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ORNITHINE DECARBOXYLASE—APPARENT LACK OF INVOLVEMENT IN THE INDUCTION OF N-ACETYLTRANSFERASE IN RAT PINEAL GLAND

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Abstract—The potential role of ornithine decarboxylase in the induction of serotonin-*N*-acetyltransferase in the rat pineal gland has been studied. The injection of isoproterenol (5 mg/kg, s.c.) resulted in an apparent induction of serotonin-*N*-acetyltransferase within 1 hr with a maximal response at 4 hr. No change in ornithine decarboxylase was observed in the first hour following isoproterenol administration. Rat pineal glands in organ culture responded to addition of 2 μM isoproterenol with a similar induction of *N*-acetyltransferase. There was a small but significant decrease in ornithine decarboxylase in the cultured rat pineal glands in response to isoproterenol. Additional experiments indicated that isoproterenol also did not elevate ornithine decarboxylase in the superior cervical ganglia under conditions known to induce tyrosine hydroxylase in this organ. The results of this study are consistent with our previous finding that ribosomal RNA synthesis is unaffected during the induction process in the pineal gland.

An increase in ornithine decarboxylase activity (EC 4.1.1.17) has been observed in a number of tissues following enhanced cyclic AMP production [1–4]. The enzyme appears to have a dual role in that it is the rate-limiting enzyme in polyamine synthesis [5–7] and has been shown recently to stimulate RNA polymerase I directly [8]. This polymerase is responsible for the formation of 45S ribosomal RNA. Thus, an increased tissue level of ornithine decarboxylase is indicative of enhanced ribosomal RNA synthesis.

Recent studies in our laboratory have attempted to disclose some of the molecular events in the apparent induction of serotonin-*N*-acetyltransferase (NAT) in the pineal gland [9]. In this system pinealocytes respond to a β agonist by an increase in the cAMP content of the cells. The generation of cAMP appears to be an obligatory event of the induction process. However, we have been unable to detect any changes in the synthesis of any class of RNA associated with the induction process. Additional investigations [10]* also were unable to correlate partial inhibition of RNA synthesis with inhibition of NAT induction. These results appear to contradict the presently accepted hypothesis that NAT induction requires stimulation of both transcription and translation [11, 12]. Therefore, it was decided to explore other regulatory aspects of RNA synthesis which might be involved in the mechanism for the induced rise in NAT. In the present experiments we examined the effect of β-receptor stimulation of cultured rat pineal glands on the activity of ornithine decarboxylase.

MATERIALS AND METHODS

Chemicals used and their sources were: Hyamine Hydroxide from Sigma Chemical Co. (St. Louis, MO), isoproterenol–HCl from Aldrich Chemicals (Milwaukee, WI), dithiothreitol (DTT), pyridoxal-5′-phosphate and EDTA from CalBiochem (San Diego, CA), Tris–HCl from Bethesda Research Laboratories (Rockville, MD), L-[1-^{14}C]ornithine from Amersham Searle (Arlington Heights, IL), BGJB Fitton-Jackson medium (with added salts and lipids) from Grand Island Biological Co. (Grand Island, NY) and liquifluor from NEN (Boston, MA).

Animals. Male Sprague–Dawley rats (150–170 g) were purchased from Taconic Farms Inc., Germantown, NY. The animals were housed under diurnal lighting conditions (12 hr light, 0600–1800 hr) for at least 6 days prior to experimental use.

Pineal gland culture. Animals were decapitated in the early afternoon to eliminate the presence of residual RNA coded for NAT synthesis. After decapitation of the animals, the pineal glands were removed and cultured as described by Morrissey and Lovenberg [9].

