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Adenosine 3′,5′-Monophosphate Concentrations and Isoproterenol-Induced Synthesis of Deoxyribonucleic Acid in Mouse Parotid Gland

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SUMMARY

We studied the relationship between the concentration of adenosine 3′,5′-monophosphate (cyclic 3′,5′-AMP) and the rate of synthesis of DNA in the mouse parotid gland. Isoproterenol produced a dose-related increase in the concentration of cyclic 3′,5′-AMP. This increase was maximal within 10 min and persisted longer than 2 hr. The rise of cyclic 3′,5′-AMP in parotid gland was several times greater and longer-lasting than that seen in lung and heart, even though the concentration of isoproterenol in the parotid gland was lower than in lung and heart. Preliminary treatment of mice with aminophylline potentiated the isoproterenol-induced increase of cyclic 3′,5′-AMP in parotid gland. Isoproterenol also increased the incorporation of [^{3}H]thymidine into DNA and the total amount of DNA per gland as measured 28 hr after the injection. These increases were potentiated by prior treatment of the animals with aminophylline. In parotid gland we found a positive relationship between the concentration of cyclic 3′,5′-AMP and the synthesis rate of DNA, whether isoproterenol was administered alone or in conjunction with aminophylline. Moreover, the increased concentration of cyclic 3′,5′-AMP induced by these drugs preceded any changes in the synthesis of DNA. These results suggest that in parotid gland cyclic 3′,5′-AMP may play a role in the acceleration of DNA synthesis elicited by isoproterenol.

INTRODUCTION

In mice or rats, large doses of isoproterenol cause hyperplasia of the salivary glands (1–3). This occurs after a lag period of 24–48 hr and is preceded by several changes of salivary gland biochemistry. An increase of membrane-bound adenylate cyclase activity occurs a few minutes after the intraperitoneal injection of isoproterenol (4) and is followed by a cascade of changes. Within 30 min, the synthesis of acidic nuclear proteins increases (5). This precedes the increased synthesis of RNA (6, 7), nuclear histones (5), and DNA (1–3), and the appearance of an increased number of mitoses (2–3, 8).

Several drugs modify these effects of isoproterenol. Various metabolic inhibitors, such as actinomycin, 5-azacytidine, and cycloheximide, can block this sequence of biochemical events by acting selectively at one of the listed steps above (5, 9). Dichloroisoproterenol and propranolol, but not phenoxybenzamine (2, 10), inhibit the isoproterenol-induced enlargement of salivary glands, suggesting that *beta* receptors are involved. On the other hand, theophylline, an inhibitor of phosphodiesterase activity (11), potentiates the effect of isoproterenol on DNA synthesis (4).

Thus the isoproterenol-stimulated en-

largement of the salivary gland provides a useful model for studying the influence of the sympathetic nervous system on mitotic activity. Indirectly, this model may also provide some information on how catecholamine-induced changes in the properties of cell membranes are linked with the control of biochemical events that occur in the cytoplasm and nuclei.

It has been suggested that adenosine cyclic 3′,5′-monophosphate (cyclic 3′,5′-AMP), through an activation of histone kinase, can modify the activity of repressing and derepressing proteins that control DNA template activity and DNA synthesis (5, 12–14). Since the activation of adenylate cyclase is the first demonstrable biochemical change seen after the injection of isoproterenol, and since in erythroblasts and thymocytes cyclic 3′,5′-AMP has already been shown to influence the synthesis of DNA (15–17), we have studied the quantitative relationships that exist between the concentration of cyclic 3′,5′-AMP in parotid gland and the rate of synthesis of DNA in this gland.

MATERIALS AND METHODS

Male, Swiss, Webster mice (30 g) were allowed free access to food and water until 2 hr before being used in these studies.

The mice were killed by decapitation. The parotid glands were removed, freed from lymph nodes and adhering fat tissue, and frozen on Dry Ice within 30 sec after death.[1] The lungs were rapidly removed and frozen in 5–7 sec. The heart was frozen *in situ* according to the procedure described by Namm and Mayer (18).

