pound has been identified as a major benzo[*a*]pyrene metabolite bound in vivo to DNA (*7, 8, 8a*). With microsomes, however, other metabolites such as the K-region epoxide and the 9-phenol are also bound to DNA (*8a, 17*). Furthermore, a detailed study of the binding of benzo[*a*]-pyrene in vivo to the DNA of different tissues has not been reported. Our findings that β-glucuronidase catalyzes the binding of benzo[*a*]pyrene-3-glucuronide suggest the possibility that the latter type of metabolite may be an important intermediate of benzo[*a*]pyrene carcinogenesis. It could function as a carrier of a benzo[*a*]pyrene metabolite to distal sites such as the bladder, kidney, or intestines where glucuronidase action may induce its binding to DNA. Renwick and Drasar (*18*) have reported the bacterial hydrolysis of benzo[*a*]pyrene conjugates by gut bacteria and have suggested that this may be a mechanism of retoxification of the polycyclic hydrocarbons. Our studies suggest that the hydrolysis of phenols or diols may yield a reactive intermediate that binds to DNA and be carcinogenic. Another possibility is that the 7,8-diol glucuronide is transported either intracellularly or to a distal organ site where it can be hydrolyzed to the diol and serve as a substrate for diol epoxide formation. Recent reports have indicated a nuclear mixed function oxidase activity which may be responsible for this activation (*19*).

NADAO KINOSHITA*
HARRY V. GELBOIN†
Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

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* Present address: Chemistry Department, School of Health Sciences, Kyushu University 90, Maidashi, Higashi-ku, Fukuoka 812, Japan.
† Reprint requests should be addressed to H.V.G.

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Pineal Serotonin *N*-Acetyltransferase Activity: Abrupt Decrease in Adenosine 3′,5′-Monophosphate May Be Signal for "Turnoff"

Abstract. *Dispersed pinealocytes have been used to study the role of adenosine 3′,5′-monophosphate (cyclic AMP) in the "turnoff" of* N*-acetyltransferase activity. Activity was first stimulated 100-fold by treating cells with* l*-norepinephrine.* l*-Propranolol acted stereospecifically to rapidly reverse this, resulting in a 70 percent loss of enzyme activity within 15 minutes. An even more rapid* l*-propranolol-induced decrease in cyclic AMP also occurred. This together with the observation that the inhibitory effect of* l*-propranolol on* N*-acetyltransferase was blocked by dibutyryl cyclic AMP and phosphodiesterase inhibitors indicate that an abrupt decrease in cyclic AMP may be the signal for the rapid decrease in pineal* N*-acetyltransferase activity.*

One of the rapid transmitter-regulated changes in enzyme activity is the decrease [halving time ($t_{1/2}$), 3 minutes] in rat pineal serotonin *N*-acetyltransferase activity which occurs when an animal is exposed to light after a period of darkness at night (*1*). A similar "turnoff" of enzyme activity occurs at night as a result of *l*-propranolol treatment and when adrenergically stimulated pineal glands are treated with this blocking agent in vitro (*2, 3*). Although it is clear that the adrenergically induced increase in *N*-acetyltransferase activity is mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*3-6*), this second messenger has not been implicated in either the maintenance of increased *N*-acetyltransferase activity or in the rapid "turnoff" of enzyme activity.

We have examined this problem using a new pinealocyte culture method (*7*) and a sensitive technique for the assay of cyclic AMP (*8*), and here report that the rapid *l*-propranolol-induced decrease in *N*-acetyltransferase activity is accompanied by an even more rapid decrease in cellular cyclic AMP. This and associated observations provide evidence that the signal for "turnoff" of pineal *N*-acetyltransferase activity may be the rapid decrease in cyclic AMP to a critical low level.

Cells isolated from pineal glands of 2-day-old rats were incubated under control conditions for 24 hours. During this period a small degree of aggregation was observed; essentially no cells were attached to the plastic culture tubes. Addition of *l*-norepinephrine (1 μ*M*) resulted in a 100-fold increase in cyclic AMP and *N*-acetyltransferase activity (Fig. 1). The increase in cyclic AMP peaked at about 15 minutes and gradually decreased during the next few hours. The rate of increase of *N*-acetyltransferase activity

