Journal of Neurochemistry
Raven Press, New York
© 1983 International Society for Neurochemistry
0022-3042/83/0601-1647/$03.50/0

Regulation of Rat Pineal Hydroxyindole-*O*-Methyltransferase in Neonatal and Adult Rats

David Sugden and David C. Klein

Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, U.S.A.

Abstract: The relative importance of neural, and some nonneural, mechanisms in the control of pineal hydroxyindole-*O*-methyltransferase (HIOMT) activity during development and in the adult rat was studied. In neonatal rats, guanethidine-treatment, bilateral superior cervical ganglionectomy (SCGX), or exposure to constant light did not prevent the initial appearance of HIOMT activity, indicating that neural stimulation of the gland is not essential for the development of HIOMT activity. In adult rats, decentralization or removal of the SCG led to a slow fall in HIOMT activity, to about 30% of control activity, indicating that the enzyme is largely under neural control. Additionally, adrenalectomy or hypophysectomy had no effect on HIOMT activity, refuting the suggestion that adrenal and/or gonadal steroids are of major importance in the regulation of this enzyme. The fall in activity of the enzyme after SCGX or exposure to constant light probably does not represent a shift in the K_m of the enzyme nor the selective disappearance of a distinct molecular species. Similar changes in HIOMT activity and cyclic GMP responsiveness occur in response to alterations in the length of the daily dark period, adding further evidence to our earlier speculation that there may be a functional relationship between these two. **Key Words:** Pineal—Hydroxyindole-*O*-methyltransferase—Neural regulation—Neonatal rats. **Sugden D. and Klein D. C.** Regulation of rat pineal hydroxyindole-*O*-methyltransferase in neonatal and adult rats. *J. Neurochem.* **40**, 1647–1653 (1983).

Hydroxyindole-*O*-methyltransferase (HIOMT; EC 2.1.1.4) converts *N*-acetylserotonin to melatonin in the pineal gland (Axelrod and Weissbach, 1960). In the rat, the activity of this enzyme is under neural control. Stimulatory neural signals appear to originate in the suprachiasmatic nuclei of the hypothalamus (SCN) and are transmitted to the pineal gland each night for about a 10-h period (Klein and Moore, 1979). The SCN and pineal gland are connected by a neural circuit passing through central and peripheral structures, including the superior cervical ganglia (SCG) (Moore, 1978). Light, acting via the eye and a retinal-SCN projection, blocks transmission of signals to the pineal gland. Accordingly, exposure to constant lighting causes a gradual decrease in HIOMT activity, as does removal of the SCG or destruction of the SCN (Wurtman et al., 1963; Moore and Rapport, 1971; Klein and Moore, 1979).

Although recent studies suggest that HIOMT activity, like several other aspects of pineal biochemistry (Klein, 1983), is controlled by the transsynaptic release of norepinephrine (Sugden and Klein, 1982), there has also been considerable speculation that nonneural mechanisms may play a role in the physiological regulation of this enzyme (Preslock, 1977; Cardinali, 1981). The studies described in this report were designed to examine the relative importance of neural, and some nonneural, mechanisms in controlling the developmental appearance and amount of HIOMT activity in the rat.

EXPERIMENTAL PROCEDURES

Treatment of animals

Male Sprague-Dawley rats (Zivic Miller Co., Allison Park, PA) were used, except in developmental studies which involved mixed-sex litters. Bilateral superior cer-

Received July 12, 1982; accepted December 13, 1982.
Address correspondence and reprint requests to Dr. David Sugden, Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, Building 6, Room 1A15, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205, U.S.A.

Abbreviations used: HIOMT, Hydroxyindole-*O*-methyltransferase (EC 2.1.1.4); LD, Light-dark; LL, Constant light; NAT, *N*-Acetyltransferase (EC 2.3.1.5); SCG, Superior cervical ganglia; SCGX, Bilateral superior cervical ganglionectomy; SCN, Suprachiasmatic nuclei.

vical ganglionectomy (SCGX), decentralization of the SCG, bilateral adrenalectomy, and hypophysectomy were performed by the supplier. All hypophysectomized rats had lower body weights than sham-operated or control rats on the day of sacrifice.

Ablation of the peripheral sympathetic nervous system was accomplished by injection of guanethidine bisulfate (Ismelin, CIBA Pharmaceutical Co., Summit, NJ; 50 mg/kg i.p.) five times a week for 3 weeks beginning 4 days after birth (Johnson et al., 1976). Bilateral adrenal demedullation was performed on these animals on the last day of injection. Sham-treated rats received daily saline injections and sham adrenal demedullation. The success of the guanethidine procedure is indicated by the marked bilateral ptosis exhibited by all treated rats.

