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CONTROL OF 5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN THE RAT PINEAL GLAND BY SYMPATHETIC NERVES[1]

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The mammalian pineal gland is rich in serotonin content and this amine is present in high concentration within the sympathetic nerve endings of this organ (Quay and Halevy, 1962; Bertler *et al.*, 1963). Sympathetic denervation of the pineal gland results in a 50% decrease in its serotonin content (Bertler *et al.*, 1963; Pellegrino de Iraldi *et al.*, 1963). Recently it has been demonstrated that the pineal gland has the highest concentration of 5-hydroxytryptophan decarboxylase, the enzyme which forms serotonin, of all mammalian tissues examined (Snyder and Axelrod, 1964a). An important role of serotonin in the pineal gland is to serve as a precursor for the gonadal inhibiting hormone, melatonin (Axelrod and Weissbach, 1960; Weissbach *et al.*, 1960; Wurtman *et al.*, 1963a). The synthesis of melatonin as well as the concentration of serotonin has been shown to be influenced by environmental lighting (Wurtman *et al.*, 1963b; Quay, 1963).

Ariens-Kappers (1960) has demonstrated that the rat pineal gland is largely or wholly innervated by sympathetic nerves whose cell bodies are located in the superior cervical ganglia. The effects of changes in environmental lighting on the melatonin synthesizing enzyme in the pineal gland have been shown to be mediated by its sympathetic innervation (Wurtman *et al.*, 1964). The experiments described here will demonstrate that the sympathetic nerves to the pineal gland as well as environmental lighting markedly influence the activity of pineal 5-hydroxytryptophan decarboxylase (5-HTPD).

MATERIALS AND METHODS. H^3-norepinephrine (20 mc/mg) was obtained from New England Nuclear Corporation. Bretylium tosylate was kindly supplied by Dr. J. J. Burns of Burroughs Wellcome Company.

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[1] Some of this work has been reported in preliminary communications (Snyder and Axelrod, 1964a; Snyder *et al.*, 1964).

Sprague-Dawley female (160–180 g) rats were kept in diurnal lighting conditions with 12 hours of light (7 A.M. to 7 P.M.) and 12 hours of darkness daily and killed between 10 A.M. and noon except when otherwise specified.

Bilateral or unilateral excision of the superior cervical ganglia was performed under ether anesthesia. The eyelids of rats which had been subjected to bilateral superior cervical ganglionectomy were removed to correct for the possible effects of associated ptosis on the response to light. Celiac ganglia were removed under ether anesthesia. The abdomen was opened through a mid-line incision and the preaortic tissue from the splenic to the superior mesenteric arteries was removed. All tissue between these vessels to a point 1½ cm along the arteries was extirpated. Sham operated rats served as controls. The effectiveness of celiac denervation was established by an experiment similar to that described by Hertting *et al.* (1961). Sham operated animals and denervated animals received 10 μc of H^3-norepinephrine intravenously and were killed 1 hour later. Spleens were removed and assayed for H^3-norepinephrine by specific assay (Whitby *et al.*, 1961) (table 1). There was a marked decrease in the ability of the spleen to take up and retain H^3-norepinephrine indicating almost complete denervation of this tissue and the ileum.

In some experiments, rats were blinded by enucleation performed under light Nembutal anesthesia.

For the assay of 5-HTPD, tissues were quickly removed, chilled and homogenized in 0.05 M phosphate buffer pH 7.4 and the enzyme activity measured as previously described (Snyder and Axelrod, 1964b). Assays were always performed on the day the animals were killed.

In experiments involving exposure to constant darkness or light, rats were placed in rooms with fluorescent lighting with 100 foot-candles intensity at the level of the cages, or in complete darkness except for a 25-watt red light bulb which was used for about 20 minutes when the cages were cleaned. The rooms were equipped with double door light baffles and air conditioning. Animals were killed

TABLE 1

Lack of uptake of H^3-norepinephrine in rat spleen after celiac ganglionectomy

Group	H^3-Norepinephrine
	mμc/g
Sham operated	20.3 ± 4.3
Celiac ganglionectomy	4.0 ± 0.3
P	<.001

Rats which had undergone celiac ganglionectomy received 10 μc H^3-norepinephrine intravenously 1 week later. After 1 hour the H^3-norepinephrine content of the spleens was assayed. Results are expressed as mean ± S.E.M. for groups of 4 animals.