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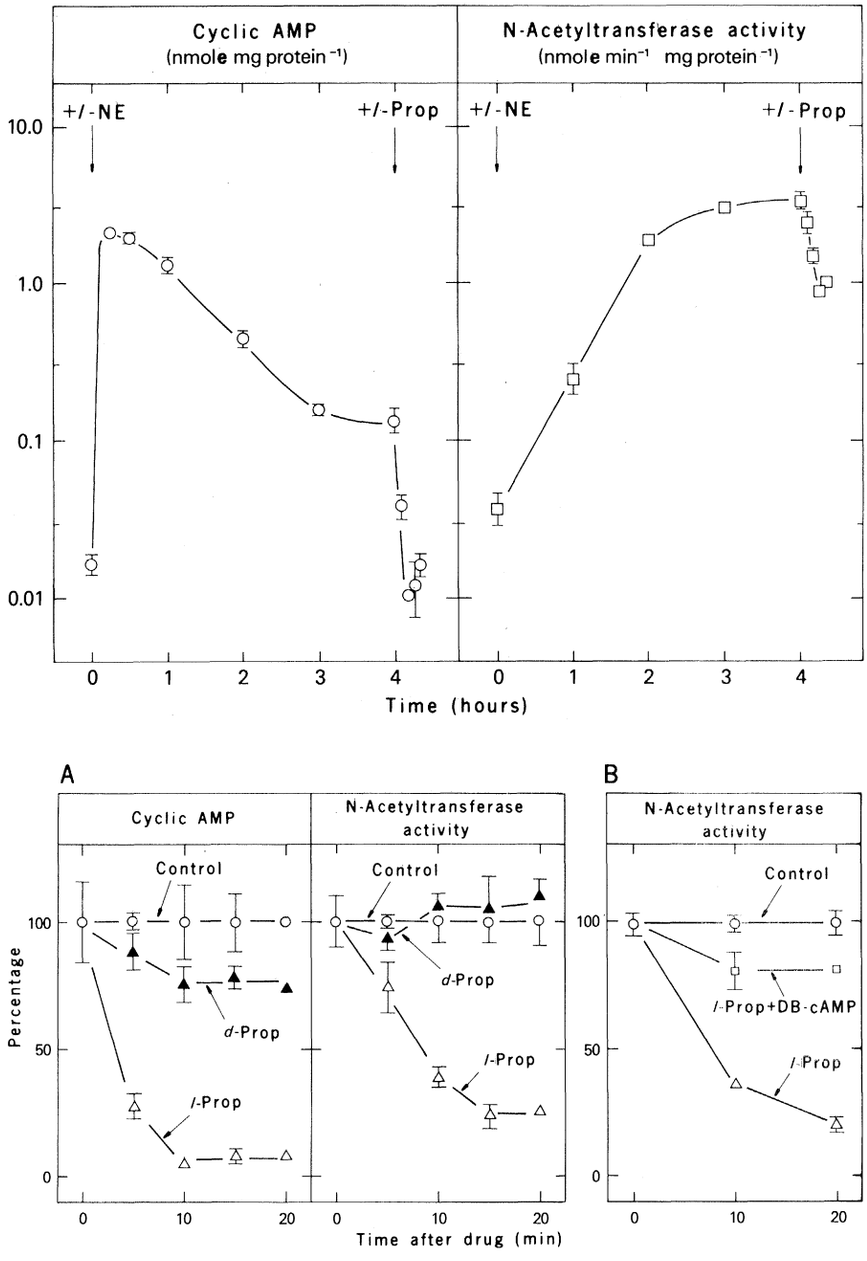