All rats had access to food and water *ad libitum* and were housed under a diurnal lighting cycle (light-dark 14:10; lights on at 0500 h) unless otherwise indicated. Light was provided by 34-W cool-white fluorescent tubes (Sylvania F40/CW/RS/SS), and its intensity was 200 μW/cm^2 at the level of the animals. Adrenalectomized rats were given sodium chloride (1% wt/vol) in the drinking water.

Rats were killed by decapitation, and the pineal glands were rapidly removed and frozen on dry ice. Glands were stored at −40°C until they were assayed for HIOMT or *N*-acetyltransferase (NAT) activity.

Cyclic GMP responsiveness was tested as described previously (Klein et al., 1981). Briefly, individual glands were incubated (37°C, 95% O_2/5% CO_2) for a 60-min control period in 0.3 ml BGJb medium (Fitton-Jackson modification) and then transferred to fresh medium containing L-norepinephrine (10^{-5} *M*) for a 10-min test period. At the end of the test period the glands were rapidly frozen in tubes on solid CO_2 and stored at −70°C until assayed for cyclic GMP.

Assays

Pineal HIOMT activity was measured by a modification of the Axelrod and Weissbach (1961) procedure. Single pineal glands were sonicated in 100 μl of sodium phosphate buffer (0.05 *M*, pH 7.9), and a sample (usually 50 μl) of the tissue homogenate was added to a microtube containing *N*-acetylserotonin and [methyl-^{14}C]*S*-adenosyl-L-methionine (ICN, Irvine, CA; specific activity, 25 mCi/mmol). The final concentrations of these substrates were 1 m*M* and 0.1 m*M*, respectively. The tubes were incubated for 30 min at 37°C; then 200 μl of sodium borate buffer (0.45 *M*, pH 10) and 1 ml of chloroform were added. The mixture was vortexed twice for 15 s to extract the product, [^{14}C]melatonin, into the chloroform. The organic layer was washed once with borate buffer, and an aliquot (500 μl) was dried and its radioactivity was measured.

NAT activity in the pineal tissue homogenate was measured by a published procedure (Parfitt et al., 1975). Pineal cyclic GMP was measured using commercially available reagents (New England Nuclear, Boston, MA) by an established radioimmunoassay method (Steiner et al., 1972). Protein was measured by a dye binding method, with bovine serum albumin as a standard (Bradford, 1976).

Statistical analysis

All data given represent the mean ± SEM and were analyzed by Student's *t* test or analysis of variance. The Scafit data processing computer program (Faden and Rodbard, 1976) was used to fit the best straight line to the HIOMT kinetic data and to determine the apparent K_m and V_{max} values and their SEM.

RESULTS

Studies with neonatal rats

The influence of neural stimulation on pineal HIOMT activity in neonatal rats was tested in two experiments. First, the developmental appearance of HIOMT activity was studied in rats housed from birth either in LD (light-dark) or in LL (constant light) and in rats that had undergone SCGX 2 days after birth (Fig. 1). A gradual increase in activity was observed to occur in all animals between day 5 and 15. This is in contrast to the abrupt increase between day 10 and 13 reported earlier (Klein and Lines, 1969). This difference may be a reflection of the different enzyme assays used; in this study substrate concentrations that saturate the enzyme were used. Exposure to LL or SCGX had no effect on

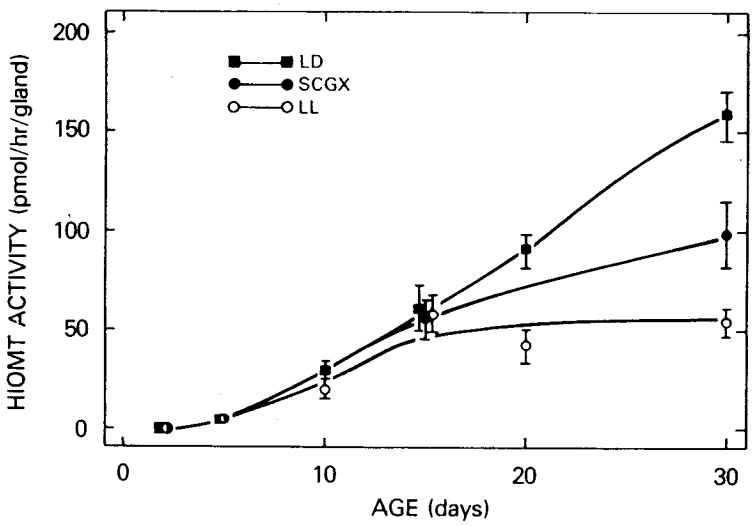

FIG. 1. Developmental appearance of pineal HIOMT activity in intact, SCGX, and LL rats. Sprague-Dawley rats of either sex were either housed in LD 14:10 or constant light (LL) from the day of birth or were bilaterally superior cervical ganglionectomized (SCGX) 2 days after birth. Rats were killed at various ages and pineal HIOMT activity determined. Each point represents the mean ± SEM of six rats.

