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*Short Communication*

# Stimulation of β-Adrenergic Receptors in the Pineal Gland Increases the Noradrenaline Stores of Its Sympathetic Nerves

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*Summary.* The administration of isoproterenol decreases the level of serotonin in the rat pineal gland and at the same time it increases pineal noradrenaline. These effects depend on the stimulation of a β-adrenergic receptor because they are blocked by pretreatment of the animals with propranolol; this drug by itself does not modify either serotonin or noradrenaline levels in the pineal. The elevation of noradrenaline produced by isoproterenol is selective for the pineal because it is not observed in the salivary gland innervated by postganglionic adrenergic fibers from the same origin as pineal nerves. Pineal serotonin is stored in equilibrium in two compartments, i.e., the parenchymal cells and the adrenergic nerves and thus is most probably reduced in both sites. Since noradrenaline and serotonin are detected in pineal nerve vesicles and may coexist in them, the diminution of intravesicular serotonin, by making more storage sites available, probably determines the selective increase of pineal noradrenaline. A similar modification in the ratio of intravesicular amines as a result of the physiological stimulation of pineal β-adrenergic receptors by the adrenergic neurotransmitter may explain some of the changes observed in the content of pineal amines.

*Key words:* Noradrenaline — Serotonin — Pineal Gland — Amine Storage.

The indole metabolism in the pineal is controlled by noradrenaline released from the postganglionic sympathetic fibers innervating the gland. The circadian variations in neurotransmitter release appear to be responsible for the 24 hr rhythm of indole metabolism in the pineal (ref. see Axelrod, 1974; Klein, 1974). However, the content of noradrenaline in the pineal increases during the night when it is presumed to be released and when its turnover rate is greatly accelerated, while pineal noradrenaline diminishes at the end of the light period when its turnover is the lowest (Wurtman and Axelrod, 1966; Brownstein and Axelrod, 1974). We thought that this puzzling situation might be explained by

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the particular mechanism of noradrenaline storage in pineal adrenergic nerves.

The pineal gland of the rat contains large amounts of serotonin which is stored both in the parenchymal cells and in the nerve fibers innervating the gland. Although these postganglionic sympathetic nerves contain noradrenaline, they have the capacity to incorporate the serotonin synthesized in the pinealocytes (Pellegrino de Iraldi *et al.*, 1963; Owman, 1964; Neff *et al.*, 1969). Cytochemical observations, subcellular distribution studies and pharmacological experiments indicate that noradrenaline and serotonin share common storage sites in the vesicles of pineal adrenergic nerves (Jaim-Etcheverry and Zieher, 1971, 1973, 1974). For example, the pharmacological depletion of neuronal serotonin produces a marked and selective rise in pineal noradrenaline (Jaim-Etcheverry and Zieher, 1971) while the depletion of noradrenaline increases neuronal serotonin (Zweig and Axelrod, 1969).

The serotonin content of the pineal is controlled by $\beta$-adrenergic receptors of the pinealocytes (Klein *et al.*, 1973; Brownstein *et al.*, 1973). During the night, when sympathetic activity increases, noradrenaline is released from the nerves and these receptors are activated. This enhances the activity of the enzyme serotonin-N-acetyltransferase and consequently the concentration of serotonin decreases due to an acceleration of its conversion to N-acetylserotonin (Axelrod, 1974; Brownstein *et al.*, 1973; Klein *et al.*, 1973).

Since parenchymal and neuronal serotonin are in equilibrium (Neff *et al.*, 1969), the depletion of total pineal serotonin produced by the activation of postsynaptic $\beta$-adrenergic receptors during the night most probably affects the content of serotonin of the nerve fibers as well. In this case, and in accordance with the proposed coexistence of amines in pineal nerve vesicles, the noradrenaline content of the gland should be expected to increase as a result of the enhanced availability of vesicular storage sites.

With the aim of demonstrating that this mechanism of amine coexistence responds to physiological changes, the activation of the $\beta$-adrenergic receptors which normally takes place at night, was mimicked by the injection of isoproterenol during the day. The content of endogenous amines was determined in the gland under these experimental conditions.