Cyclic 3′,5′-AMP assay. The extraction, purification, and assay of cyclic 3′,5′-AMP were carried out as follows. The tissues (25–100 mg) were homogenized in 500 μl of cold 0.4 N perchloric acid containing [^{3}H]cyclic 3′,5′-AMP (0.02 pmole; specific activity, 3 Ci/mmole) to correct for losses during the process of extraction and purification. Solutions of authentic cyclic 3′,5′-AMP (2.5–1000 pmoles) were carried through the method in parallel with tissue samples. Potassium bicarbonate (0.4 M) was added, and the homogenate was adjusted to pH 7.5 with 0.6 M Tris buffer. After centrifugation [the pellets were used for protein assay (19)], 1 ml of the supernatant fluid was placed on a neutral alumina column (4.5 × 0.4 cm, equilibrated with 0.06 M Tris-HCl buffer, pH 7.5) as described by White and Zenser (20). The effluent was discarded, and the cyclic 3′,5′-AMP was eluted with 2 ml of 0.06 M Tris buffer (pH 7.5). This eluate was drained directly onto a cation exchange column (AG50W-X8, 200–400 mesh, H^+ form, 3.5 × 0.4 cm). The resulting effluent was discarded, and the column was then eluted with water. The first 3 ml of eluate were discarded, and the following 2 ml were collected (21). This fraction contained 80–90% of the original [^{3}H]cyclic 3′,5′-AMP and was essentially free from other nucleosides and nucleoside mono-, di-, and triphosphates.

The purified cyclic 3′,5′-AMP was assayed by a modification (22) of the method reported by Ebadi, Weiss, and Costa (23). In this procedure, cyclic 3′,5′-AMP is converted enzymatically to ATP by the combined actions of phosphodiesterase, myokinase, and pyruvate kinase. The ATP is then assayed by the firefly luciferin-luciferase system. Each sample was divided into three portions, two containing phosphodiesterase, myokinase, and pyruvate kinase, and a blank containing myokinase, pyruvate kinase, and an inactivated phosphodiesterase preparation. The light emitted during the luciferin-luciferase reaction was recorded using a luminescence biometer (DuPont) (24). When different samples from the same tissue were carried through the procedure, the error was less than 10%.

The specificity of the method was determined as follows. (a) If the tissue extract was incubated with myokinase and pyruvate kinase but with boiled phosphodiesterase, no production of ATP was observed, and

[1] This time interval was selected because in preliminary experiments we found no significant change in the concentration of cyclic 3′,5′-AMP in mouse parotid gland frozen 15 sec or 5 min after decapitation. Moreover, these levels of cyclic 3′,5′-AMP were the same as those found in parotid glands frozen *in situ* in anesthetized animals.

the readings of the tissue extracts were similar to those obtained when a blank (H_2O) was taken through the procedure. (b) There was a linear relationship between the concentration of tissue and the concentration of cyclic 3′,5′-AMP in the extract. The extrapolation of this line through zero on both the ordinate and abscissa testified to the absence of activators or inhibitors that might interfere with the measurement of cyclic 3′,5′-AMP. (c) Portions of the cyclic 3′,5′-AMP fraction were further purified on silica gel thin-layer chromatography plates (25). The tissue concentrations of cyclic 3′,5′-AMP obtained after this purification step were the same as those obtained without silica gel chromatography.

Extraction of [^3H]isoproterenol. Isoproterenol was extracted from tissue by an adaptation of the procedure used for extracting other catecholamines (26). The tissue was homogenized in 6 volumes of 0.4 N $HClO_4$ containing sodium metabisulfite (0.5 mg/ml). After centrifugation and addition of potassium acetate to raise the pH to 4.5, a portion of the supernatant fluid was placed on a column (3 × 0.4 cm) of a cation exchange resin (Dowex 50W-X4, 200–400 mesh, buffered at pH 6.5). The column was washed with 4 ml of 0.1 M sodium acetate buffer (pH 4.5), 10 ml of the same buffer (pH 6), and 5 ml of 0.4 N HCl, and these eluates were discarded. Isoproterenol was eluted from the column with 10 ml of 1 N HCl. A portion of this fraction was added to a tube containing 500 mg of alumina suspended in 8 ml of 3 M Tris buffer, pH 8.5. These tubes were shaken and then washed twice with water, and the isoproterenol was eluted with 0.1 N HCl. A portion was added to liquid scintillation fluid and was counted for radioactivity. The recovery of standard solutions added to the tissue and carried through the procedure was about 50%.

[^3H]Thymidine incorporation into DNA. The incorporation of thymidine into DNA was studied between 1 and 3 p.m. in mice that had received [^{3}H]thymidine (0.3 μCi/g; specific activity, 19 Ci/mmole) subcutaneously 15 min before decapitation. The DNA was extracted from tissue by the procedure of Munro and Fleck (27). The concentration of DNA was determined by the method of Blobel and Potter (28). The radioactivity of the whole homogenate, the "acid-soluble fraction," and the "DNA fraction" was measured with a liquid scintillation spectrometer.