TABLE 2

Effect of superior cervical ganglionectomy on pineal 5-hydroxytryptophan decarboxylase activity

Treatment	Pineal Wt.	5-HTPD Activity
	mg	
Control (19)	0.91 ± 0.04	12.82 ± 1.31
Sham operated (5)	1.22 ± 0.16	13.32 ± 2.45
18 Hours (6)	0.89 ± 0.08	16.72 ± 1.53
40 Hours (6)	0.86 ± 0.13	11.69 ± 2.05
64 Hours (5)	1.19 ± 0.16	18.32 ± 4.19
88 Hours (6)	0.74 ± 0.17	18.70 ± 2.57[a]
1 Week (15)	0.93 ± 0.08	22.42 ± 1.99[b]
1 Month (10)	0.90 ± 0.08	22.45 ± 1.32[b]

Differs from control [a]P < .05; [b]P < .001.

Ganglionectomized rats were killed at varying time intervals after ganglionectomy together with controls, and pineal glands were assayed for 5-HTPD activity. 5-HTPD activity was expressed as mμmoles C^{14}-serotonin formed from C^{14}-5-hydroxytryptophan per mg pineal per hour ± S.E. Numbers in parentheses are the rats used in each experiment.

while still in the light or dark environments between 10 A.M. and noon.

RESULTS. *Effect of bilateral superior cervical ganglionectomy on pineal 5-HTPD.* Groups of rats in diurnal lighting conditions were killed at varying time intervals from 18 hours to 30 days after bilateral superior cervical ganglionectomy, together with sham operated or unoperated controls. Ganglionectomy was performed at intervals so that all animals were killed the same day. Following ganglionectomy there was an increase in 5-HTPD activity after 88 hours (table 2). This elevated level of enzyme activity was maintained for at least 1 month. Sympathectomy did not cause a significant change in pineal or total body weight.

In order to ascertain if an inhibitory factor was present in innervated glands, control and denervated pineal glands were combined and assayed for 5-HTPD activity. Enzyme activity of the combined glands was additive, indicating the absence of an inhibitory factor in the innervated pineal glands.

To examine the effect of sympathetic denervation on 5-HTPD activity in other organs, rats were subjected to unilateral superior cervical ganglionectomy and 5-HTPD activity was measured in submaxillary glands of the control and denervated sides 7 days after the operation. In other rats, the celiac ganglionic plexus was surgically removed and 5-HTPD of the ileum was examined in control and operated rats 7 days later. 5-HTPD activity in both salivary gland and intestinal homogenates was unaffected by sympathetic denervation (table 3).

Effect of constant light and darkness on 5-HTPD decarboxylase activity. Groups of rats were kept in constant darkness or light for 30 days or in diurnal lighting conditions. At the end of this period, animals were killed, and their pineal glands were examined for 5-HTPD activity. There was a 2-fold difference in 5-HTPD activity in rats kept in constant light compared to rats in constant darkness (table 4). 5-HTPD activity of rats in diurnal lighting conditions was intermediate between that of rats in constant darkness

TABLE 3

Effect of sympathetic denervation on salivary and intestinal 5-hydroxytryptophan decarboxylase activity

	5-HTPD Activity	
	Submaxillary gland	Ileum
Innervated	2.31 ± 0.21	1170 ± 220
Denervated	2.68 ± 0.19	938 ± 140

Groups of 6 rats, subjected to unilateral superior cervical ganglionectomy or to celiac ganglionectomy, were killed 7 days later and tissues assayed for 5-HTPD activity.

5-HTPD was expressed as mμmoles of serotonin formed per gram tissue per hour ± S.E.M.

TABLE 4

Effect of constant light or darkness on pineal 5-hydroxytryptophan decarboxylase activity

Group	Pineal Wt. (mg)	5-HTPD Activity per Pineal	5-HTPD Activity per mg Pineal
Constant light	0.96 ± 0.07	17.7 ± 1.2	18.4 ± 1.0
Diurnal	1.15 ± 0.09	15.4 ± 1.1	13.4 ± 1.5[a]
Constant darkness	1.22 ± 0.08[a]	12.6 ± 1.3[a]	10.3 ± 1.1[b]

Differs from constant light [a]P < .02; [b]P < .001.

Rats were placed in constant light or dark environments or in diurnal lighting conditions. Rats were killed 30 days later and pineal glands were assayed for 5-HTPD activity. Each group contained 20 rats. Enzyme activity was expressed as mμmoles of serotonin formed per hour from 5-hydroxytryptophan ± S.E.M.

or light. In confirmation of previous reports (Fiske *et al.*, 1960; Wurtman *et al.*, 1963b) constant light also resulted in a significant decrease in pineal gland weight. The increase in 5-HTPD activity of pineal glands of rats in constant light was statistically significant whether expressed per mg weight or per whole pineal gland. There was no significant difference in total body weight of rats exposed to constant light, constant darkness or diurnal lighting conditions.