Fig. 1 (top). Effects of adrenergic drugs on *N*-acetyltransferase activity and cyclic AMP in cultured pineal cells. Pineal cells were prepared by enzymatic (0.25 percent trypsin, 0.04 percent deoxyribonuclease) and mechanical treatment of pineal glands removed from 2-day-old rats (*5*). After debris was removed the cells were distributed into individual culture vessels, each containing approximately 200,000 cells in 0.5 ml of Dulbecco's modified Eagle's medium containing 10 percent fetal calf serum. The cells were cultured for 24 hours (37°C; 95 percent air and 5 percent CO_2) and then treated with 1 μM *l*-norepinephrine (*l-NE*). Four hours later *l*-propranolol (*l-Prop*) was added to a final concentration of 1 μM. Cells were collected by centrifugation (8000*g*, 30 seconds) at room temperature, the medium was rapidly removed, and the remaining cell pellet was frozen immediately. For the determination of *N*-acetyltransferase activity, a cell pellet was disrupted by sonication in 50 μl of an enzyme assay mixture [for other details of this enzyme method see (*6*)]. The data are means ± standard error (S.E.) of four determinations. The cyclic AMP present in the cell pellet was extracted by sonication of cells in 0.5 ml of 5 percent trichloroacetic acid. The extract was centrifuged at 8000*g* for 2 minutes. The precipitate was dissolved in 1*N* NaOH and a portion was used for the determination of protein; bovine serum albumin was used as a standard (*12*). Cyclic AMP was assayed without purification by radioimmunoassay (*8*); purification on a Dowex-1 column did not change the cyclic AMP measurements. Samples containing low levels of cyclic AMP were assayed after succinylation. Values are means (± S.E.) of duplicate determinations of four individual cultures. The absence of error bars indicates that the S.E. values fell within the area covered by the symbols. Fig. 2 (bottom). Relative effect of drugs on *N*-acetyltransferase and cyclic AMP in norepinephrine-treated pinealocytes. Results are expressed as percentages of values at each time point in cells treated only with norepinephrine (*Control*). Each value is the mean (± S.E.) of four determinations. The absolute values of cyclic AMP and *N*-acetyltransferase are consistent with those of similarly treated cells in Fig. 1. The absence of error bars indicates that the S.E. values fell within the area covered by the symbols. (A) Cells were treated with 1 μM *l*-norepinephrine for 4 hours as described in Fig. 1 and then the indicated drug treatments were initiated (*l-Prop*, 1 μM *l*-propranolol; *d-Prop*, 1 μM *d*-propranolol). (B) Cells were treated with 0.1 μM norepinephrine for 3 hours and then the indicated drug treatments were initiated (*l-Prop*, 1 μM *l*-propranolol; *DB-cAMP*, 1 m*M* dibutyryl cyclic AMP).

was slower; a plateau was reached after 3 to 4 hours. The time courses of these effects are consistent with earlier observations made on intact pineal glands in organ culture (*5*).

The addition of *l*-propranolol (1 μM) after 4 hours of treatment with *l*-norepinephrine caused a 90 percent decrease in cyclic AMP ($t_{1/2}$ = 3 minutes) and a 70 percent decrease in *N*-acetyltransferase activity ($t_{1/2}$ = 6 to 8 minutes) within the first 20 minutes of treatment with the antagonist. During the next 40 minutes of treatment with *l*-propranolol, *N*-acetyltransferase activity decreased gradually and cyclic AMP remained relatively constant (*9*). The effect of *l*-propranolol appears to be stereospecific because *d*-propranolol (1 μM) had a much smaller and slower effect, one which was statistically significant ($P > .01$) only at the 20-minute point (Fig. 2A). The small decrease in cyclic AMP produced by *d*-propranolol apparently was not associated with a decrease in *N*-acetyltransferase activity. Further evidence that *l*-propranolol (1 μM) was acting as a specific adrenergic inhibitor of enzyme activity in this system, and not as a nonspecific metabolic inhibitor, is provided by our finding that it could not reverse the stimulatory effects of dibutyryl cyclic AMP (0.5 m*M*, 3 hours) treatment (*9*).

Although these studies demonstrate that *l*-propranolol causes rapid concurrent decreases in both cyclic AMP and *N*-acetyltransferase activity, they do not prove that a decrease in cyclic AMP is functionally involved in "turnoff." Support for this point, however, is accumulating (*9*). We found that the large *l*-propranolol-induced decrease in *N*-acetyltransferase activity could be blocked by about 80 percent if dibutyryl cyclic AMP is added with *l*-propranolol (Fig. 2B); the small decrease that occurred was statistically significant ($P > .01$) only at 20 minutes after the addition of both drugs. In other experiments in which dibutyryl cyclic AMP was added 5 minutes prior to *l*-propranolol, no significant de-

crease in enzyme activity occurred (*9*). Apparently this 5-minute time period is required for the cyclic nucleotide to reach an intracellular concentration that will maintain *N*-acetyltransferase activity. This finding of complete antagonism of the effects of *l*-propranolol by dibutyryl cyclic AMP suggests to us that all the effects of *l*-propranolol on *N*-acetyltransferase activity are through an adrenergic receptor and are mediated by cyclic AMP. In addition, if we inhibit the *l*-propranolol-induced decrease in cyclic AMP by treating cells with either theophylline (10 m*M*) or isobutylmethylxanthine (0.5 m*M*), compounds which inhibit cyclic nucleotide phosphodiesterase activity (*10*), *N*-acetyltransferase activity also does not decrease.