Materials. Cyclic 3′,5′-nucleotide phosphodiesterase (EC 3.1.4.c), *l*-isoproterenol bitartrate, aminophylline (theophylline ethylenediamine), cyclic 3′,5′-AMP, and other nucleotides and nucleosides were purchased from Sigma Chemical Company. Myokinase (ATP:AMP phosphotransferase, EC 2.7.4.3) and pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) were purchased from Boehringer/Mannheim. Luciferin-luciferase (firefly) was obtained from du Pont.

Analytical grade cation exchange resins, Dowex 50W-X8 and Dowex 50W-X4 (200–400 mesh, H^+ form), were purchased from Bio-Rad Laboratories. Neutral alumina oxide for chromatography was obtained from Alupharm Chemicals (New Orleans). [8-^{3}H]-Cyclic 3′,5′-AMP (specific activity, 3 Ci/mmole) and [7-^{3}H]*dl*-isoproterenol (specific activity, 2 Ci/mmole) were obtained from Amersham/Searle. (*methyl*-^{3}H]Thymidine (19 Ci/mmole) was purchased from Schwarz BioResearch. All the other reagents were obtained commercially in the purest form available.

Statistical methods. Lines of regression were evaluated statistically according to the analysis of variance test. The difference between two regression lines was determined according to the covariance analysis test (29).

RESULTS

Distribution of [^3H]isoproterenol in various mouse tissues. Within 1 min after an intraperitoneal injection of [^{3}H]isoproterenol (600 μmoles/kg) there were measurable concentrations of radioactive drug in the lung, heart, and parotid gland (Fig. 1). In lung and heart the highest levels were found 5 min after the injection, whereas in parotid gland the peak concentration of isoproterenol was found 10 min after the injection. The maximum concentration of isoproterenol obtained in parotid gland was only about one-half that of heart and one-third that of lung. In all tissues the drug levels

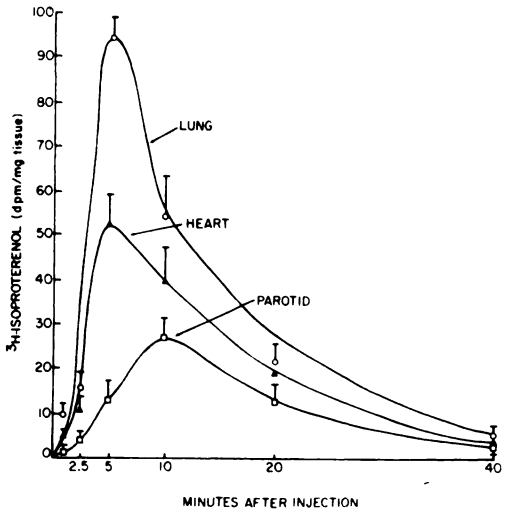

FIG. 1. *Concentration of [^{3}H]isoproterenol in mouse parotid glands, lungs, and hearts*

Mice were treated intraperitoneally with [^{3}H]-isoproterenol (600 μmoles/kg, 0.16 mCi/mmole) and were killed at various times after the injection. Each point represents the mean of three to five experiments. Vertical brackets indicate standard errors.

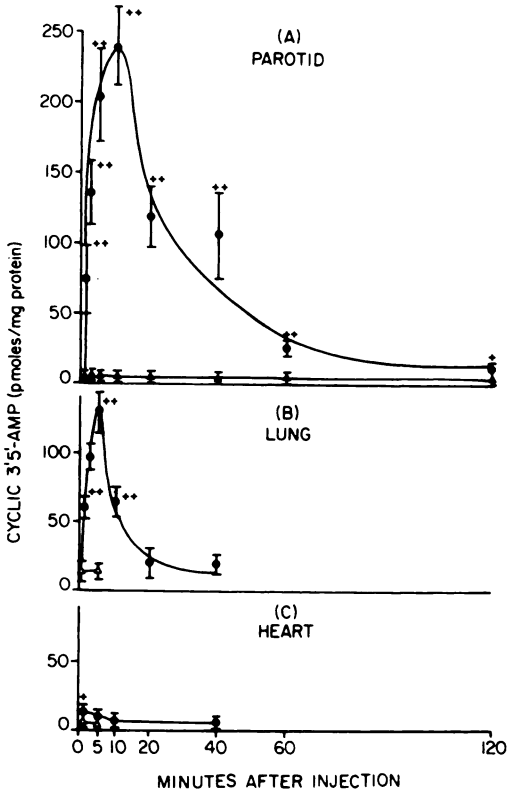

FIG. 2. *Effect of isoproterenol and pilocarpine on concentration of cyclic 3',5'-AMP in mouse parotid glands, lungs, and hearts*

A. Cyclic 3',5'-AMP concentration in mouse parotid gland. Each point represents the mean value of at least six experiments. The levels of cyclic 3',5'-AMP at different times after pilocarpine were not different from those of 0.9% NaCl-treated or untreated mice (5.2 ± 0.5 pmoles/mg of protein; n = 17).