Ornithine decarboxylase activity. The pineal gland or superior cervical ganglia were placed in a 9 cm × 2 cm test tube which contained 0.25 ml of buffered solution consisting of (at final concentrations): Tris–HCl (pH 7.5) 50 mM, L-[1-^{14}C]ornithine (56 μCi/μmole) 20 μM, pyridoxal-5′-phosphate 40 μM, EDTA 4 mM, and dithiothreitol 5 mM. Liver tissue was prepared by homogenizing at 20 per cent (w/v) in buffered solution containing Tris–HCl (pH 7.5) 50 mM, DTT 5 mM, and pyridoxal-5′-phosphate 40 μM. After centrifugation at 48,000 rev/min for 30 min, the supernatant fraction was removed and 50 μl was added to 200 μl of buffered solution (indicated above) in a 9 cm × 2 cm test tube. After a 5-sec sonication period the tubes were capped with serum stoppers with attached plastic cups containing 0.1 ml of Hyamine Hydroxide. The samples were incubated anerobically at 37° with gentle mixing for 1 hr, at which point 0.1 ml of 9 N H_2SO_4 was added to stop the reaction. To allow complete absorption of $^{14}CO_2$ into

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the Hyamine Hydroxide, the capped tubes were kept at room temperature overnight. Then the plastic cups were removed and placed in 12 ml of liquid scintillation solution (3.64 liters toluene + 160 ml liquifluor + 25 ml methanol) and counted.

N-*acetyltransferase activity*. NAT activity was determined as described by Morrissey and Lovenberg [9] except that the glands were sonicated and incubated in a total volume of 100 μl.

RESULTS

Similar to results shown by other investigators [13–15], the injection of isoproterenol (5 mg/kg, s.c.) caused a rapid rise in the level of NAT (Fig. 1). While the first significant increase in enzyme activity was observed at 1 hr, maximal increase occurred at 4 hr. At this time NAT activity had increased 45-fold compared to the control. From these results it was felt that, if ornithine decarboxylase participated in some manner in the induction of NAT, it must necessarily occur within hr 1. Thus, new r-RNA specifically coded for synthesis of NAT would precede the appearance of new molecules of the enzyme. Analysis of ornithine decarboxylase activity during hr 1 after the injection of isoproterenol (Table 1) revealed no significant increase in the activity of this enzyme.

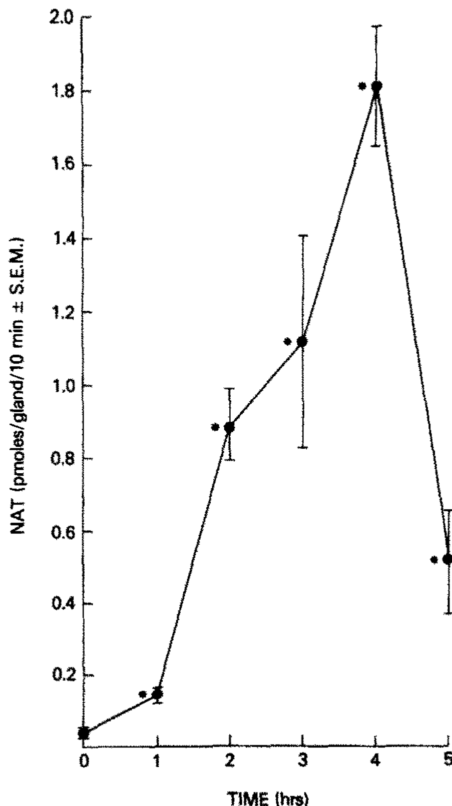

Fig. 1. NAT activity in pineal glands at various intervals after the injection of isoproterenol (5 mg/kg, s.c.). Each value represents the average ± S. E. M. of four separate glands. An asterisk indicates P < 0.05 compared to pineals from untreated animals.

Table 1. Effects of isoproterenol (5 mg/kg, s.c.) on ornithine decarboxylase in rat pineal gland*

Time after injection (min)	Ornithine decarboxylase activity (pmoles/gland/hr ± S. E. M.)
0	6.61 ± 0.79
10	6.98 ± 1.10
20	7.15 ± 1.30
30	8.85 ± 1.80
45	7.06 ± 1.20
60	7.22 ± 0.60

* Ornithine decarboxylase activity was determined as described in Materials and Methods. Each value represents the average ± S. E. M. of eight separate glands.

