

STIMULATION OF [¹⁴C] TRYPTOPHAN 5-HYDROXYLATION
BY NOREPINEPHRINE AND DIBUTYRYL ADENOSINE
3', 5' MONOPHOSPHATE IN RAT PINEAL ORGAN CULTURES

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Abstract. Both norepinephrine and dibutyryl adenosine 3', 5' monophosphate (DAMP) accelerate the synthesis of [¹⁴C] serotonin from [¹⁴C] tryptophan by cultured rat pineals. When pineals are incubated with [¹⁴C] 5-hydroxytryptophan instead of [¹⁴C] tryptophan, neither norepinephrine nor DAMP influences the rate of [¹⁴C] serotonin synthesis. These data indicate that one locus at which norepinephrine and DAMP influence the biosynthesis of pineal indoles is the enzymatic hydroxylation of [¹⁴C] tryptophan. Since the enzymes which catalyze serotonin biosynthesis in brain and pineal appear to be similar, our data raise the possibility that noradrenergic neurons in brain which make functional synaptic contact with serotonergic neurons might stimulate serotonin synthesis in these neurons.

In the rat pineal grown in organ culture, the synthesis of serotonin and melatonin from tryptophan proceeds by the same steps as in the innervated pineal gland (1): tryptophan → 5-hydroxytryptophan → serotonin → N-acetylserotonin → melatonin. Serotonin is metabolized in the pineal cultures, as in vivo, by deamination and oxidation to form 5-hydroxy-indoleacetic acid. The rate-limiting step for serotonin biosynthesis in pineal cultures (2), as in intact brain (3), appears to be the formation of 5-hydroxytryptophan (2). The enzymes which catalyze serotonin biosynthesis

in pineal cultures remain active for at least two days after explantation (1, 2) and appear to be identical with the corresponding tryptophan hydroxylase and aromatic l-amino acid decarboxylase in the brain (4, 5). Addition of either norepinephrine or dibutyryl adenosine 3', 5' monophosphate (DAMP) to the nutrient medium of pineal cultures stimulates the synthesis of both [^{14}C] serotonin and [^{14}C] melatonin from [^{14}C] tryptophan (6-9). The acceleration of [^{14}C] melatonin synthesis by norepinephrine or DAMP is due in part to the stimulation of the enzymatic N-acetylation of [^{14}C] serotonin (9). Norepinephrine released from pineal sympathetic nerve endings in vivo has been shown to control the activity of the pineal enzyme hydroxyindole O-methyltransferase (HIOMT), which converts N-acetylserotonin to melatonin (10).

In contrast to these findings that clarify the mechanisms by which noradrenaline and DAMP stimulate pineal [^{14}C] melatonin biosynthesis, the mechanisms for the stimulation of [^{14}C] serotonin synthesis remain undetermined. This report will show that both norepinephrine and DAMP stimulate pineal [^{14}C] serotonin synthesis by enhancing the enzymatic 5-hydroxylation of [^{14}C] tryptophan.

Materials and Methods

Pineal glands were removed between 11 a. m. and 1 p. m. from adult female Sprague-Dawley rats previously housed under diurnal (14 hours light, 10 hours dark) lighting conditions. Each pineal was clotted individually to the walls of a Wasserman tube, and 0.5 ml of nutrient medium (2) was added. The nutrient medium contained [^{14}C] dl-tryptophan or [^{14}C] 5-hydroxytryptophan (0.5mCi in a 10^{-4}M solution) and, where indicated, 1-norepinephrine or DAMP. The cultures were sealed with a rubber stopper and incubated on a roller wheel

at 37°C for 48 hours. Each treatment group contained ten individual pineal gland cultures. Control groups contained the complete incubation mixtures but no pineal glands. At the end of the incubation period, the nutrient media were assayed individually for [^{14}C] serotonin as previously described (1).

