Cyclic Adenosine Monophosphate: Stimulation of Melatonin and Serotonin Synthesis in Cultured Rat Pineals

Abstract. *Dibutyryl cyclic adenosine monophosphate, like norepinephrine, stimulates the synthesis of labeled melatonin and serotonin from tryptophan labeled with carbon-14 by rat pineals in organ culture. Unlike norepinephrine, dibutyryl cyclic adenosine monophosphate does not enhance the accumulation of labeled tryptophan or protein within the pineal. These findings are compatible with the hypothesis that cyclic adenosine monophosphate mediates some, but not all, of the effects of norepinephrine.*

The concentrations of melatonin and serotonin in the rat pineal are influenced by signals transmitted to the organ by way of its sympathetic nerves (*1*). The terminal boutons of these neurons contain both norepinephrine and serotonin (*2*); however, recent studies in vitro (*3, 4*) have provided evidence that norepinephrine, and not serotonin, is the neurotransmitter which controls pineal indole content. Norepinephrine added to rat pineal organ cultures stimulates the synthesis of labeled melatonin and serotonin from tryptophan labeled with C^{14} (*3*), and also enhances the accumulation of labeled tryptophan and protein within the pineal itself (*4*). In contrast, serotonin is without effect in this system (*3, 4*).

The biochemical mechanisms by which norepinephrine stimulates synthesis of C^{14}-indoles in the pineal have not as yet been elucidated. However, numerous actions of norepinephrine in other organs appear to be mediated by 3′,5′-adenosine monophosphate (cyclic AMP), a compound whose synthesis from adenosine triphosphate (ATP) is catalyzed by the enzyme adenyl cyclase (*5*). Moreover, norepinephrine stimulates adenyl cyclase activity in rat pineal homogenates (*6*). We now report that a derivative of cyclic AMP, dibutyryl cyclic AMP (DAMP), stimulates the synthesis of C^{14}-melatonin and C^{14}-serotonin in rat pineal organ cultures. However, this compound differs from norepinephrine in its inability to enhance the accumulation of C^{14}-tryptophan and C^{14}-protein within pineal cells.

Pineal glands were removed between 11 a.m. and 1 p.m. from adult female Sprague-Dawley rats previously housed under diurnal lighting conditions. Each pineal was clotted individually to the walls of a Wasserman tube, and 0.5 ml of nutrient medium (*7*) was added. The nutrient medium contained C^{14}-DL-tryptophan (0.5 μc in a $10^{-4}M$ solution) and, where indicated, DAMP. The cultures were sealed with a rubber stopper and incubated on a roller wheel at 37°C. In most experiments, the period of incubation was 48 hours. Each treatment group contained six to eight individual pineal gland cultures. Control groups were prepared as described (*4, 8*).

At the end of the incubation period, the nutrient media were assayed individually for C^{14}-melatonin, C^{14}-serotonin, and C^{14}-labeled 5-hydroxyindoleacetic acid (*8*). The pineals were then analyzed for C^{14}-protein and C^{14}-tryptophan (*4*).

The addition of DAMP ($3 \times 10^{-4}M$ to $3 \times 10^{-3}M$) to pineal cultures incubated for 48 hours was associated with a marked stimulation of synthesis of labeled melatonin and serotonin (Table 1). In concentrations of $3 \times 10^{-3}M$ or $10^{-3}M$, addition of DAMP was associated with an increase in C^{14}-melatonin synthesis greater than 300 percent and an increase in C^{14}-serotonin synthesis greater than 200 percent. Addition of DAMP in lower concentration ($3 \times 10^{-4}M$) caused a smaller but still significant increase in the synthesis of both of these C^{14}-indoles (Table 1). At all three concentrations, DAMP had no effect on the amounts of C^{14}-labeled 5-hydroxyindoleacetic acid (5-HIAA) in the media, or on the contents of C^{14}-labeled protein or tryptophan in the pineals (Table 1).