## Methods

Female Wistar rats of 150—200 g were kept under diurnal lighting with the lights on from 07.30 hr to 19.30 hr for at least a week before the experiments. Drugs were dissolved in saline and injected subcutaneously; dosages of drugs refer to salts. (±)-Isoproterenol HCl (Sigma Chemical Co.) was injected at 07.25 hrs

(15 mg/kg body weight), 11.00 hr (10 mg/kg) and 13.30 hr (10 mg/kg) and the animals were were killed by decapitation at 15.30 hr. In other groups, (±)-propranolol HCl (Ayerst Laboratories) was injected in a dose of 30 mg/kg at 07.00 hr, 10.30 hr and 13.00 hr either alone or before the injection of isoproterenol. The drugs administered before 07.30 hr were injected in the dark with the aid of a dim red light. Control rats were injected with saline.

Pineals from 6–8 groups of rats for each treatment schedule (each group consisting of 6–8 rats) were homogenized in 10 ml of ice-cold 0.4 N perchloric acid containing $Na_2S_2O_5$ and EDTA (Atack, 1973). From the supernatant of the centrifuged homogenate, noradrenaline and serotonin were separated by column chromatography according to the procedure of Atack and Magnusson (1970). Mean recoveries were of 95% for noradrenaline and 70% for serotonin; experimental values were not corrected for recovery. On the corresponding eluates, the concentration of noradrenaline and serotonin was assayed fluorometrically according to the procedures of Häggendal (1963) and Magnusson (1973) respectively. Thus, both amines were determined in the same pineal extract. The noradrenaline content of the salivary glands from control and treated rats (6–8 experiments for each group) was also determined. The significance of differences between values obtained was analyzed with Student's *t*-test.

## Results

As shown in Fig. 1, injections of isoproterenol during the day produced a marked depletion of pineal serotonin. This is known to be the result of an increase in N-acetyltransferase activity (Brownstein *et al.*, 1973; Klein *et al.*, 1973). Propranolol by itself did not modify the content of serotonin in the pineal. This β-adrenergic blocker prevents the nocturnal rise in the activity of N-acetyltransferase and the corresponding decrease in serotonin (ref. Axelrod, 1974). Thus, its failure to affect the diurnal content of serotonin indicates that the sympathetic tone is maintained at subthreshold levels during daytime. However, administration of propranolol prevented the fall of serotonin induced by isoproterenol, confirming that β-adrenergic receptors mediate this effect as has been previously demonstrated (Brownstein *et al.*, 1973; Klein *et al.*, 1973). On the other hand, noradrenaline levels increase in the gland following the injection of isoproterenol as seen in Fig. 1 and this increase was prevented by propranolol given before the β-adrenergic agonist. Also in this case the administration of propranolol alone did not change the pineal noradrenaline content. The increase in noradrenaline produced by isoproterenol injection was selective for the pineal gland since it was not observed in other structures innervated by postganglionic fibers from the same origin such as the salivary glands. In these organs the content of noradrenaline was not significantly modified by any of the different treatments. The mean content of noradrenaline in the salivary glands expressed as nmoles/g weight ± SEM was: controls 9.25 ± 0.76, isoproterenol 9.18 ± 0.86, propranolol 7.92 ± 1.27 and propranolol plus isoproterenol 8.01 ± 0.66.

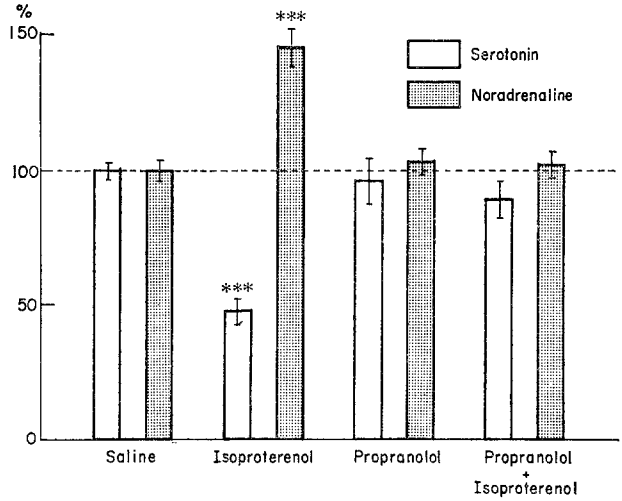


Fig. 1. Changes in the content of serotonin and noradrenaline in the pineal gland of rats injected with isoproterenol, propranolol and propranolol plus isoproterenol. Absolute values for serotonin in control glands were 329 $\pm$ 9.62 pmoles/pineal and for noradrenaline 26.07 $\pm$ 1.10 pmoles/pineal. These are mean values $\pm$ SEM of 6—8 experiments for each treatment. The mean weight of the pineal gland in the saline treated group was 1.25 $\pm$ 0.02 mg and was not modified significantly by the drugs used. *** $P < 0.001$ when compared with control values. The values in the group treated with propranolol plus isoproterenol differ significantly ($P < 0.001$) from those in the group treated with isoproterenol alone