Results and Discussion

To determine whether DAMP or norepinephrine stimulates [^{14}C] serotonin synthesis by influencing the rate of hydroxylation, we examined their effect on the conversion of [^{14}C] 5-hydroxytryptophan, the hydroxylated derivative of tryptophan, to [^{14}C] serotonin. The addition of DAMP ($3 \times 10^{-3}\text{M}$) or norepinephrine ($3 \times 10^{-4}\text{M}$) to pineal cultures incubated with [^{14}C] tryptophan resulted in a highly significant ($p < 0.001$) increase in [^{14}C] serotonin synthesis (Table 1); this increase ranged in different experiments from 50 to 150 percent. By contrast, in parallel cultures using [^{14}C] 5-hydroxytryptophan instead of [^{14}C] tryptophan as a substrate neither DAMP nor norepinephrine increased [^{14}C] serotonin synthesis (Table 1).

These data indicate that both norepinephrine and DAMP lose their capacity to stimulate [^{14}C] serotonin synthesis when the 5-hydroxylation of [^{14}C] tryptophan is experimentally "by-passed" (i. e. by substitution of [^{14}C] hydroxytryptophan for [^{14}C] tryptophan as the labeled substrate). Accordingly, the mechanism whereby norepinephrine and DAMP increase pineal [^{14}C] serotonin synthesis from its amino acid precursor must involve actions either on tryptophan hydroxylation, per se, or on an earlier step in the pathway, i. e. the uptake of the amino acid into pineal parenchymal cells. We have previously observed that DAMP has no effect on the steady-state labeling of pineal tryptophan (8); hence the acceleration of [^{14}C] tryptophan hydroxylation by DAMP does not appear to be a pool effect. The steady-state labeling

TABLE 1

The effect of norepinephrine and dibutyryl adenosine 3', 5' mono-phosphate (DAMP) on pineal [¹⁴C] serotonin synthesis when pineals are incubated with either [¹⁴C] 5-hydroxy-tryptophan or [¹⁴C] tryptophan

Labeled Substrate		Norepinephrine Concentration	DAMP Concentration	[¹⁴ C] Serotonin
[¹⁴ C] Tryptophan	[¹⁴ C] 5-hydroxy- tryptophan	(M)	(M)	(c. p. m.)
+	0	0	0	5847 _± 437
+	0	3x10 ⁻⁴	0	8749 _± 384*
0	+	0	0	7070 _± 838
0	+	3x10 ⁻⁴	0	6851 _± 383
+	0	0	0	5757 _± 200
+	0	0	3x10 ⁻³	8978 _± 555*
0	+	0	0	5279 _± 276
0	+	0	3x10 ⁻³	4757 _± 295

*p < .001, differs from control cultures incubated with [¹⁴C] tryptophan but without norepinephrine or DAMP.

Groups of ten culture tubes, each containing a rat pineal gland, were incubated with [¹⁴C] tryptophan (1x10⁻⁴M) or [¹⁴C] 5-hydroxytryptophan (1x10⁻⁴M) in the presence or absence of norepinephrine and (in a separate experiment) in the presence or absence of DAMP for 2 days at 37°C. Results are expressed as means _± S. E. M. of c. p. m. of [¹⁴C] radioactivity

of pineal tryptophan (i. e., in organs incubated with [¹⁴C] tryptophan) is enhanced by norepinephrine (11); however, the true uptake of the labeling amino acid is unaffected by norepinephrine (12). Hence our present data most likely reflect primary actions of norepinephrine and DAMP on the enzymatic conversion of [¹⁴C] tryptophan to [¹⁴C] 5-hydroxytryptophan. These actions may involve changes in the amount of tryptophan hydroxylase, in cofactor levels, or in a variety of other biochemical factors.

The finding that both norepinephrine and DAMP stimulate pineal serotonin synthesis by an action at the same step in the indole pathway suggests that adenosine 3', 5' monophosphate (cyclic AMP) may mediate norepinephrine-induced stimulation of pineal serotonin synthesis in vivo.

Inasmuch as there appear to be no fundamental differences between the mechanisms of serotonin biosynthesis in brain and pineal (4, 5), the possibility should be considered that norepinephrine released at brain synapses might also enhance serotonin synthesis in post-synaptic serotonergic neurons. It is of interest in this connection that mescaline, a phenylethylamine hallucinogen related structurally to norepinephrine, produces increased brain serotonin and 5-hydroxyindoleacetic acid concentrations in vivo (13), and also stimulates pineal [¹⁴C] serotonin synthesis in vitro via acceleration of [¹⁴C] tryptophan 5-hydroxylation (14).

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