Mediation by the sympathetic nervous system of the effects of light on pineal 5-HTPD activity. Previous work in this laboratory indicated that the pathway by which light affects hydroxyindole-O-methyl transferase activity in the pineal gland involves the eyes and the superior cervical ganglia (Wurtman *et al.*, 1964). Accordingly, groups of rats were placed in continuous light or darkness 1 day after bilateral orbital enucleation or bilateral superior cervical ganglionectomy and their pineal glands examined for 5-HTPD activity. Animals were kept in the light or dark environments for 30 days along with unoperated control animals. Both blinding and superior cervical ganglionectomy prevented the rise in pineal 5-HTPD of rats in constant light (table 5). In blinded and ganglionectomized rats, there was no difference in pineal gland weight between animals kept in constant light or darkness.

The effect of bretylium and guanethidine on the response of pineal 5-HTPD to constant light. The effect of drugs which interfere with sympathetic nerve impulses on the response of pineal 5-HTPD to constant light was examined in the following experiment. Groups of rats were placed in continuous light or dark environments 2 days after beginning daily intraperitoneal injections of

TABLE 5

Effect of ganglionectomy and blinding on the response of 5-hydroxytryptophan decarboxylase activity in the rat pineal gland to constant light or darkness

Group	Pineal Wt. (mg)	5-HTPD Activity per Pineal	5-HTPD Activity per mg Pineal
Sham operated			
Dark	1.26 ± 0.08	12.6 ± 1.5	10.1 ± 1.6
Light	1.02 ± 0.08[a]	18.8 ± 1.6[b]	18.4 ± 2.0[c]
Ganglionectomy			
Dark	1.04 ± 0.09	12.4 ± 1.6	11.8 ± 1.2
Light	1.15 ± 0.07	15.4 ± 1.5	13.7 ± 1.5
Blinded			
Dark	1.14 ± 0.09	11.7 ± 1.8	9.9 ± 1.3
Light	1.28 ± 0.06	13.1 ± 1.3	9.2 ± 1.2

Differs from dark value [a]P < .05; [b]P < .02; [c]P < .01.

Bilaterally superior cervical ganglionectomized rats and blinded rats were placed in constant light or darkness together with sham operated controls. Each group contained 8 to 10 rats. Rats were killed after 30 days, pineal glands removed and assayed for 5-HTPD activity. 5-HTPD activity is expressed as mμmoles serotonin formed per hour ± S.E.M.

bretylium 20 mg/kg or guanethidine 20 mg/kg. Drug treatment was continued for 30 days in the light or dark environments. Bretylium blocked the elevation of 5-HTPD in pineal glands of rats in constant light but guanethidine had no effect (table 6). Both drugs, however, prevented the decrease in pineal weight normally induced by exposure to constant light.

The possibility of the presence of an inhibitory or stimulatory factor produced by environmental light was examined by combining pineal glands of rats exposed to constant light or dark for 30 days and measuring 5-HTPD activity of the combined pineal glands. Under these conditions, enzyme activity was additive indicating the absence of an inhibitory or stimulatory factor.

DISCUSSION. The results described here clearly demonstrate that the activity of 5-hydroxytryptophan decarboxylase in the pineal gland is regulated in part by the sympathetic nerves to this organ.

Since the enzyme is not specific and can decarboxylate other aromatic amino acids, such as dihydroxyphenylalanine, histidine, tyrosine, and phenylalanine (Lovenberg *et al.*, 1962) it is possible that the sympathetic nerves to the pineal gland may be affecting the formation of other biogenic amines besides serotonin.

Sympathetic denervation of the pineal gland

TABLE 6

Effect of bretylium and guanethidine on the response of rat pineal 5-hydroxytryptophan decarboxylase to constant light or darkness

Treatment	Pineal Wt. (mg)	5-HTPD Activity per Pineal	5-HTPD Activity per mg Pineal
Control			
Dark	1.30 ± 0.08	13.7 ± 1.4	10.5 ± 1.1
Light	0.98 ± 0.05[b]	19.4 ± 1.5[b]	19.7 ± 1.4[c]
Bretylium			
Dark	1.35 ± 0.19	16.9 ± 3.3	11.8 ± 1.7
Light	1.18 ± 0.11	17.3 ± 3.0	14.2 ± 2.1
Guanethidine			
Dark	1.27 ± 0.10	13.0 ± 2.4	9.5 ± 2.2
Light	1.24 ± 0.07	26.0 ± 4.0[a]	19.8 ± 1.9[b]

Differs from dark value [a]$P < .02$; [b]$P < .01$; [c]$P < .001$.

