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INCREASE IN RAT PINEAL MELATONIN CONTENT FOLLOWING L-DOPA ADMINISTRATION

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Summary

L-Dopa in saline suspension, administered subcutaneously to rats, caused a 3 to 6-fold increase in pineal melatonin content. Injection of the saline vehicle alone resulted in a modest (i.e., 30-50%) increase in melatonin. The effect of L-dopa on pineal melatonin content is potentiated in animals whose postganglionic sympathetic neurons have been damaged by pretreatment with 6-hydroxydopamine.

Introduction

The mammalian pineal, a neuroendocrine transducer (1), responds to its postganglionic sympathetic input by synthesizing and presumably secreting the hormone, melatonin (2). Norepinephrine released from these nerve terminals (3) acts via β-receptors (4) to activate an adenylate cyclase (5); the consequent increase in pineal cyclic 3',5'-AMP content apparently mediates the enhancement of melatonin biosynthesis (6). Cyclic changes in ambient illumination act via retinal photoreceptors (7,8,9) to produce corresponding alterations in the sympathetic tone to the pineal, and thus modulate the rate of melatonin biosynthesis; light inhibits and darkness enhances this rate.

The sympathetic input to the rat pineal can be experimentally modified by a variety of surgical, chemical, and environmental

manipulations. For example, the postganglionic neurons can be damaged by superior cervical ganglionectomy (2) or treatment with 6-hydroxydopamine (10); their preganglionic inputs can be interrupted by surgical decentralization (11) or their tonic activity can be supressed by exposing animals to light (12).

Deguchi and Axelrod have recently shown that L-dopa administration causes marked increases in the activity of the pineal enzyme serotonin N-acetyltransferase, and that this effect is blocked by concurrent treatment with β-adrenergic blocking agents or by drugs that interfere with decarboxylation of the L-dopa (13). These observations suggest that the enzyme response results from the stimulation by a decarboxylation product of dopa, presumably a catecholamine, of receptors on pineal parenchymal cells. Pineal sympathetic denervation potentiates the enzyme response to L-dopa treatment; this suggests that dopamine formed intravascularly, rather than norepinephrine, may mediate the L-dopa effects.

The present study shows that L-dopa administration also causes profound increases in pineal melatonin content and, presumably, melatonin biosynthesis. This response is also potentiated by sympathetic denervation of the pineal.

Materials and Methods

Male Sprague-Dawley rats weighing 160-180 g (Charles River Laboratories, Wilmington, Mass.) were maintained under diurnal lighting conditions (lights on from 6 a.m. to 6 p.m.) for at least one week prior to use. Illumination, measuring 30 footcandles at the level of the cages, was supplied by "Vita-Lite" fluorescent tubes (Duro-Test Manufacturing Co., North Bergen, N. J.). Big Red Laboratory Animal Diet and water were available *ad libitum*.

A partial chemical sympathectomy was performed by use of a series of four intravenous (tail vein) injections of 6-hydroxydopamine (6-OH-DA) (14) dissolved in 0.001 N HCl. The first two injections, spaced 24 hours apart, each provided 34 mg/kg of the 6-OH-DA (corresponding to 2×50 mg/kg of 6-OH-DA-hydrobromide); the last two injections, 8 and 9 days after the first, each provided 68 mg/kg. The pineal melatonin response to L-dopa treatment was measured 5 to 7 days after the last dose of 6-OH-DA.

L-Dopa (L-3,4-dihydroxyphenylalanine) suspended in saline was injected subcutaneously in a dose of 300 mg/kg. Control animals received only saline or no injections. Animals were subjected to the various treatments and killed by decapitation during the light phase of the daily lighting cycle.

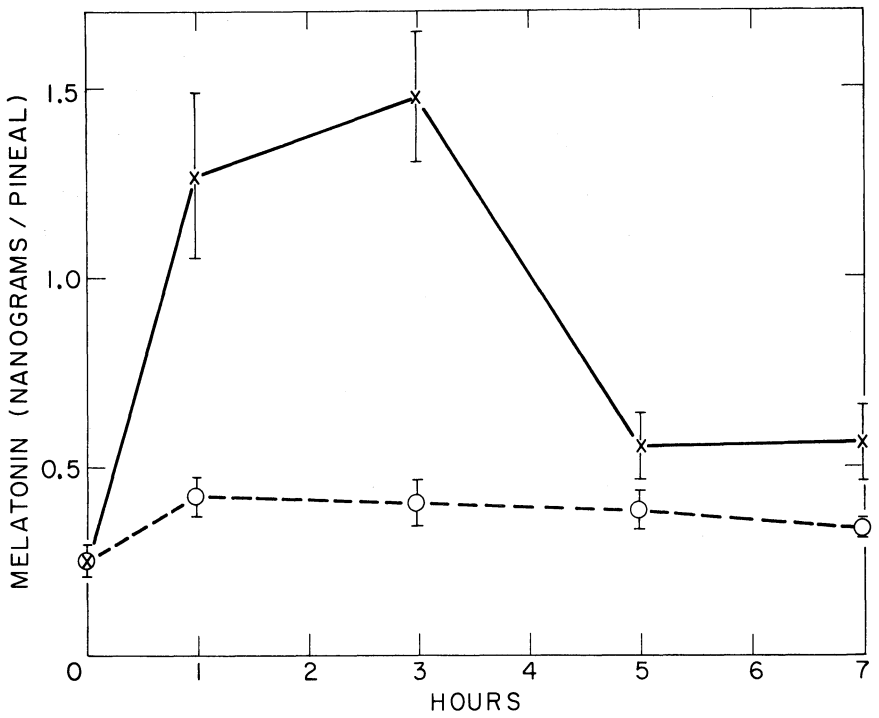
Pineal melatonin content was measured by bioassay (15). Pineal organs were homogenized in deionized water. Light-adapted Rana pipiens larvae (tadpoles that have the melanin granules maximally dispersed in their dermal melanophores) were placed in the dilute homogenates. The melatonin content of the pineal was then estimated by comparing the extent of melanin reaggregation that resulted in these animals with that observed in tadpoles similarly exposed to known concentrations of authentic melatonin.