B. Cyclic 3',5'-AMP concentration in mouse lung. Each point represents the mean value of six experiments. Control values = 15 ± 1 pmoles/mg of protein; n = 6.

C. Cyclic 3',5'-AMP concentration in mouse heart. Each point represents the mean value of three experiments. Control values = 4.9 ± 0.4 pmoles/mg of protein; n = 3.

The vertical brackets indicate standard errors. ●——●, isoproterenol (600 μmoles/kg intraperitoneally); ▲——▲, pilocarpine (250 μmoles/kg intraperitoneally); △——△, 0.9% NaCl.

$^+ p < 0.05$ compared with control animals.

$^{++} p < 0.01$ compared with control animals.

decreased markedly from their peak values by 40 min. Prior treatment of mice with aminophylline (200 μmoles/kg intraperitoneally) altered neither the distribution of isoproterenol nor the concentration of total radioactivity.

Effect of isoproterenol on concentration of cyclic 3',5'-AMP in various mouse tissues. Within 1 min after the intraperitoneal administration of isoproterenol (600 μmoles/kg) (a dose producing marked salivation) there was a significant elevation in the concentration of cyclic 3',5'-AMP in mouse parotid gland (Fig. 2). A maximum increase of cyclic 3',5'-AMP, about 50 times higher than controls, was seen 10 min after the injection. Even after 2 hr there was still a significant (2-fold) increase in the concentration of cyclic 3',5'-AMP in the parotid gland. Pilocarpine, given in doses that induce profuse salivary secretion (250 μmoles/kg intraperitoneally), had no significant effect on the concentration of cyclic 3',5'-AMP of mouse parotid gland.

Isoproterenol also increased the concentration of cyclic 3',5'-AMP in lung and heart. However, in these tissues the extent and time course of the increase were quite different from those observed in the parotid gland. In lung the maximum increase of cyclic 3',5'-AMP was seen 5 min after the

injection, and was 10 times the control value. No significant increase was seen after 20 min. In heart the concentration of cyclic 3′,5′-AMP increased about 2-fold 1 min after the injection of isoproterenol, but the levels returned to control values within 5 min.

The data plotted in Fig. 3 show the relationship between the dose of isoproterenol and the concentration of cyclic 3′,5′-AMP in mouse parotid gland measured at the time of the peak effect. Doses of 9.4 μmoles/kg, intraperitoneally, caused a significant elevation in the concentrations of cyclic 3′,5′-AMP. A maximum response was seen at 300μ moles/kg. Higher doses of isoproterenol induced a smaller elevation in the concentration of cyclic 3′,5′-AMP.

Effect of aminophylline on isoproterenol-induced elevation of cyclic 3′,5′-AMP in mouse parotid gland. Aminophylline, in doses of 50 μmoles/kg intraperitoneally, failed to increase significantly the concentration of cyclic 3′,5′-AMP in mouse parotid gland (Fig. 4). Doses of 200 μmoles/kg produced a 2-fold increase ($p < 0.01$) in the concentration of cyclic 3′,5′-AMP at 20 min after the injection; after 40 min the concentration of cyclic 3′,5′-AMP was in the range of control values. Doses of 350 μmoles/kg produced a similar elevation in the concentration of cyclic 3′,5′-AMP, but the peak effect was reached at 10 min and the increase was longer-lasting.

To determine the effect of aminophylline on the isoproterenol-induced elevation of cyclic 3′,5′-AMP, we administered the methylxanthine 30 min before injecting various concentrations of isoproterenol. This treatment potentiated the increase of cyclic 3′,5′-AMP concentrations elicited by isoproterenol (Fig. 5). For example, whereas isoproterenol at doses of 2.3 μmoles/kg induced no change in the concentration of cyclic 3′,5′-AMP when administered alone, it produced almost a 20-fold elevation of cyclic 3′,5′-AMP when administered to mice that had previously received 200 μmoles/kg of aminophylline.

