ADENYL CYCLASE: RELATIONSHIP TO STIMULATED DNA SYNTHESIS IN PAROTID GLANDS

Daniel Malamud

Fels Research Institute and Department of Pathology
Temple University School of Medicine
Philadelphia, Pennsylvania

Received May 8, 1969

SUMMARY

Parotid adenyl cyclase increases 3-fold within 2.5 minutes after injection of isoproterenol (IPR). Theophylline, an inhibitor of cyclic 3', 5' AMP breakdown, potentiates the effect of IPR on DNA synthesis in parotid glands, suggesting that cyclic 3',5' AMP is involved in the stimulation of DNA synthesis.

Isoproterenol (IPR) stimulates adenyl cyclase activity in a variety of tissues (9). Since this compound also stimulates DNA synthesis in salivary glands of rats and mice (1,2), it was of interest to determine if adenyl cyclase was activated in salivary glands after IPR, and if this activation was related to subsequent DNA synthesis.

MATERIALS AND METHODS

For determination of adenyl cyclase activity by the methods of Streeto and Reddy (8) and Krishna et al. (10), parotid glands from 2 male Swiss mice were homogenized in 0.25 M sucrose. An aliquot of the homogenate was incubated in 0.4 ml of reaction mixture containing 0.06 M Tris HCl buffer, pH 7.8, 6.7×10^{-2} M caffeine, 10^{-2} M $MgSO_4$, 5.7×10^{-3} M phosphoenolpyruvic acid, 1 unit pyruvate kinase, 1.1×10^{-3} M ATP, and 1.25 µC 8-^{14}C-ATP giving a final volume of 0.8 ml. Following incubation at 30°, 0.3 ml of a solution containing 0.2 µC ^{3}H-cyclic 3',5' AMP, 150 µg 3',5' AMP, and 150 µg AMP was added. Tubes were placed in boiling water for 3 min and then

centrifuged for 15 min at 2,000 x g (8). Isolation of 3',5' AMP was carried out on Dowex 50-H^+ columns followed by 2 precipitations of the 3',5' AMP fractions with $ZnSO_4$ and $Ba(OH)_2$ to remove trace contaminants (5). An aliquot of the supernatant was used for scintillation counting in Bray's solution (3) with efficiencies of 15% and 40% for ^3H and ^{14}C respectively. The total amount of ^{14}C- 3',5' AMP was calculated from ^3H counts recovered. The reaction was linear with time for at least 20 min at protein concentrations from 0.5-3.5 mg per flask.

Cyclic 3',5' AMP phosphodiesterase activity was assayed by the method of Weiss and Costa (10). Methods for determination of ^3H-thymidine incorporation into DNA and ^3H-phenylalanine incorporation into protein of salivary glands have been previously described (4,7).

RESULTS

Figure 1 shows the changes in parotid adenyl cyclase with time after injection of IPR. Assay of 3',5' AMP phosphodiesterase showed no changes

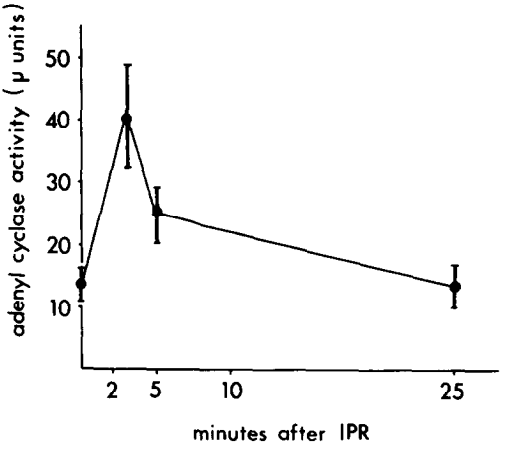

Figure 1. Adenyl cyclase activity in mouse parotid glands after IPR. Mice were injected intraperitoneally with IPR (1 μmole/g) and killed at the times indicated. Adenyl cyclase activity was determined as described in the text. A unit of activity is equal to 1 μmole/mg protein/min. Each point represents the mean ± S.E. for 5 determinations. Values are normalized to the mean of the controls.

in activity of this enzyme up to 30 minutes after injection of IPR, indicating that increased levels of 3',5' AMP were due to an increase in adenyl cyclase activity rather than a decreased phosphodiesterase activity.