TABLE 1. *Effect of neonatal ablation of the sympathetic nervous system on the development of rat pineal NAT and HIOMT activities*

Treatment	NAT (nmol/h/mg protein)		HIOMT (nmol/h/mg protein)	
	Day	Night	Day	Night
Control	2.4 ± 0.6	202.8 ± 25.2	2.30 ± 0.22	2.93 ± 0.24
Guanethidine	4.8 ± 0.6[a]	3.0 ± 0.6[b]	1.11 ± 0.10[b]	1.32 ± 0.13[b]

For treatment of rats see Experimental Procedures. Rats were killed at 4 weeks of age at either 1200 (day) or 2400 h (night). NAT and HIOMT activities and the protein content of each gland were assayed. All the values represent the mean ± SEM of 10 to 14 rats.

[a] $p < 0.01$.

[b] $p < 0.001$ compared with the appropriate control value.

enzyme activity up to day 15. However, at day 30 enzyme activity in both groups was considerably lower than that seen in control animals.

In a second experiment, adrenergic stimulation of the gland was blocked by a combination of two procedures. Neonatal animals were treated with guanethidine for 3 weeks and then subjected to adrenal demedullation. This schedule of guanethidine treatment has been shown to produce a complete and permanent peripheral sympathectomy (Johnson et al., 1976). Analysis of pineal NAT, which normally exhibits a large day-night difference, indicated that the dark-induced elevation in the activity of this enzyme was blocked in the guanethidine-treated rats at 28 days of age (Table 1). This is evidence that the sympathetic input to the pineal gland was destroyed in these animals. However, HIOMT activity was clearly present in guanethidine-treated rats, indicating that destruction of the sympathetic system did not prevent the developmental appearance of HIOMT.

Studies with adult rats

Effect of SCGX and SCG decentralization. The rate of decrease in HIOMT activity following removal of the SCG was compared with that following decentralization of the SCG (Fig. 2). Activity decreased more rapidly in decentralized animals. However, 4 weeks after surgery, enzyme activity in both groups appeared to plateau at about 25% of that seen in the unoperated group housed in LD.

Relationship between the duration of the dark period, HIOMT activity, and cyclic GMP responsiveness. Although daily neural stimulation appears to be required for maintaining HIOMT activity at normal levels, the exact relationship between the length of the dark period and enzyme activity has not been established. This was studied (Fig. 3), and it was found that 10 days of exposure to no dark period (LL), or to a dark period of 1 h a day (LD 23:1) resulted in a similar fall in HIOMT activity. The normal value of HIOMT seen in animals in LD 14:10 was produced by exposing animals to only 5 h of darkness a day (LD 19:5) for a period of 10 days.

We have speculated that there is a functional relationship between HIOMT activity and cyclic GMP (Sugden and Klein, 1983). This was based on the observations that both HIOMT activity and cyclic GMP responsiveness to adrenergic stimulation fell gradually as a result of interruption of neural stimulation by SCGX or LL, and that both could be restored by daily norepinephrine injections (Klein et al., 1981; Sugden and Klein, 1983). To examine this potential relationship further, the effect of daily dark periods of different lengths (0, 1, 5, or 10 h) on cyclic GMP responsiveness was determined (Fig. 4). A 10-day exposure to LL or LD 23:1 severely reduced the cyclic GMP response to norepineph-

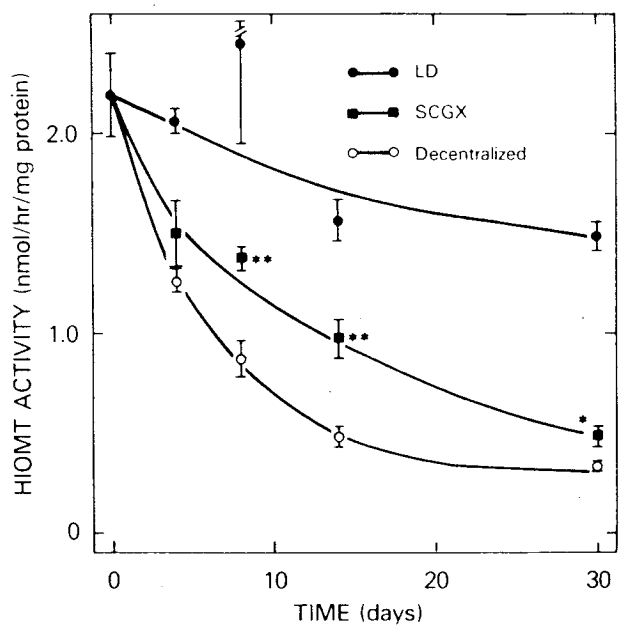

FIG. 2. Time course of the decline in pineal HIOMT activity after SCGX or decentralization of the SCG. All rats were killed during the light period, and pineal HIOMT activity was determined. Each point represents the mean ± SEM of six rats at the 0, 4, 8, and 14 day time points and 10 rats at the 30 day time point. Single asterisk indicates $p < 0.05$, double asterisk $p < 0.01$ compared with the decentralized group at that time point.