Effect of isoproterenol and aminophylline on incorporation of thymidine into DNA of mouse parotid gland. To study the effect of isoproterenol on the incorporation of thymidine into DNA, mice were treated with various intraperitoneal doses of isoproterenol 28 hr before the administration of [^{3}H]-thymidine. We selected this time interval because preliminary experiments had shown that the incorporation of thymidine was maximal at this time both in mice receiving isoproterenol alone and in animals receiving isoproterenol in conjunction with aminophylline. The mice were killed 15 min after the labeled injection, and the incorporation

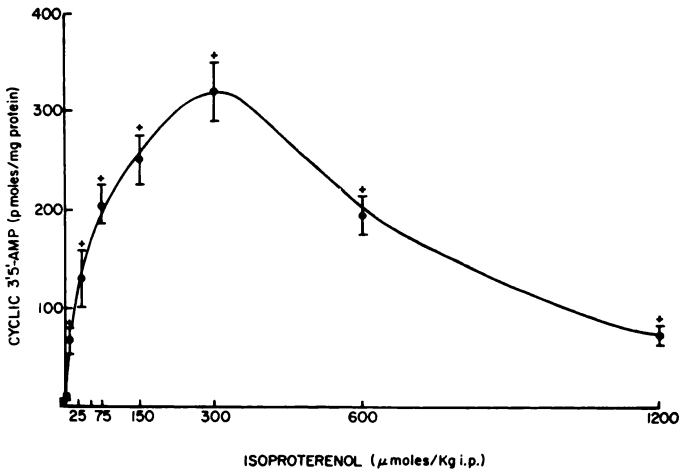

FIG. 3. *Effect of various doses of isoproterenol on concentration of cyclic 3′,5′-AMP in mouse parotid gland*
Mice received isoproterenol intraperitoneally and were killed 10 min later. Each point represents the mean of three to six experiments. Vertical brackets indicate standard errors.
$^+ p < 0.01$ compared with control animals.

of [^{3}H]thymidine into DNA was determined. Increasing amounts of isoproterenol induced a progressive increase in the incorporation of [^{3}H]thymidine into DNA (Fig. 6). Aminophylline, in doses of 200 μmoles/kg, produced no significant effect by itself, but increased the incorporation of [^{3}H]thymidine into DNA when given 30 min before isoproterenol (Fig. 6). Neither drug significantly changed the radioactivity of the "acid-soluble fraction," suggesting that the amount of precursor available for DNA synthesis was unaltered.

The DNA content of parotid glands, calculated on the basis either of wet weight or of protein content, was not changed by any treatment. However, since the weight of the glands was increased after isoproterenol and was increased further after treatment with isoproterenol plus aminophylline, there was a rise in the content of DNA when calculated per parotid gland (see Fig. 7).

The specific activity of DNA of parotid glands, measured 28 hr after the administration of pilocarpine (250 μmoles/kg intraperitoneally), was not significantly different from that of the control values (controls = 8 ± 0.9 dpm/μg of DNA; pilocarpine = 9 ± 1 dpm/μg of DNA). Moreover, pilocarpine failed to alter the weight of the

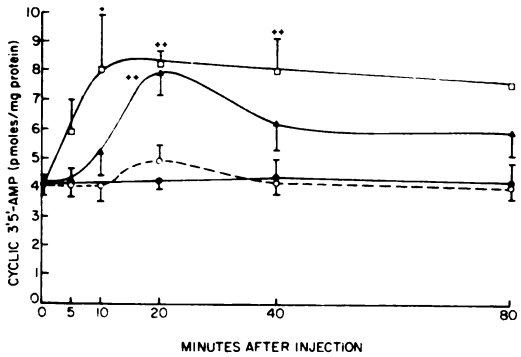

FIG. 4. *Effect of aminophylline on concentration of cyclic 3',5'-AMP in mouse parotid gland*

Each point represents the mean value of three to six experiments. Vertical brackets indicate standard errors. ●——●, 0.9% NaCl; ○- - -○, aminophylline (50 μmoles/kg intraperitoneally); ▲——▲, aminophylline (200 μmoles/kg intraperitoneally); □——□, aminophylline (350 μmoles/kg intraperitoneally).

$^+ p < 0.05$ compared with control animals.

$^{++} p < 0.01$ compared with control animals.