## Discussion

Isoproterenol does not affect directly the amines stored in adrenergic nerve endings because it is not taken up by them (Hertting, 1964). Thus, the stimulation of the postsynaptic $\beta$-adrenergic receptor in pineal cells is the event which triggers the fall in pineal serotonin and the selective rise in pineal noradrenaline. The participation of such receptors was further confirmed by the blockade of both actions by pretreatment with propranolol. These findings suggest that not only parenchymal but also neuronal serotonin is depleted because there was a similar selective increase of pineal noradrenaline when the intraneuronal concentration of serotonin was pharmacologically depleted (Jaim-Etcheverry and Zieher, 1971). Such an elevation of pineal noradrenaline levels has been interpreted as a consequence of an increased availability of intravesicular storages sites. Probably these are normally saturated in the pineal gland because the amine must be present in very high concentrations around the endings and inside the vesicles in order to be detected

cytochemically inside the vesicles of adrenergic nerves (Zieher and Jaim-Etcheverry, 1971).

The decrease in the absolute content of pineal serotonin produced by isoproterenol injection is very pronounced in comparison with the relatively slight absolute increase of noradrenaline stores. It is known that under normal conditions approximately only 30% of pineal serotonin is localized in adrenergic nerves (Neff *et al.*, 1969; Tilders *et al.*, 1974). Even if it is assumed that following isoproterenol both compartments of serotonin are depleted to the same extent, the difference, although smaller, still persists. Thus, other factors should be considered to explain this discrepancy. One of them is the time course of noradrenaline elevation. Previous studies have shown that the increase in noradrenaline lags behind serotonin depletion, i.e., even if the decrease of serotonin is rapid and marked, the highest increase of noradrenaline is observed only after several hours (Jaim-Etcheverry and Zieher, 1971). Moreover, not only noradrenaline but other amines also present in pineal nerves may increase in their vesicles when storage sites become available after serotonin depletion. This is what occurs with octopamine which increases in the pineal about four fold following neuronal serotonin depletion while in the salivary gland the concentration of octopamine does not change under the same conditions (Jaim-Etcheverry and Zieher, 1975).

Several studies have shown that during the night pineal serotonin decreases while the noradrenaline content of the gland increases (Quay, 1963; Snyder *et al.*, 1965; Illnerova, 1971; Brownstein and Axelrod, 1974). During nighttime the increased discharge of noradrenaline from the nerves (Brownstein and Axelrod, 1974) produces an enhanced synthesis of the N-acetylating enzyme (Klein *et al.*, 1971; Deguchi and Axelrod, 1972). In view of the results reported, the released neurotransmitter most probably decreases the content of serotonin not only in the pinealocytes but also in adrenergic nerves. Thus, since vesicular storage sites in sympathetic nerves seem to be shared in the pineal by noradrenaline and serotonin during the day, additional storage sites for noradrenaline are probably made available by the reduction of neuronal serotonin due to stimulation of pineal postsynaptic $\beta$-adrenergic receptors by noradrenaline physiologically released at night. This situation was mimicked in our experiments by the injection of isoproterenol.

The ratio between serotonin and noradrenaline within the vesicles of pineal adrenergic nerves seems to be regulated in part by the biochemical reactions in the parenchymal cells which are in turn controlled by a $\beta$-adrenergic-cyclic AMP mechanism (ref. Axelrod, 1974; Klein, 1974). The reciprocal storage relationship between noradrenaline and serotonin may participate in the modulation of neurotransmitter action and provide the basis for a feedback mechanism between sympathetic

nerves and pineal parenchymal cells. For example, it could be possible that when serotonin increases in the pinealocytes at the end of the dark period as a result of changes in noradrenaline turnover as well as in the responsiveness of postsynpatic receptors (Axelrod, 1974), serotonin may enter in nerve vesicles thus contributing to modify the net effect of nerve stimulation.

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