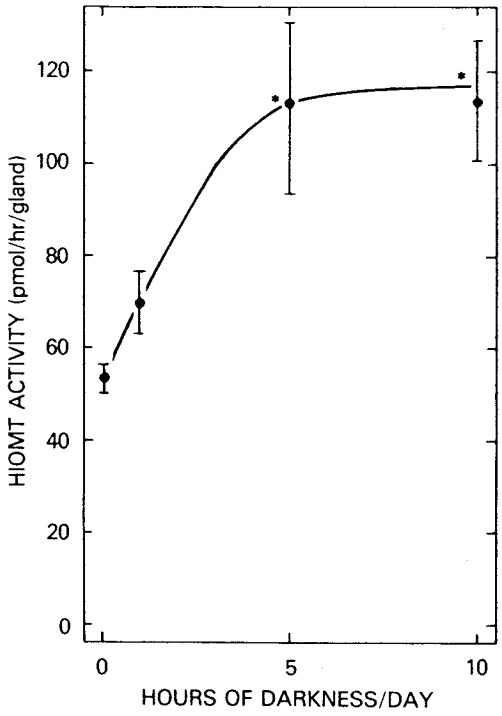

FIG. 3. Effect of the duration of the dark period on rat pineal HIOMT activity. For a period of 10 days, groups of rats were housed in constant light (LL), 1 h of darkness a day (from 23:30 to 00:30 h), 5 h of darkness a day (21:30 to 02:30 h), or 10 h of darkness a day (from 1900 to 0500 h). At the end of this period the rats were killed and pineal HIOMT activity determined. Each point represents the mean ± SEM of six to seven rats. Asterisk indicates $p < 0.01$ compared with the LL group.

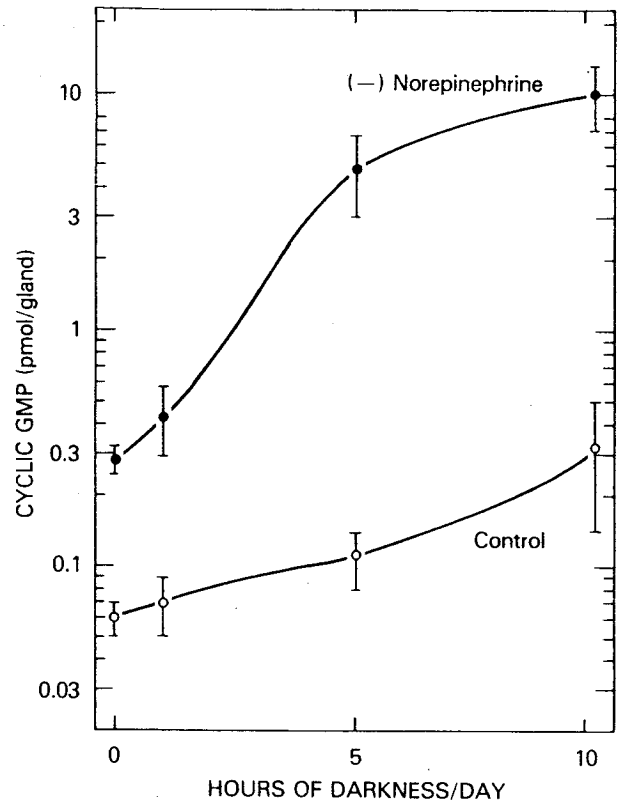

FIG. 4. Effect of duration of the dark period on rat pineal cyclic GMP responsiveness. For details see the legend to Fig. 3 and Experimental Procedures.

rine. Maximum responsiveness was found in rats exposed to either LD 19:5 or LD 14:10.

Effects of adrenalectomy and hypophysectomy. The effects of removal of the adrenal or the pituitary gland on HIOMT activity were examined 9 days after surgery. During this 9-day period, half the animals were housed in LL and the remainder in LD 14:10. Exposure to LL tended to decrease HIOMT activity in all groups (Table 2). In LD, hypophysectomy appeared to decrease HIOMT activity, expressed per gland, but also significantly reduced pineal protein content (unoperated, 123 ± 7 μg/gland; hypophysectomy, 65 ± 16 μg/gland, $p < 0.01$). Analysis of variance of the data (expressed either per gland or per milligram protein) failed to reveal a significant effect of any surgical treatment in either lighting condition (HIOMT activity/gland: LD, $F = 1.30$, df 4, 20; LL, $F = 2.19$, df 4, 24.