To confirm these findings, the study was extended to pineal glands incubated in culture medium. Using these conditions, isoproterenol (2 μM) caused a significant increase in NAT activity within 2–3 hr, while the maximal increase occurred at 5–6 hr [9, 16]. Therefore, we analyzed ornithine decarboxylase activity every hour over a 4-hr period in pineal glands incubated in culture media with or without isoproterenol. As shown in Table 2, ornithine decarboxylase activity in pineal glands did not change over the entire time period examined. However, when isoproterenol was present in the culture medium, the activity of the enzyme unexpectedly decreased. Insel and Fenno [17] also observed a decrease in ornithine decarboxylase activity in lymphoma cells incubated with N^6,$O^{2'}$-dibutyryl cyclic AMP.

For a comparative analysis, ornithine decarboxylase was examined in the superior cervical ganglia after injection of isoproterenol (i.p.). This drug causes a rapid and significant increase in cAMP in this tissue [18]. Therefore, it was felt that ornithine decarboxylase activity might be affected. Enzyme activity was examined at each hour over a 5-hr period, since ornithine decarboxylase has been shown in numerous tissues to increase significantly within the first 4–5 hr [19–23]. Surprisingly, no significant change in basal activity (9.24 ± 0.25 pmoles/mg/hr) of the enzyme was observed in the superior cervical ganglia.

DISCUSSION

In the present study the activity of the enzyme ornithine decarboxylase showed no increase when pineal glands were examined either after injection of isoproterenol (5 mg/kg) or when maintained in culture medium containing isoproterenol. This is consistent with unpublished observations of T. Oka and D. C. Klein (personal communication). An increase in enzyme activity would have suggested enhanced synthesis of 45S ribosomal RNA, since ornithine decarboxylase has been proposed to modulate the activity of RNA polymerase I. Thus, the present results support, at least in part, the findings of Morrissey and Lovenberg [9, 10] and Miller and Lovenberg. *These latter studies did not detect new RNA synthesis specific for NAT induction

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Table 2. Effects of incubation of pineal glands in culture medium with or without isoproterenol (2 μM) on ornithine decarboxylase activity*

Period of incubation (hr)	Ornithine decarboxylase activity (pmoles/gland/hr ± S. E. M.)	
	Without isoproterenol	With isoproterenol
0	6.00 ± 0.68	6.11 ± 0.64
0.5	5.68 ± 1.77	4.51 ± 0.78
1	4.81 ± 0.76	4.16 ± 0.69
2	5.81 ± 0.74	3.29 ± 0.43†
3	4.44 ± 0.51	3.95 ± 0.38
4	4.89 ± 1.06	5.46 ± 0.45

* Ornithine decarboxylase activity was determined as described in Materials and Methods. Each value represents the average ± S. E. M. of four separate glands.

† $P < 0.02$, compared to unincubated sample.

and did not correlate partial inhibition of synthesis of different RNA species with inhibition of the induced rise in NAT.

Addition of isoproterenol to culture medium containing pineal glands or injection of the drug into rats leads to an increase in cAMP in this gland [23]. Because a similar rise in cAMP in other tissues led to a subsequent rise in ornithine decarboxylase activity, Byus and Russell [20] suggested that ornithine decarboxylase activity is regulated by cAMP levels. Thus, it was surprising in the present study to find that the enzyme was unaffected in the pineal gland. However, the proposal that the increase in cAMP is an essential component of the mechanism for ornithine decarboxylase induction has been challenged by the recent results of Mufson *et al.* [24]. In those studies the application of 12-O-tetradecanoyl phorbol-13-acetate caused a 200- to 400-fold induction of ornithine decarboxylase in mouse epidermal preparations while no changes were observed in the basal level of 3′:5′-cyclic AMP. In addition, i.p. injection of isoproterenol led to an accumulation of cAMP in epidermal tissue but to no change in enzyme activity. These results led the authors to conclude that the regulation of ornithine decarboxylase induction by cAMP may be a tissue-specific mechanism and not a general phenomenon. In support of this are the results of Richman *et al.* [25] using the thyroid gland and the studies of Chen and Canellakis [26] using neuroblastoma cells.

Finally, it has been proposed that the increase in ornithine decarboxylase activity is an early marked event in tissues stimulated to hypertrophy, to undergo cell mitosis or to differentiate [20]. Present results suggest that the induction of ornithine decarboxylase may not be necessary to turn on or off a differentiated function such as the induction of NAT.

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