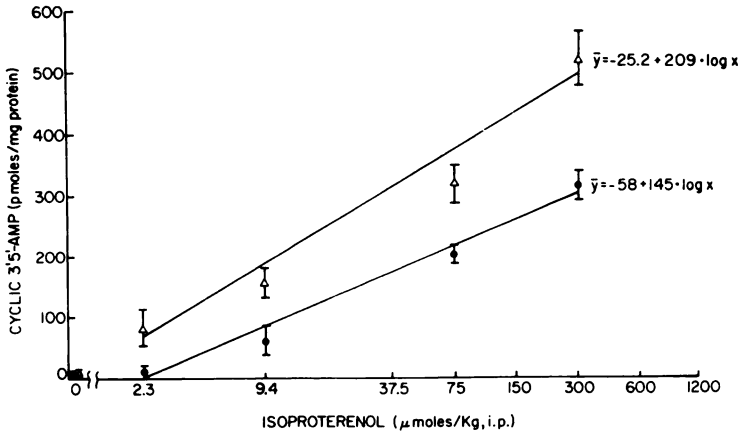

FIG. 5. *Effect of isoproterenol and aminophylline on concentration of cyclic 3',5'-AMP in mouse parotid gland*

Mice were treated intraperitoneally with 0.9% NaCl (10 ml/kg) or aminophylline (200 μmoles/kg). After 30 min the animals were administered isoproterenol and were killed 10 min later. Each point represents the mean of three to six experiments. Vertical brackets indicate standard errors. The concentration of cyclic 3',5'-AMP in parotid glands of mice receiving 0.9% NaCl (4.9 ± 0.5 pmoles/mg of protein) was not significantly different from that of mice receiving aminophylline (6.2 ± 0.6 pmoles/mg of protein). The concentration of cyclic 3',5'-AMP in parotid glands of mice receiving isoproterenol plus aminophylline was statistically higher ($p < 0.001$) than that of mice given isoproterenol alone (covariance analysis test; see MATERIALS AND METHODS). ●——●, without aminophylline; △——△, with aminophylline.

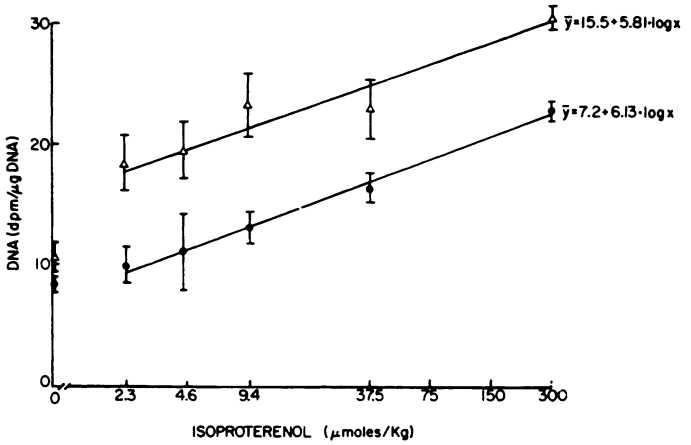

FIG. 6. *Effects of isoproterenol and aminophylline on incorporation of [³H]thymidine into DNA of mouse parotid gland*

Mice were treated by intraperitoneal injection of either 0.9% NaCl (10 ml/kg) or aminophylline (200 μmoles/kg). After 30 min the animals were given various intraperitoneal doses of isoproterenol; 28 hr later [³H]thymidine (0.3 mCi/kg subcutaneously) was injected. The mice were killed 15 min after the administration of [³H]thymidine. Each point represents the mean value of five experiments. Vertical brackets indicate standard errors. The specific activity of DNA in the parotid glands of mice receiving 0.9% NaCl (8.5 ± 0.7 dpm/μg of DNA) was not significantly different from that of mice receiving aminophylline (10.5 ± 1.5 dpm/μg of DNA). The specific activity of DNA in the glands of mice receiving isoproterenol plus aminophylline was statistically higher ($p < 0.001$) than that of mice receiving isoproterenol alone (covariance analysis test; see MATERIALS AND METHODS). ●——●, without aminophylline; △——△, with aminophylline.