TABLE 2. *Effect of bilateral adrenalectomy and hypophysectomy on rat pineal HIOMT activity*

	HIOMT Activity			
	pmol/h/gland		nmol/h/mg protein	
Surgical treatment	LL	LD 14:10	LL	LD 14:10
None	92.8 ± 7.4[b]	194.0 ± 12.1	0.95 ± 0.05[b]	1.59 ± 0.08
Sham adrenalectomy	92.4 ± 10.1[b]	206.2 ± 19.0	0.72 ± 0.05[b]	1.54 ± 0.12
Adrenalectomy	86.3 ± 6.4[b]	193.8 ± 18.0	0.92 ± 0.04[b]	1.49 ± 0.12
Sham hypophysectomy	126.4 ± 14.2	170.4 ± 23.7	1.05 ± 0.14[a]	1.57 ± 0.15
Hypophysectomy	80.0 ± 8.3	122.5 ± 23.5	0.91 ± 0.08	2.06 ± 0.64

Rats were placed in a LD 14:10 schedule or constant light (LL) the day after surgery. Adrenalectomized rats received sodium chloride (1% wt/vol) in their drinking water. Nine days after operation all rats were killed and pineal HIOMT activity and protein were determined. Each value represents the mean ± SEM of six or seven rats.

[a] $p < 0.05$, [b] $p < 0.001$ compared with LD rats undergoing the same treatment.

TABLE 3. *Time course of the effect of hypophysectomy on HIOMT activity*

Surgical treatment	Time after surgery (days)	HIOMT Activity	
		pmol/h/gland	nmol/h/mg protein
Sham hypophysectomy	3	210.7 ± 17.9	1.49 ± 0.14
Hypophysectomy		110.0 ± 22.0[a]	1.01 ± 0.20
Sham hypophysectomy	6	176.7 ± 10.6	1.17 ± 0.07
Hypophysectomy		129.0 ± 18.4[a]	1.19 ± 0.12
Sham hypophysectomy	11	187.9 ± 21.0	1.13 ± 0.11
Hypophysectomy		103.6 ± 34.2[a]	0.85 ± 0.21

Sham-hypophysectomized or hypophysectomized rats were housed in a LD 14:10 schedule until killing. Pineal HIOMT activity and protein were determined. Each value represents the mean ± SEM for five to seven rats.

[a] $p < 0.05$ compared with the sham-hypophysectomized rats killed on the same day.

HIOMT activity/mg protein: LD, $F = 0.08$, df 4, 20; LL, $F = 0.01$, df 4, 24).

In a further experiment (Table 3), hypophysectomy significantly reduced HIOMT activity (expressed per gland) 3, 6 and 11 days after surgery; but when enzyme activity was expressed per milligram of protein, these changes were not statistically significant, indicating that the effect reflects a nonspecific loss of pineal protein.

Kinetic analysis of HIOMT. A kinetic analysis of HIOMT prepared from the pineal glands of rats maintained in LD, LL, and also from SCGX rats was performed (Fig. 5). SCGX and LL produced a large decrease in the V_{max} values; however, the apparent K_m values for *N*-acetylserotonin and *S*-adenosyl-L-methionine were similar in the LD, LL, and SCGX groups (Table 4).

DISCUSSION

The results of these investigations provide some interesting insights into both the development and regulation of HIOMT activity, and indicate that both neural and nonneural factors play a role in the control of this enzyme in neonatal and adult rats.

