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MEDIATION BY β-ADRENERGIC RECEPTORS OF EFFECT OF NOREPINEPHRINE ON PINEAL SYNTHESIS OF [^{14}C]SEROTONIN AND [^{14}C]MELATONIN

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Abstract—Rat pineal organs maintained in organ culture converted [^{14}C]tryptophan to [^{14}C]serotonin and [^{14}C]melatonin. The synthesis of both indoles was stimulated by the presence of norepinephrine or dibutyryl adenosine 3′,5′-monophosphate. This effect of norepinephrine could be blocked by the β-adrenergic blocking drug, propranolol, but was not modified by the α-adrenergic blocking agent, phenoxybenzamine. Neither blocking agent modified the pineal response to dibutyryl adenosine 3′,5′-monophosphate. Unlike dibutyryl adenosine 3′,5′-monophosphate, the naturally occurring adenosine phosphates did not stimulate synthesis of [^{14}C]melatonin *in vitro*.

THE SYMPATHETIC innervation to the rat pineal gland mediates the daily rhythmic fluctuations in the activities of pineal enzymes and contents of pineal indoles that occur in response to environmental lighting (FISKE, 1964; WURTMAN, AXELROD and FISCHER, 1964; AXELROD, WURTMAN and SNYDER, 1965; SNYDER, ZWEIG, AXELROD and FISCHER, 1965; WURTMAN, AXELROD and KELLY, 1968*a*). Organ culture of this gland provides a useful system for examining *in vitro* the mechanisms by which norepinephrine, the neurotransmitter probably released at pineal sympathetic nerve endings (AXELROD, SHEIN and WURTMAN, 1969), controls pineal indole metabolism.

The cultured pineal gland of the rat is able to convert [^{14}C]tryptophan to characteristic [^{14}C]indoles and to [^{14}C]protein (WURTMAN *et al.*, 1968*a*; WURTMAN, SHEIN, AXELROD and LARIN, 1969). The indoles, which include [^{14}C]serotonin, [^{14}C]melatonin and [^{14}C]5-hydroxyindoleacetic acid, are released into the incubation medium; the [^{14}C]proteins are largely retained within the organ. The addition of norepinephrine to the culture medium markedly enhances the rates at which all of the [^{14}C]tryptophan derivatives are synthesized (AXELROD *et al.*, 1969; WURTMAN *et al.*, 1969). Dibutyryl adenosine 3′,5′-monophosphate (DAMP) reproduces the stimulation of [^{14}C]indole synthesis (but not of [^{14}C]protein synthesis) by norepinephrine (SHEIN and WURTMAN, 1969). Since norepinephrine increases adenyl cyclase activity in homogenates of rat pineal (WEISS and COSTA, 1967), we have suggested that some of the effects of the catecholamine on pineal biosynthetic activity *in situ* might normally be mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (SHEIN and WURTMAN, 1969; SHEIN, 1971). The effect of norepinephrine on pineal adenyl cyclase is blocked by β-adrenergic blocking agents (WEISS, 1970). This report describes studies on the pharmacology of the norepinephrine receptor in cultured pineal organs, and on the specificity of the stimulation of [^{14}C]indole synthesis by DAMP.

Abbreviations used: DAMP (dibutyryl cyclic AMP), dibutyryl adenosine 3′,5′-monophosphate; cyclic AMP, adenosine 3′,5′-monophosphate.

MATERIALS AND METHODS

Pineal glands, taken between 1100 and 1300 h from adult female Sprague–Dawley rats (160–180 g) previously housed under diurnal lighting conditions, were clotted to the walls of Wasserman tubes containing 0·5 ml of nutrient medium, as previously described (SHEIN, WURTMAN and AXELROD, 1967). Each culture tube contained DL-[3-^{14}C]tryptophan (New England Nuclear Corp., Boston, Mass; initial specific radio activity 20 mCi/mmol; 0·5 μCi in an 0·1 mM solution) (SHEIN and WURTMAN, 1969) and, where indicated, norepinephrine, various adenosine derivatives, DAMP, the α-blocking agent, phenoxybenzamine (Dibenzylene; Smith, Kline and French, Philadelphia, Pa.), or the β-blocking agent, propranolol (Inderal; Ayerst Laboratories, New York, N.Y.). The tubes were then sealed with a rubber stopper and incubated at 37°C on a roller wheel for 48 h. Each treatment group contained six to eight individual pineal cultures and each experiment was performed three or four times. Control groups were prepared as previously described (WURTMAN, LARIN, AXELROD SHEIN and ROSASCO, 1968*b*; WURTMAN *et al.*, 1969). At the end of the incubation period, portions of the nutrient media were assayed for [^{14}C]serotonin and [^{14}C]melatonin after extraction of these compounds into organic solvents (WURTMAN *et al.*, 1968*b*). Each sample was evaporated under N_2, and its radioactivity was counted by liquid scintillation spectrophotometry. The efficiency of counting, checked for each sample, ranged from 59 to 62 per cent; hence no correction was made for counting efficiency. Data were presented as means ± S.E.M., and analysed by *t*-test.