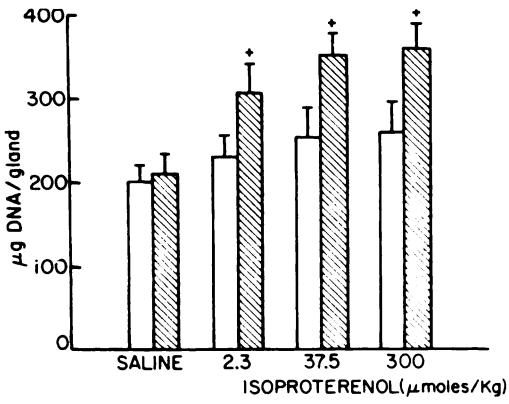

FIG. 7. *Effect of isoproterenol and aminophylline on DNA content of mouse parotid gland*

Mice were treated by intraperitoneal injection of either 0.9% NaCl (10 ml/kg) or aminophylline (200 μmoles/kg). After 30 min the animals were administered isoproterenol and were killed 28 hr later. Each point represents the mean values of 5–10 experiments. Vertical brackets indicate standard errors. Open bars, without aminophylline, hatched bars, with aminophylline.

⁺ $p < 0.05$ compared with animals treated with 0.9% NaCl.

gland or the total amount of DNA in each gland.

DISCUSSION

Studies *in vitro* and *in vivo* on bone marrow cells, lymphoblasts, and inactive lymphocytes of rat and mice show that the elevation of cyclic 3′,5′-AMP induced by catecholamines or other drugs is responsible for the increased synthesis of DNA in eukaryotic cells (15–17). Other lines of evidence suggest that either sympathetic nerve activity or catecholamines can affect the processes that control protein synthesis (12, 30, 31). Moreover, isoproterenol increases the mitotic activity of the acinar cells of rodent parotid gland (3). These findings prompted us to ask whether cyclic 3′,5′-AMP plays a role in the isoproterenol-induced hyperplasia of mouse parotid glands. The results of the present report show that the administration of isoproterenol *in vivo* raises the endogenous concentrations of cyclic 3′,5′-AMP in parotid gland. The concentration of cyclic 3′,5′-AMP reaches a peak in 10 min and

returns to control values in about 2 hr; thus the elevation of cyclic 3′,5′-AMP concentrations shown in this report precedes the acceleration in the synthesis rate of DNA and the increased weight of the gland (3).

The elevation in the concentration of cyclic 3′,5′-AMP seen in the parotid gland lasted longer and was greater than that seen in lung and heart. In an attempt to understand the reason for these differences, we measured the uptake of isoproterenol by lung, heart, and parotid gland. We found that the concentrations of isoproterenol in heart and lung were higher than those in parotid; therefore the higher concentration of cyclic 3′,5′-AMP in parotid gland was not due to a higher concentration of isoproterenol in this tissue. On the other hand, the greater increase in the concentration of cyclic 3′,5′-AMP in parotid gland compared with heart or lung may be attributed to differences in the relative activities of adenylate cyclase and phosphodiesterase. Indeed, phosphodiesterase activity of the salivary gland is among the lowest of all mammalian tissues studied (32). This could explain the marked and long-lasting rise in the intracellular levels of cyclic 3′,5′-AMP following isoproterenol injection.

The increase of cyclic 3′,5′-AMP in parotid gland was proportional to the dose of isoproterenol administered, up to 300 μmoles/kg. With doses of 600 and 1200 μmoles/kg, the levels of cyclic 3′,5′-AMP were lower than those found after 300 μmoles/kg (Fig. 6). This inversion of the dose-response relationship may have been due to toxic (circulatory and metabolic) effects of the high doses of isoproterenol. However, similar inversions of the responses to catecholamines have been noted *in vitro* (33), and it is possible that this is a paradoxical effect of high doses of catecholamines on adenylate cyclase activity (34).

Our main objective was to determine whether there was a correlation between the concentration of cyclic 3′,5′-AMP and the synthesis of DNA in the mouse parotid gland. Several lines of evidence resulting from our experiments indicate that such a correlation exists. The threshold dose of isoproterenol required to increase the concentration of cyclic 3′,5′-AMP in parotid gland was the same as the threshold dose necessary to accelerate the synthesis of DNA in the gland. Moreover, increasing doses of isoproterenol produced a progressive rise of cyclic 3′,5′-AMP and a parallel increase in the incorporation of [^{3}H]thymidine into DNA.

Whether this incorporation of [^{3}H]thymidine into DNA is a valid measure of the synthesis of DNA has been the subject of several investigations. Barka (1, 2) and Pegoraro and Baserga (35) showed that the increased incorporation of thymidine into DNA, after isoproterenol injection, represents an authentic increase in the synthesis of DNA in nuclei. Our experiments showing that in [^{3}H]thymidine-treated mice isoproterenol failed to produce significant changes in the total radioactivity of the "acid-soluble fraction" suggest that the catecholamine does not alter the concentration of the precursor for the synthesis of DNA. Although we have not determined the effect of isoproterenol on the activity of thymidine kinase or DNA polymerase, we have found that isoproterenol, especially when injected with aminophylline, produced a significant increase in the amount of DNA per gland. Therefore, even in the absence of direct measurement of the enzyme involved in DNA synthesis, we can conclude that the increased incorporation of thymidine into DNA represents an authentic increase of DNA synthesis.