The time course of the effect of DAMP on pineal indole synthesis was examined by incubating cultures for 4, 16, or 48 hours (Table 2). After 4 hours and throughout the incubation periods, C^{14}-melatonin synthesis was stimulated in the presence of DAMP. An increase in the C^{14}-serotonin content of the medium was first apparent after 16 hours of incubation with DAMP. However, synthesis of C^{14}-serotonin must also have been accelerated in samples incubated for 4 hours, inasmuch as this indole is an intermediate in the conversion of labeled tryptophan to labeled melatonin (*3, 8*).

These data indicate that DAMP mimics some of the effects of norepinephrine on pineal C^{14}-tryptophan metabolism, but not others. It stimulates the synthesis and release of labeled serotonin and melatonin, but does not enhance the accumulation of labeled tryptophan or protein within pineal cells. Norepinephrine apparently exerts two kinds of actions on receptors in pineal cells (Fig. 1). (i) It acts directly at a membrane level to enhance the cellular uptake of C^{14}-tryptophan (*4*), and perhaps of other important substances; and (ii) it acts on a membrane enzyme (*5, 6*), adenyl cyclase, to accelerate the production of

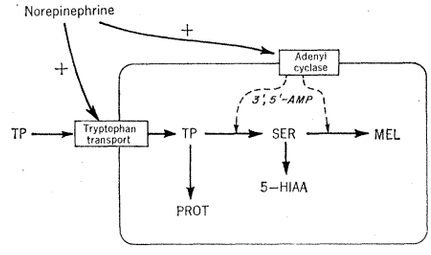

Fig. 1. Norepinephrine effects on pineal tryptophan metabolism. Norepinephrine apparently acts (+) on two receptors in the plasma membrane of the pineal parenchymal cell: (i) It stimulates tryptophan (*TP*) transport into the cell (*4*). (ii) It enhances the activity of adenyl cyclase (*6*) and thereby stimulates synthesis of cyclic AMP (3′5′-AMP). This "second messenger" acts at one or more unidentified intracellular loci to accelerate the formation of serotonin (*SER*) and melatonin (*MEL*).

Table 1. Effects of various doses of dibutyryl cyclic AMP (DAMP) on pineal synthesis of C^{14}-indoles and on pineal content of C^{14}-protein and C^{14}-tryptophan. Groups of six culture tubes containing a rat pineal gland were incubated with C^{14}-tryptophan ($10^{-4}M$) for 2 days at 37°C. Results are expressed as counts per minute of C^{14} radioactivity; 5-HIAA is 5-hydroxyindoleacetic acid.

DAMP concentration (*M*)	Radioactivity (count/min)				
	Melatonin	Serotonin	5-HIAA	Protein content	Tryptophan content
0	374 ± 57	348 ± 26	87 ± 31	222 ± 11	449 ± 74
3×10^{-4}	677 ± 106*	617 ± 78†	51 ± 41	246 ± 25	478 ± 36
10^{-3}	1464 ± 140‡	870 ± 115‡	63 ± 16	230 ± 22	473 ± 35
3×10^{-3}	1264 ± 55‡	1006 ± 122‡	37 ± 26	193 ± 14	533 ± 81

* $P < .05$. † $P < .01$. ‡ $P < .001$, differs from controls without DAMP.

Table 2. Time course of effects of dibutyryl cyclic AMP (DAMP) on pineal synthesis of C^{14}-melatonin and C^{14}-serotonin. Groups of six culture tubes each containing a rat pineal gland were incubated with C^{14}-tryptophan ($10^{-4}M$) in the presence or absence of $3 \times 10^{-3}M$ DAMP for 4, 16, or 48 hours at 37°C. Results are expressed as counts per minute of C^{14} radioactivity.

DAMP (+ or 0)	Melatonin (count/min)	Serotonin (count/min)
	4 hours	
0	125 ± 36	182 ± 52
+	309 ± 57*	233 ± 35
	16 hours	
0	484 ± 43	601 ± 25
+	1770 ± 276†	1272 ± 165‡
	48 hours	
0	591 ± 52	636 ± 45
+	2424 ± 215†	1751 ± 28†

* $P < .05$. † $P < .001$, differs from controls without DAMP. ‡ $P < .01$.

cyclic AMP. This substance then works as a "second messenger" to mediate such intracellular effects of the catecholamine as stimulation of labeled serotonin and melatonin synthesis.