It is clear from the developmental studies that

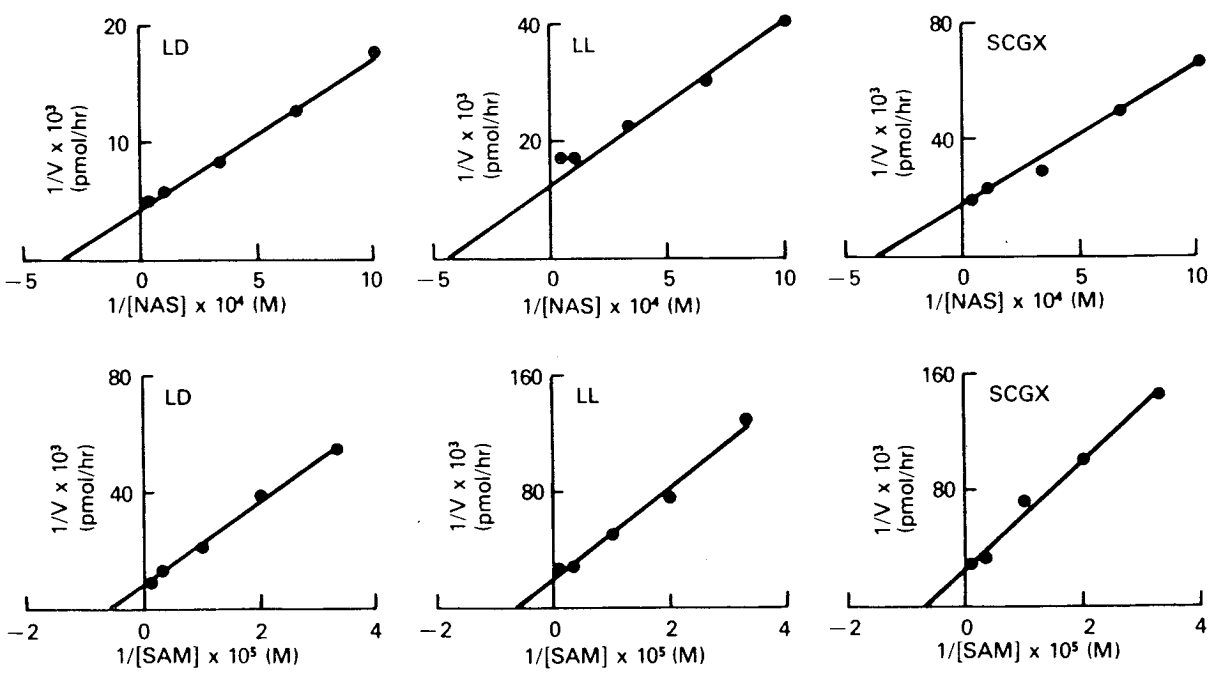

FIG. 5. Lineweaver-Burk plots of 1/*V* against 1/*S* for *N*-acetylserotonin (NAS) and *S*-adenosyl-L-methionine (SAM) for pineal HIOMT from rats housed in LD or LL, and SCGX rats. For the determination of the apparent K_m for NAS, the SAM concentration was fixed at 200 μM. For the SAM K_m determination, the NAS concentration was 1 m*M*. All points represent the mean of duplicate determinations which differed by less than 10%. The best straight line was fitted to the data by the Scafit data processing computer program (Faden and Rodbard, 1976) and gave the apparent K_m and V_{max} values shown in Table 4. Velocities are expressed in pmol of melatonin produced/h/gland. Two different groups of LD rats were used for SAM and NAS K_m determinations. Rats were used 70–80 days after exposure to LL or SCGX.

TABLE 4. *Apparent* K_m *(μM) and* V_{max} *(pmol/h/gland) values ± SEM for S-adenosyl-L-methionine and N-acetylserotonin calculated from data in Fig. 5*

Experiment	Substrate		LD	LL	SCGX
I	SAM	K_m (μM)	20.4 ± 2.4	12.7 ± 0.2	17.4 ± 2.1
		V_{max} (pmol/h/gland)	135.1 ± 10.0	44.5 ± 4.3	44.2 ± 3.3
II	NAS	K_m (μM)	28.0 ± 2.4	15.1 ± 1.3	27.3 ± 3.4
		V_{max} (pmol/h/gland)	225.8 ± 9.7	65.0 ± 2.2	59.8 ± 3.7

For further experimental details see legend to Fig. 5.
SAM = *S*-adenosyl-L-methionine; NAS = *N*-acetylserotonin.

HIOMT activity appears in the absence of normal neural stimulation of the gland, which is known to start at about day 5. Innervation by sympathetic neurons from the SCG is first detected at 1 day of age as catecholamine-containing fibers on the surface of the gland; a marked invasion and progressive maturation of these fibers follows, and the innervation achieves an adult appearance by the 2nd or 3rd week of life (Hakanson et al., 1967). In our experiments, neural stimulation of the gland during development was severely disrupted in three different ways: guanethidine treatment, SCGX, and exposure to LL. In all cases HIOMT activity was clearly present at the end of the 4th week of life, albeit at about 50% of that seen in control animals (Table 1; Fig. 1). Thus, innervation and the normal pattern of neural stimulation of the pineal gland apparently are not essential during development for the initial appearance of HIOMT activity.

There are many factors that could trigger the expression of HIOMT activity during development. One candidate is circulating catecholamines, which are known to stimulate the neonatal rat pineal gland (Yuwiler et al., 1977). The lack of an effective uptake mechanism in the immature sympathetic neurons of the pineal appears to permit norepinephrine and epinephrine in the fetal circulation (Ben-Jonathan, 1978) to act as hormones to stimulate NAT, and perhaps HIOMT, in the neonatal pineal gland.