If cyclic 3′,5′-AMP mediates the effect of isoproterenol, this action should be potentiated by compounds which prevent the hydrolysis of cyclic 3′,5′-AMP. Indeed, aminophylline, which inhibits phosphodiesterase activity of mouse parotid gland,[2] potentiated the effects of isoproterenol both in raising the concentration of cyclic 3′,5′-AMP and in increasing the incorporation of thymidine into DNA. For example, 2.3 μmoles/kg of isoproterenol did not change the concentration of cyclic 3′,5′-AMP or the amount of thymidine incorporated into DNA. However, the same amount of isoproterenol injected with aminophylline markedly increased the concentration of cyclic 3′,5′-AMP and the incorporation of

[2] Unpublished observations.

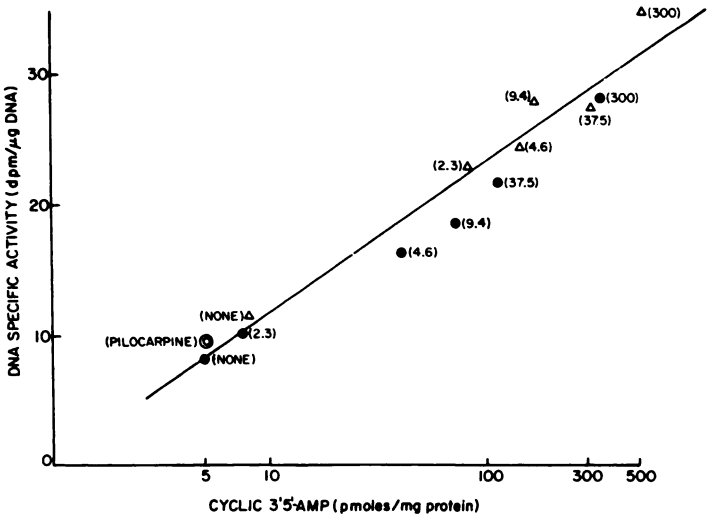

FIG. 8. *Relationship between concentration of cyclic 3',5'-AMP and specific activity of DNA of mouse parotid gland*

●, animals treated with isoproterenol after 0.9% NaCl; △, animals treated with isoproterenol after aminophylline (200 μmoles/kg intraperitoneally); ○, animals treated only with pilocarpine (250 μmoles/kg intraperitoneally). Numbers in parentheses refer to the dosage of isoprotorenal (micromoles per kilogram intraperitoneally). The data were taken from Figs. 5 and 6. Note the linear relationship between the concentration of cyclic 3',5'-AMP and the specific activity of DNA.

[^{3}H]thymidine into DNA. By plotting the specific activity of DNA against the logarithm of the concentration of cyclic 3',5'-AMP, we obtained a linear regression between the two variables (Fig. 8). As can be seen, this relation is independent of the amount of isoproterenol injected.

Several investigators favor the proposal that the process of salivary secretion is dependent upon an increase in the concentration of cyclic 3',5'-AMP (36, 37), and it could be hypothesized that the increased synthesis of DNA following isoproterenol is a consequence of the stimulation of salivary secretion. However, pilocarpine—which also induces salivation and the secretion of large amounts of α-amylase, proteins, and electrolytes (38–40)—fails (a) to change significantly the concentration of cyclic 3',5'-AMP in mouse parotid gland, (b) to induce the early increase in protein synthesis (5), and (c) to change the synthesis of DNA or the weight of the gland.

Speculating on the mechanism whereby cyclic 3',5'-AMP may increase the synthesis of DNA in parotid gland, we may note that Stein and Baserga (5) reported that 30 min after the injection of isoproterenol there was an increased formation of those acidic nuclear proteins which are thought to be involved in regulating DNA synthesis. Since our experiments showed that isoproterenol causes a long-lasting rise in the concentration of cyclic 3',5'-AMP in parotid gland, and since recent evidence implicates cyclic 3',5'-AMP in the phosphorylation of proteins (12–14), which may in turn influence the activity of RNA polymerase, we propose as a working hypothesis that isoproterenol elicits the delayed proliferative response in parotid gland cells through the elevation of cyclic 3',5'-AMP content.

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