RESULTS

In confirmation of previous findings (AXELROD *et al.*, 1969), the addition of norepinephrine (0·3 mM) to the pineal organ cultures greatly stimulated ($P<0·01$) synthesis of [^{14}C]serotonin and [^{14}C]melatonin (Table 1). This effect of norepinephrine was not inhibited by the presence of phenoxybenzamine in concentrations of 1–100 μM; however, the effect was significantly suppressed by propranolol in concentrations of 10 μM or 100 μM (Table 1). Like norepinephrine, mM-DAMP caused a significant stimulation ($P<0·01$) of pineal synthesis of [^{14}C]serotonin and [^{14}C]melatonin (SHEIN and WURTMAN, 1969) that was not altered by the presence of phenoxybenzamine (Table 2). In contrast to the effect of norepinephrine, however, the stimulation of pineal [^{14}C]indole synthesis by DAMP was also unaffected by propranolol at all concentrations tested (Table 2). Propanolol also had no effect on the synthesis of [^{14}C]indoles by unstimulated pineals (i.e. those incubated without norepinephrine or DAMP). Pineal organs were incubated with AMP, ADP, ATP, cyclic AMP or DAMP in 3 mM concentrations. DAMP significantly enhanced, and cyclic AMP significantly

TABLE 1.—EFFECT OF PHENOXYBENZAMINE OR PROPRANOLOL ON PINEAL RESPONSES TO NOREPINEPHRINE

Norepinephrine (M)	Phenoxybenzamine (M)	Propranolol (M)	[^{14}C]Melatonin (c.p.m./medium)	[^{14}C]Serotonin (c.p.m./medium)
—	—	—	1107* ± 97	332* ± 28
3×10^{-4}	—	—	2174 ± 154	874 ± 62
3×10^{-4}	10^{-6}	—	1852 ± 186	787 ± 71
3×10^{-4}	10^{-5}	—	2285 ± 115	939 ± 90
3×10^{-4}	10^{-4}	—	2388 ± 142	1046 ± 152
3×10^{-4}	—	10^{-6}	1852 ± 95	787 ± 48
3×10^{-4}	—	10^{-5}	1398* ± 84	585* ± 40
3×10^{-4}	—	10^{-4}	940* ± 96	332* ± 48

* Differs significantly ($P<0·01$) from group receiving only norepinephrine. Values represent means (± S.E.M.) for six to eight observations per experimental group.

TABLE 2.—EFFECT OF PHENOXYBENZAMINE OR PROPRANOLOL ON PINEAL RESPONSES TO DIBUTYRYL CYCLIC AMP

Dibutyryl cyclic AMP (M)	Phenoxybenzamine (M)	Propranolol (M)	[^{14}C]Melatonin (c.p.m./medium)	[^{14}C]Serotonin (c.p.m./medium)
—	—	—	320* ± 46	963* ± 86
10^{-3}	—	—	1549 ± 129	2461 ± 196
10^{-3}	10^{-6}	—	1220 ± 134	2480 ± 201
10^{-3}	10^{-5}	—	1588 ± 162	3138 ± 246
10^{-3}	10^{-4}	—	1483 ± 141	2702 ± 182
10^{-3}	—	10^{-6}	1453 ± 108	2324 ± 230
10^{-3}	—	10^{-5}	1657 ± 108	2542 ± 174
10^{-3}	—	10^{-4}	1566 ± 124	2854 ± 195

* Differs significantly ($P < 0.01$) from group receiving only dibutyryl cyclic AMP. Values represent means (± S.E.M.) for six to eight observations per experimental group.