Our studies on adult rats revealed a clear difference in the rate of decrease in HIOMT activity following removal of the SCG, as compared with that seen following decentralization (Fig. 2). These studies were performed on normal rats, and are in general agreement with a previous study on blind rats (Moore and Rapport, 1971). We believe a more rapid decrease in HIOMT activity occurs in rats with decentralized SCG, because the uptake mechanism for catecholamines in presynaptic nerve endings remains unaffected by decentralization, but is absent in SCGX rats. The uptake mechanism prevents circulating catecholamines from stimulating the pinealocytes (Parfitt and Klein, 1976). In addition, after SCGX, but not decentralization, norepinephrine in the degenerating nerve endings is slowly released, and could act transsynaptically to stimulate HIOMT activity (Sugden and Klein, 1983).

In this and other studies we have observed that HIOMT activity does not disappear completely after neural stimulation is blocked for long periods. The HIOMT activity which remains might reflect a low level of stimulation of the pineal gland by circulating catecholamines, by a basal release of norepinephrine from pineal nerve endings, or other agents.

However, we feel it is more important to emphasize that interruption of the neural pathway to the pineal gland caused HIOMT activity to decrease to about 25% of control values (Fig. 2). This supports the conclusion that neural stimulation is the major factor maintaining HIOMT activity in the adult rat.

This conclusion is also supported by our failure to show any effect of adrenalectomy or hypophysectomy on pineal HIOMT activity (Table 2). Sandrock et al. (1980) reported that hypophysectomy reduced pineal HIOMT activity (expressed per gland) and that dexamethasone administration reversed this decline, suggesting that the enzyme was controlled by adrenal glucocorticoids. Our hypophysectomy experiments (Tables 2 and 3) show that this fall in enzyme activity may be a nonspecific effect, due simply to a reduction in the size of the gland, as HIOMT activity was unaltered when expressed per milligram of protein. Furthermore, the lack of effect of adrenalectomy argues against glucocorticoid control. A stimulatory role for gonadal steroids in the control of pineal HIOMT is controversial (Preslock, 1977), and it is not supported by the results of our hypophysectomy experiments. Our inability to show any effect of adrenal or pituitary removal indicates that adrenal and gonadal steroids are not of major importance in the physiological regulation of HIOMT in adult rats.

The kinetic analysis performed on pineal HIOMT in LD, LL, and SCGX rats was done to determine if two forms of the enzyme were present in LD animals, with only one form remaining after prolonged exposure to LL or after chronic SCGX. This possibility was attractive, because an enzyme has been detected in the rat Harderian gland which can *O*-

methylate *N*-acetylserotonin to form melatonin (Vlahakes and Wurtman, 1972). However, unlike pineal HIOMT, this enzyme requires divalent cations and has a much higher apparent K_m for *N*-acetylserotonin (1.2×10^{-3} *M*), suggesting that this indole is not its physiological substrate (Cardinali and Wurtman, 1972). Also, multiple forms of pineal HIOMT have been described (Jackson and Lovenberg, 1971; Cremer-Bartels, 1979).

In the current study we failed to discern any marked differences in the apparent K_m values of HIOMT for either substrate (Table 3; Fig. 5). Accordingly, we believe that the decrease in enzyme activity is not due to shifts in the K_m of the enzyme for either substrate, nor to the selective disappearance or suppression of a distinct molecular species of the enzyme. Rather, the decrease is probably due to a decrease in the number of active molecules of HIOMT (Yang and Neff, 1976).

Finally, the similar characteristics of the changes in pineal HIOMT activity and cyclic GMP responsiveness to norepinephrine, seen when the length of the daily dark period is altered, add further circumstantial evidence to the suggestion that there is a functional relationship between them (Figs. 3 and 4). The correctness of such a speculation awaits more direct testing.

In summary, our experiments indicate that the initial developmental appearance of HIOMT activity is not the result of transsynaptic stimulation of the pineal gland. However, it is clear that in the adult rat transsynaptic stimulation of the gland is the most important specific physiological factor maintaining HIOMT activity at normal levels.

REFERENCES

Axelrod J. and Weissbach H. (1960) Enzymatic *O*-methylation of *N*-acetylserotonin to melatonin. *Science* **131**, 1312.

Axelrod J. and Weissbach H. (1961) Purification and properties of hydroxyindole-*O*-methyltransferase. *J. Biol. Chem.* **236**, 211–213.

Ben-Jonathan N. (1978) Plasma catecholamines in fetal and neonatal rats. *Life Sci.* **23**, 39–44.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Cardinali D. P. (1981) Hormone effects on the pineal gland, in *The Pineal Gland: I. Anatomy and Biochemistry* (Reiter R. J., ed), pp. 243–272. CRC Press, Boca Raton, Florida.