Rats were placed in constant light or dark environments 2 days after beginning daily intraperitoneal injections of bretylium 20 mg/kg or guanethidine 20 mg/kg along with controls which were injected with saline. Drug treatment was continued for 30 days in the experimental lighting conditions. Rats were then killed and pineal glands assayed for 5-HTPD activity. Each group contained 20 rats. 5-HTPD activity was expressed as mμmoles serotonin formed per hour ± S.E.M.

almost doubles this enzymatic activity within 4 days under diurnal lighting conditions. This observation was reported in a preliminary communication (Snyder and Axelrod, 1964a) and was subsequently confirmed in another laboratory (Pellegrino de Iraldi and Arnaiz, 1964). Innervated pineal glands do not appear to contain an inhibitor of enzyme activity which is lost upon denervation. However, the increase in enzyme activity may result from the removal of factors present in the sympathetic nerves which might normally inhibit the synthesis of the enzyme. The inhibitor could be serotonin, which is highly localized in the sympathetic nerves and whose concentration decreases after denervation, or perhaps other compounds present in these nerves.

In rats exposed to constant light there is a doubling in pineal 5-HTPD activity. Quay and Halevy (1962) have shown that the serotonin content of the pineal gland of rats exposed to constant light is reduced by about 50%. These data, as well as the increase in pineal 5-HTPD after superior cervical ganglionectomy, suggest an inverse relationship between serotonin content and 5-HTPD activity.

In diurnal lighting conditions, ganglionectomy causes a rise in 5-HTPD activity (table 2) whereas in constant light it does not (table 5). Ganglionectomy appears to act on pineal 5-HTPD of rats in constant light by preventing the elevating effects of constant light on this enzyme. Other results have also implicated a discrepancy between the effects of constant light exposure and diurnal lighting conditions. Quay (1963) has shown the presence of a circadian rhythm in pineal serotonin content of rats under diurnal lighting conditions with a peak of 90 nanograms per gland at noon and a trough of 10 nanograms per gland at midnight. Constant light exposure, however, results in a 50% decrease in pineal serotonin content (Quay and Halevy, 1962). Experiments are underway in this laboratory to examine the possibility of a diurnal variation in pineal 5-hydroxytryptophan decarboxylase activity.

Previous work in this laboratory (Wurtman *et al.*, 1963b, 1964) has shown that exposure to constant light markedly reduces the activity of the melatonin forming enzyme, hydroxyindole-O-methyl transferase, in the rat pineal gland, and that the effects of light on this enzyme are mediated *via* the eyes and the sympathetic nervous system. In contrast to the melatonin forming enzyme, constant light exposure increases rat pineal 5-HTPD activity. Blinding and sympathetic denervation eliminate the effects of environmental lighting on pineal 5-HTPD. These results demonstrate that the pathway by which light affects pineal 5-HTPD involves the eyes and the sympathetic nervous system. It is possible that the sympathetic nerves influence pineal 5-HTPD by the release of a neurohumor. Such a mechanism is supported by the observation that bretylium, a drug which blocks the release of norepinephrine from sympathetic nerve endings (Boura and Green, 1959), also prevents the light induced increase in pineal 5-HTPD. Guanethidine, a compound which, in addition to blocking sympathetic nerve impulses, also depletes catecholamines from peripheral tissues (Cass *et al.*, 1960), had no effect on the light induced increase in pineal 5-HTPD.

Exposure to constant light has been shown to reduce pineal gland weight (Fiske *et al.*, 1960), and blinding and superior cervical ganglionectomy have been shown to prevent the action of light on pineal weight (Wurtman *et al.*, 1964). Bretylium and guanethidine also block the effect of light on pineal weight. It would be highly unlikely that pineal serotonin and melatonin content and the enzymes involved in their metabolism would influence pineal weight to any

measurable degree. The fact that drugs which affect the sympathetic nervous system also affect pineal weight suggests that the sympathetic innervation of the pineal gland may influence many other biochemical events in this organ.

SUMMARY

Sympathetic denervation of the rat pineal gland causes an elevation of 5-hydroxytryptophan decarboxylase activity in this gland. The pineal glands of rats kept in constant light environments have about twice as much 5-hydroxytryptophan decarboxylase activity as the pineal glands of litter-mates kept in constant darkness. Blinding or sympathetic denervation prevents the elevating effect of constant light exposure on this enzymatic activity. Bretylium, but not guanethidine blocks the increase in 5-hydroxytryptophan decarboxylase activity in pineals of rats kept in constant light. Exposure of rats to continuous light causes a reduction in pineal weight. Bretylium and guanethidine block this reduction in weight.

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