It would appear from these observations that hours after the start of adrenergic stimulation, the low concentration of cyclic AMP that persists serves to maintain *N*-acetyltransferase activity at increased levels; the "turnoff" of enzyme activity could primarily be a result of a decrease in cyclic AMP below a critical intracellular concentration necessary for maintenance of the enzyme in an active state. This decrease might in part be due to a decrease in synthesis; alternatively, the decrease in cyclic AMP might reflect its release from a bound form and subsequent metabolism.

Previous experiments have indicated that a cessation of protein synthesis does not produce a rapid decrease in *N*-acetyltransferase activity (*2*, *3*). Hence, it seems improbable that "turnoff" is a result of a halt in the synthesis of a specific protein; the rapid rate of disappearance of enzyme activity is more consistent with an inactivation mechanism that might involve rapid reversal of the adrenergic-cyclic AMP-induced changes in membrane potential required for stimulation of *N*-acetyltransferase (*6*). It is interesting that induced *N*-acetyltransferase activity is highly unstable in broken cell preparations, and that it is stabilized by acetyl coenzyme A (*11*), and also that depolarizing agents can, albeit slowly, turn off *N*-acetyltransferase activity (*3*). In view of this, it seems possible that cyclic AMP, perhaps acting in part by way of its effects on membrane physiology (*6*), might facilitate the interaction between acetyl coenzyme A and enzyme molecules to maintain the enzyme in the active and stable form; alternatively, cyclic AMP might accomplish this by stimulating the phosphorylation of *N*-acetyltransferase molecules by protein kinase, a generally occurring mode of enzyme regulation.

The pinealocyte preparation in these experiments can be used to study the mechanism through which cyclic AMP acts to mediate rapid transmitter-regulated changes in enzyme function. Further information about this mechanism might be helpful in understanding the nature of rapid changes in neurochemical transduction, a fundamental issue in neurobiology.

D. C. KLEIN
M. J. BUDA
Neuroendocrinology Unit, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014
C. L. KAPOOR
G. KRISHNA
Section on Drug-Tissue Interaction, Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20014

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Regulatory Role of Guanosine 3′,5′-Monophosphate in Adrenocorticotropin Hormone-Induced Steroidogenesis

Abstract. *The relation between steroidogenesis induced by adrenocorticotropic hormone and the concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) was studied at different time intervals in isolated adrenal cells. Submaximal and supramaximal steroidogenic concentrations of the hormone did not cause detectable changes in cyclic AMP during the first 30 minutes, whereas there was an increase in the concentration of cyclic GMP that was accompanied by phosphorylation and steroidogenesis. It is therefore suggested that cyclic GMP, rather than cyclic AMP, is the physiological mediator of adrenocorticotropic hormone–induced adrenal steroidogenesis.*

Isolated adrenal cells consisting mainly of fasciculata cells (*1*) respond to microunit amounts of adrenocorticotropin hormone (ACTH) (*1*) in the synthesis of corticosterone, have undetectable phosphodiesterase activity (*2*), and can convert the exogenously added precursors, (20*S*)-20-hydroxycholesterol, pregnenolone, progesterone, and deoxycorticosterone to corticosterone (*3*, *4*). This system thus permits direct investigation of ACTH action in an isolated fasciculata cell.

Previous studies (*4*) with this system provided evidence that was not compatible with the hypothesis (*5*) that adenosine 3′,5′-monophosphate (cyclic AMP) is the sole obligatory mediator of ACTH-induced steroidogenesis. Indirect evidence bringing into question the mediatory role of cyclic AMP in adrenal steroidogenesis has also been presented by other laboratories (*6*). In more direct studies (*7*), we showed that the physiological concentration of ACTH, less than 10 μU, stimulated phosphorylation and corticosterone synthesis but did not cause the corresponding increment in cyclic AMP synthesis. Under identical conditions, however, guanosine 3′,5′-monophosphate (cyclic GMP) concentrations were raised with the corresponding increase in the protein kinase activity and corticosterone synthesis (*7*).

To demonstrate unequivocally the correlation of cyclic GMP levels with the ACTH-induced steroidogenesis, we adopted a simple, sensitive, and specific assay for this nucleotide (*8*). We used crude *Escherichia coli* fractions instead of the highly purified *E. coli* polypeptide chain elongation factor Tu (EF-Tu) used by Arai *et al.* (*9*). This method, which is based on the highly specific binding property of the EF-Tu with guanosine diphosphate (GDP) includes the con-

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