The enhanced accumulation of C^{14}-protein in pineals incubated with norepinephrine probably reflects an increase in the intracellular specific activity of its precursor, C^{14}-tryptophan, secondary to the increased uptake of the amino acid (*4*). In contrast, the acceleration of C^{14}-indole synthesis induced by norepinephrine is not simply a "pool effect," inasmuch as the concentrations of labeled serotonin and melatonin in the medium show a much greater proportionate increase than the concentration of labeled tryptophan in the pineal (*3, 4*).

The pattern of changes that norepinephrine induces in the synthesis of pineal indoles (that is, increases in labeled serotonin and melatonin, no change in labeled 5-hydroxyindoleacetic acid) is essentially the same as the pattern produced by adding DAMP to the medium; moreover the time courses of these changes are similar (*3*). Since norepinephrine elevates the activity of adenyl cyclase in pineal homogenates (*6*), it seems likely that the effects of DAMP are not simply pharmacological, but reproduce the actions of endogenous cyclic AMP, which is produced in response to norepinephrine or to sympathetic nervous stimulation. The precise mechanisms by which DAMP enhances the syntheses of labeled serotonin and melatonin have not yet been identified. However, the stimulation of C^{14}-melatonin synthesis probably involves an increase in the activity of hydroxyindole-*O*-methyl transferase, the enzyme that catalyzes the formation of melatonin from *N*-acetyl serotonin (*9*), inasmuch as sympathetic nervous function controls this enzyme in vivo (*1*), and norepinephrine enhances its activity in vitro (*10*).

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Oculomotor Neurons in Fish: Electrotonic Coupling and Multiple Sites of Impulse Initiation

Abstract. *Oculomotor neurons are electrotonically coupled in three teleosts. Electron microscopy revealed axosomatic synapses with close appositions of pre- and postsynaptic membranes. Similar junctions are associated with electrotonic coupling in many other cases. Stimulation of the ipsilateral eighth nerve usually initiated impulses at sites distant from the cell bodies; stimulation of the ipsilateral ophthalmic nerve initiated impulses close to the cell bodies. Electrotonic coupling may synchronize impulses arising near the cell bodies to generate synchronous muscle contractions. Impulses arising distant from the cell bodies may lead to contractions of graded strength.*

Neurons in nuclei controlling synchronously acting effector organs such as electric organs are electrotonically coupled in fishes in many instances (*1–5*). The basic experimental observation is that current passed through an electrode in one cell produces a potential in a neighboring cell that is larger than can result from current passing through extracellular space surrounding the cells. The functional significance of electrotonic coupling in these systems is that it provides rapidly acting positive feedback between cells which synchronizes their firing. A cell that is relatively more depolarized advances spike initiation in neighboring cells; a cell that is relatively less depolarized retards spike initiation in neighboring cells. Thus, these electrotonic synapses are at the same time excitatory for less depolarized cells and inhibitory for more depolarized cells and have been termed synchronizing synapses (*6*). Mutually excitatory synapses that transmit chemically could not produce the highly synchronous discharges observed because of the relatively long delay associated with this mode of transmission (usually about 0.5 msec in fishes). Electrically transmitting synapses can exhibit a much briefer delay (less than 0.05 msec). Electrically mediated transmission from higher to lower level neurons is also known in a number of systems controlling escape reflexes (*7*). The functional significance in these instances appears to be a reduction in reflex latency leading to more rapid escape.

There is considerable evidence that electrotonic coupling between cells is mediated by specialized junctions where cell membranes are so closely apposed as to occlude most or all of the extracellular space between them. These junctions have been found in every instance where electrotonically coupled cells have been studied at the ultrastructural level and are rare or absent where cells are not coupled (*3–5*). In a given nucleus, neurons can be coupled by dendrodendritic junctions or through presynaptic fibers that form junctions on more than one cell. Experimental treatments that disrupt and then rejoin the junctions lead to loss and then recovery of electrotonic coupling (*8*).

We undertook this study to determine if electrotonic coupling was involved in the control of more ordinary organs than the highly specialized ones cited above. The oculomotor system in fish was an attractive subject because of the requirement for synchronous activity in saccadic eye movements and in the quick phase of nystagmus, because of the technical simplicity of working with these animals, and be-