TABLE 3.—EFFECT OF ADENINE NUCLEOTIDES ON PINEAL [^{14}C]INDOLE SYNTHESIS

Nucleotide	[^{14}C]Serotonin (c.p.m./medium)	[^{14}C]Melatonin (c.p.m./medium)
Control	615 ± 70	358 ± 31
AMP	526 ± 15	254 ± 26
ADP	1016* ± 80	341 ± 37
ATP	740 ± 94	245 ± 30
Cyclic AMP	391* ± 16	198* ± 10
DAMP	1033* ± 89	1230* ± 89

Individual pineal organs were incubated with the indicated nucleotide (3 mM) and [^{14}C]tryptophan for 48 h. Values represent means (± S.E.M.) for six to eight observations per experimental group.
* Differs significantly ($P < 0.01$) from control.

depressed, the syntheses of both [^{14}C]serotonin and [^{14}C]melatonin. ADP stimulated the formation of [^{14}C]serotonin but not of [^{14}C]melatonin. AMP and ATP did not effect [^{14}C]indole biosynthesis (Table 3).

DISCUSSION

The norepinephrine-induced stimulation of pineal synthesis of [^{14}C]melatonin and [^{14}C]serotonin is inhibited by a β-adrenergic blocking agent (propranolol) but not by an α-adrenergic blocking agent (phenoxybenzamine). This observation indicates that the mechanism of stimulation involves interaction with 'classic' β-adrenergic receptors. In contrast, the mechanism by which DAMP stimulates pineal synthesis of [^{14}C]melatonin and [^{14}C]serotonin apparently does not involve either α- or β-receptors, inasmuch as neither type of blocking agent suppressed its effect. The ability of a β-adrenergic blocking agent to block the stimulatory effects of norepinephrine on pineal [^{14}C]indole synthesis is compatible with the hypothesis (SHEIN and WURTMAN, 1969; SHEIN, 1971) that such stimulation is mediated by the activation of adenyl cyclase

and by the consequent increase in the intracellular concentration of cyclic AMP. In various tissue preparations other than the pineal the enhanced accumulation of cyclic AMP produced by catecholamines is selectively inhibited by β-blocking agents (KAKAIUCHI and RALL, 1968; RALL and GILMAN, 1970). This observation suggests that the catecholamine-stimulated adenyl cyclase is associated with β-receptors.

In the stimulation by norepinephrine of pineal [^{14}C]indole synthesis, the combination of norepinephrine with the β-receptor apparently precedes both the change in intracellular content of cyclic AMP and the acceleration of synthesis of [^{14}C]serotonin and [^{14}C]melatonin, inasmuch as doses of propranolol which inhibit the acceleration produced by norepinephrine fail to block the effects of DAMP. This selective blockade by propranolol of the effects of norepinephrine, but not of DAMP, is compatible with the widely-held view that the metabolic receptor for norepinephrine, but not for cyclic AMP, lies on the outer surface of the responding cell. Unlike DAMP, cyclic AMP (the naturally occurring mononucleotide) did not stimulate pineal [^{14}C]indole synthesis; in fact, at the dosage tested, cyclic AMP actually inhibited the formation of [^{14}]serotonin and [^{14}C]melatonin. The mechanism of this inhibition is not clear, nor is it understood how ADP increased [^{14}C]serotonin (but not [^{14}C]melatonin) synthesis. Other investigators (KLEIN, BERG, WELLER and GLINSMANN, 1970*a*) have also observed the lack of stimulation of pineal [^{14}C]indole synthesis by cyclic AMP.

The precise biochemical loci at which DAMP acts to enhance the syntheses of [^{14}C]serotonin and [^{14}C]melatonin await clarification. DAMP could, at least theoretically, act by stimulating one or more of the following steps in the indole pathway: (1) the conversion of tryptophan to serotonin, (2) the *N*-acetylation of serotonin or (3) the *O*-methylation of *N*-acetylserotonin. KLEIN, BERG and WELLER (1970*b*) have recently presented evidence that DAMP acts at the stage of the *N*-acetylation of serotonin; the addition of DAMP to pineal cultures causes a marked enhancement of serotonin *N*-acetyltransferase activity, as measured in homogenates. It is also well established that the flow of sympathetic nervous impulses to the pineal regulates the activity *in vivo* of hydroxyindole *O*-methyl transferase (the terminal enzyme in melatonin biosynthesis) probably by regulating the amount of norepinephrine liberated (WURTMAN *et al.*, 1968*a*).

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