzyme have emerged. First, it would appear that, unlike the liver enzyme, the pineal arylamine *N*-acetyltransferase does not *N*-acetylate indoleamines. This adaptation precludes the participation of the arylamine *N*-acetyltransferase in melatonin synthesis, and channels the serotonin → melatonin pathway exclusively through the arylalkylamine *N*-acetyltransferase. The presence of these two independently controlled *N*-acetyltransferases with marked substrate specificity appears as an efficient means of independently maintaining both the rhythmic *N*-acetylation of serotonin, necessary for the special function of the pineal gland, and the unvarying *N*-acetylation of potentially toxic arylamines. A second noteworthy characteristic of the pineal arylamine *N*-acetyltransferase is the high K_m (app) for AcCoA using *p*-phenetidine as substrate. This observation raises the possibility that another acyl coenzyme A with a higher affinity for the enzyme is the natural substrate. Further investigation of this possibility as well as the attempt to identify an endogenous acyl acceptor might lead to the description of a specific function of this enzyme. Third, the pineal arylamine *N*-acetyltransferase is labile whereas the liver enzyme is apparently stable in the cold.

As a final comment, it is of interest to raise the question of whether pineal arylamine and arylalkylamine *N*-acetyltransferase are related. It may be possible that both enzymes are derived from the same gene, and that post-transcriptional or post-translational processing produces differences in stability, specificity, and quaternary structure. Alternatively, these enzymes might reflect the expression of two genes. This is supported by the finding that, in the rat, isoproterenol alters the activity of the arylalkylamine *N*-acetyltransferase only. This regulation is thought to involve gene expression (32). However, another possibility to be considered is that the gene expressed as a result of isoproterenol treatment codes for a regulatory enzyme which acts on a *N*-acetyltransferase pre-enzyme to yield the arylalkylamine *N*-acetyltransferase. If this is the case, then the single gene-two *N*-acetyltransferase hypothesis might apply. Further investigations in this area should answer these interesting questions.

REFERENCES

1. Klein, D. C., Auerbach, D. A., Namboodiri, N. A. A., and Wheler, G. H. T. (1981) in *The Pineal Gland* (Reiter, R. J., ed) Vol. 1, pp. 199-227, CRC Press Inc., Boca Raton, Florida
2. Reiter, R. J. (1980) *Endocrine Rev.* **1**, 109-131
3. Goldman, B. D., Carter, D. S., Hall, V. D., Roychoudhury, P., and Yellon, S. M. (1982) in *Melatonin Rhythm Generating System* (Klein, D. C., ed), pp. 210-231, Karger, Basel
4. Klein, D. C. (1979) in *Endocrine Rhythms* (Krieger, D. T., ed) pp. 203-223, Raven Press, New York
5. Weber, W. W. (1971) *Methods Enzymol.* **17**, 805-811
6. Yang, H. T., and Neff, N. H. (1976) *Neuropharmacology* **15**, 561-564
7. Deguchi, T. (1975) *J. Neurochem.* **24**, 1083-1085
8. Binkley, S., Klein, D. C., and Weller, J. L. (1976) *J. Neurochem.* **26**, 51-55
9. Howd, R. A., Seo, K. S., and Wurtman, R. J. (1976) *Biochem. Pharmacol.* **25**, 977-978
10. Morrissey, J. J., Edwards, S. B., and Lovenberg, W. (1977) *Biochem. Biophys. Res. Commun.* **77**, 118-123
11. Ellison, N., Weller, J. L., and Klein, D. C. (1972) *J. Neurochem.* **19**, 1335-1341
12. Rudeen, K. P., Reiter, R. J., and Vaughan, M. K. (1975) *Neurosci. Lett.* **1**, 225-229
13. Mefford, I. N., Chang, P., Klein, D. C., Namboodiri, M. A. A., Sugden, D., and Barchas, J. (1983) *Endocrinology* **113**, 1582-1586
14. Deguchi, T., and Axelrod, J. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69**, 2208-2212
15. Sugden, D., Namboodiri, M. A. A., Weller, J. L., and Klein, D. C. (1983) in *Methods in Biogenic Amine Research* (Parvez, S., Nagatsu, T., Nagatsu, I., and Parvez, H., eds) pp. 567-572, Elsevier Scientific Publishing Co., Amsterdam
16. Neff, N. H., and Golidis, C. (1972) *Adv. Biochem. Psychopharmacol.* **5**, 307-323
17. Erwin, V. G., and Deitrich, R. A. (1971) *Mol. Pharmacol.* **7**, 219-228
18. Namboodiri, M. A. A., Nakai, C., and Klein, D. C. (1979) *J. Neurochem.* **33**, 807-810
19. Klein, D. C., and Notides, A. (1969) *Anal. Biochem.* **31**, 480-483
20. Namboodiri, M. A. A., and Klein, D. C. (1979) *Anal. Biochem.* **93**, 244-247
21. Munson, P. J., and Rodbard, D. (1980) *Anal. Biochem.* **107**, 220-239
22. Namboodiri, M. A. A., Brownstein, M., Weller, J. L., and Klein, D. C. (1982) *Fed. Proc.* **41**, 2188 (abstr.)
23. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254
24. Namboodiri, M. A. A., and Klein, D. C. (1981) in *Monoamine Enzymes* (Usdin, E., Weiner, N., and Youdim, M. B. H., eds) pp. 701-714, Macmillan Journals Ltd., London
25. Buda, M., and Klein, D. C. (1978) *Endocrinology* **103**, 1483-1493
26. Gollamudi, R., Muniraju, B., and Schreiber, E. L. (1980) *Enzyme (Basel)* **25**, 309-315
27. Weber, W. W., Cohen, S. N., and Steinberg, M. S. (1968) *Ann. N. Y. Acad. Sci.* **151**, 734-741
28. Steinberg, M. S., Cohen, S. N., and Weber, W. W. (1969) *Biochim. Biophys. Acta* **184**, 210-212
29. Hein, D. W. (1982) Ph.D. dissertation, University of Michigan
30. Hein, D. W., Omichinsky, J. G., Brewer, J. A., and Weber, W. W. (1982) *J. Pharmacol. Exp. Ther.* **220**, 8-15
31. Hearse, D. J., and Weber, W. W. (1973) *Biochem. J.* **132**, 519-526
32. Morrissey, J. J., and Lovenberg, W. (1978) *Biochem. Pharmacol.* **27**, 551-555