Results

Animals injected at 8 a.m. (2 hr after the onset of light) with L-dopa or its vehicle were killed in groups of eight after 1, 3, 5, or 7 hr. L-Dopa treatment caused an abrupt increase in pineal melatonin content; maximum values (approximately 6 times those observed at the start of the experiment) were present after 3 hr (Fig. 1). Thereafter, melatonin content fell abruptly, but was still significantly elevated after 7 hr. The subcutaneous

administration of saline alone elicited a small, but statistically significant, increase in pineal melatonin content, that was maximal after 1 hr (Fig 1).

FIG. 1



Time-course of pineal melatonin response to L-dopa. Vertical bars indicate standard errors of the mean. The solid line represents pineal melatonin content of rats killed at intervals after subcutaneous injection of L-dopa (300 mg/kg of body weight) in saline suspension. The dotted line represents pineal melatonin content of rats receiving only the vehicle.

To determine whether prior sympathetic denervation modified the increase in pineal melatonin following L-dopa, groups of intact and 6-OH-DA sympathectomized rats were killed 3 hr after receiving L-dopa, its vehicle, or no treatment. Basal melatonin levels were slightly, but not significantly, higher in the pineals of 6-OH-DA-treated rats. After 3 hr L-dopa treatment caused a

3-fold increase in pineal melatonin content in intact animals, and almost a 6-fold increase in the melatonin content of denervated pineals. Three hours after saline administration, pineal melatonin levels were not significantly elevated in either group of animals (table 1).

TABLE 1

Melatonin contents of pineals from intact and chemically sympathectomized rats. All animals were killed at the same time of day, three hours following the subcutaneous administration of L-dopa (300 mg/kg of body weight in saline supension) or its vehicle.

Treatment	Intact Rats (ng/pineal ± S.E.M.)	Denervated Rats (ng/pineal ± S.E.M.)
L-Dopa	1.02 ± 0.20 (7)[a]	2.12 ± 0.18 (7)[a,b]
0.9% NaCl	0.22 ± 0.04 (7)	0.33 ± 0.05 (6)
No Injection	0.26 ± 0.03 (6)	0.36 ± 0.07 (6)

Figure in parentheses indicates the number of animals in that experimental group.

[a] $P < 0.005$ differs from animals not treated with L-dopa.

[b] $P < 0.005$ differs from L-dopa treated intact animals.

Discussion

These observations demonstrate that L-dopa administration, which has previously been shown to increase pineal serotonin-N-acetyltransferase activity (13), also causes a marked rise in pineal melatonin content. This effect may not be mediated solely by the norepinephrine released from pineal sympathetic nerve terminals, inasmuch as it is actually potentiated when these terminals have been destroyed. The effects observed probably also

involve dopamine formed intravascularly from the exogenous L-dopa.

The addition of dopamine to pineals in organ culture accelerates the synthesis of [^{14}C]melatonin from [^{14}C]tryptophan; L-dopa itself does not (3). Since L-dopa is known to be decarboxylated to dopamine within many cells, including those of the capillary endothelium (16), our data are most easily explained by assuming that dopamine formed from the exogenous L-dopa acted on receptors in pineal parenchymal cells to accelerate melatonin biosynthesis. It is also possible, of course, that 6-OH-DA and L-dopa treatment might have modified pineal melatonin content by altering the release of the hormone, or that some of the effects observed are attributable to blood-borne catecholamines of adrenal medullary origin. Studies are in progress to test these hypotheses.

Exogenous melatonin has been reported to exert beneficial effects on patients suffering from Parkinson's disease (17). Our data raise the possibility that one mechanism through which L-dopa might modify brain function is by altering the rate of pineal melatonin biosynthesis.

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