Cardinali D. P. and Wurtman R. J. (1972) Hydroxyindole-*O*-methyltransferase in rat pineal, retina and Harderian gland. *Endocrinology* **91**, 247–252.

Cremer-Bartels G. (1979) The effects of pteridines on multiple forms of hydroxyindole-*O*-methyltransferase (HIOMT) in the retina and the pineal gland. *Prog. Brain Res.* **52**, 231–239.

Faden V. B. and Rodbard D. (1976) Scafit, Scatchard Data Processing, ERRB, NICHHD, NIH, Bethesda, Maryland.

Hakanson R., Lombard Des Gouttes M-N., and Owman Ch. (1967) Activities of tryptophan hydroxylase, DOPA decarboxylase and monoamine oxidase as correlated with the appearance of monoamines in developing rat pineal gland. *Life Sci.* **6**, 2577–2585.

Jackson R. L. and Lovenberg W. (1971) Isolation and characterization of multiple forms of hydroxyindole-*O*-methyltransferase. *J. Biol. Chem.* **246**, 4280–4285.

Johnson E. M., O'Brien F., and Werbitt R. (1976) Modification and characterization of the permanent sympathectomy produced by the administration of guanethidine to newborn rats. *Eur. J. Pharmacol.* **37**, 45–54.

Klein D. C. (1983) Pineal gland: Adrenergic regulation of function, in *Catecholamines II* (Trendelenburg U. and Weiner N., eds), in press. Springer Verlag, New York.

Klein D. C. and Lines S. V. (1969) Pineal hydroxyindole-*O*-methyltransferase activity in the growing rat. *Endocrinology* **84**, 1523–1525.

Klein D. C. and Moore R. Y. (1979) Pineal *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase: Control by the retinohypothalamic tract and the suprachiasmatic nucleus. *Brain Res.* **174**, 245–262.

Klein D. C., Auerbach D. A., and Weller J. L. (1981) Seesaw signal processing in pineal cells: Homologous sensitization of adrenergic stimulation of cyclic GMP accompanies homologous desensitization of β-adrenergic stimulation of cyclic AMP. *Proc. Natl. Acad. Sci. USA* **78**, 4625–4629.

Moore R. Y. (1978) The innervation of the mammalian pineal gland, in *The Pineal Gland and Reproduction* (Reiter R. J., ed), pp. 1–29. Karger, Basel.

Moore R. Y. and Rapport R. L. (1971) Pineal and gonadal function in the rat following cervical sympathectomy. *Neuroendocrinology* **7**, 361–374.

Parfitt A. G. and Klein D. C. (1976) Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in *N*-acetyltransferase (E.C. 2.3.1.5) activity. *Endocrinology* **99**, 840–851.

Parfitt A. G., Weller J. L., Sakai K. K., Marks B. H., and Klein D. C. (1975) Blockade by ouabain or elevated potassium ion concentration of the adrenergic and cyclic 3′,5′-monophosphate-induced stimulation of pineal serotonin *N*-acetyltransferase activity. *Mol. Pharmacol.* **11**, 241–255.

Preslock J. P. (1977) Gonadal steroid regulation of pineal melatonin synthesis. *Life Sci.* **20**, 1299–1304.

Sandrock A. W. Jr., Leblanc G. G., Wong D. L., and Ciaranello R. D. (1980) Regulation of rat pineal hydroxyindole-*O*-methyltransferase: Evidence of *S*-adenosylmethionine-mediated glucocorticoid control. *J. Neurochem.* **35**, 536–543.

Steiner A. L., Parker C. W., and Kipnis D. M. (1972) Radioimmunoassay for cyclic nucleotides. *J. Biol. Chem.* **247**, 1106–1113.

Sugden D. and Klein D. C. (1983) Adrenergic stimulation of rat pineal hydroxyindole-*O*-methyltransferase. *Brain Res.* (in press).

Vlahakes G. J. and Wurtman R. J. (1972) A Mg^{2+}-dependent hydroxyindole-*O*-methyltransferase in rat Harderian gland. *Biochim. Biophys. Acta* **261**, 194–197.

Wurtman R. J., Axelrod J., and Phillips L. S. (1963) Melatonin synthesis in the pineal gland: Control by light. *Science* **142**, 1071–1073.

Yang H-Y. T. and Neff N. H. (1976) Hydroxyindole-*O*-methyltransferase: An immunochemical study of the neuronal regulation of the pineal enzyme. *Mol. Pharmacol.* **12**, 433–439.

Yuwiler A., Klein D. C., Buda M., and Weller J. L. (1977) Adrenergic control of pineal *N*-acetyltransferase activity: Developmental aspects. *Am. J. Physiol.* **233**, E141–E146.