Theophylline inhibits cyclic 3',5' AMP phosphodiesterase, thus preventing the breakdown of cyclic 3',5'AMP. If elevated levels of 3',5' AMP are related to subsequent DNA synthesis, theophylline should potentiate the stimulation of DNA synthesis induced by IPR. As shown in Table 1, theophylline alone resulted in a significant increase in parotid DNA synthesis 26 hours later. Furthermore, pretreatment with theophylline

Table 1. Effect of IPR and Theophylline on DNA Synthesis in Mouse Parotid Glands

TREATMENT	No. of Mice	DNA Specific Activity CPM/mg DNA	p
Control	13	821 ± 101	<.005
Theophylline only	10	1,741 ± 278	
IPR only	10	2,251 ± 137	<.005
Theophylline plus IPR	11	3,392 ± 339	

Mice injected intraperitoneally with IPR (.003 μmoles/g), theophylline (.1 mg/g) or theophylline 30 min before IPR. All mice injected subcutaneously with 10 μC ^{3}H-thymidine (6.7 C/mmole) after 26 hours and killed 30 min later. DNA synthesis determined as described previously (4). Values represent mean ± S.E. Probability (p) determined by Student's t test.

increased the stimulation of DNA synthesis resulting from a low dose of IPR. Figure 2 demonstrates the ability of increasing amounts of theophylline to potentiate IPR-stimulated DNA synthesis.

Since theophylline exerted only a modest effect on parotid DNA synthesis, it appeared that there might be a secondary effect of this drug. As shown in Figure 3, ^{3}H-phenylalanine incorporation into parotid gland protein was decreased for 3-5 hours after injection of theophylline.

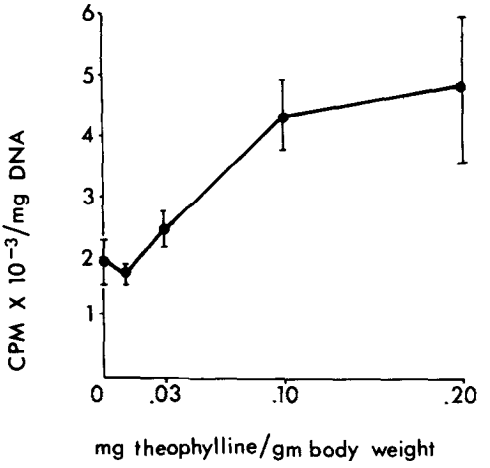

Figure 2. Potentiation of IPR-stimulated DNA synthesis by pretreatment with theophylline.
Mice injected intraperitoneally with IPR (.003 μmoles/g) alone, or 30 min earlier with theophylline. All mice injected subcutaneously with 10 μC ^{3}H-thymidine (6.7 C/mmole) after 26 hours and killed 30 min later. DNA synthesis determined as described previously (4). Values represent mean ± S.E. for 3 mice. Control mice showed a DNA specific activity of 557 ± 27 CPM/mg DNA.

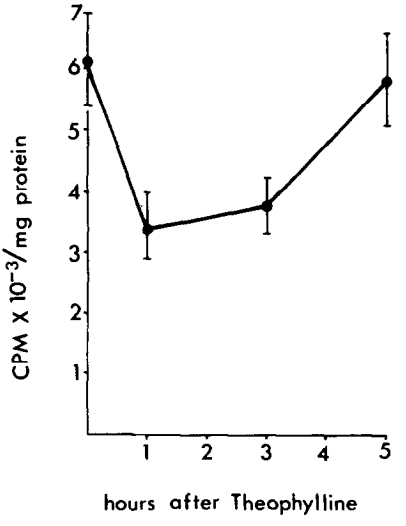

Figure 3. Effect of theophylline on ^{3}H-phenylalanine incorporation into parotid gland protein.
Mice injected intraperitoneally with theophylline (0.1 mg/gm)$_3$and at times indicated injected subcutaneously with 50 μC ^{3}H-phenylalanine (5.3 C/mmole) and killed 45 min later. Protein specific activity determined as previously described (7). Each point is the mean ± S.E. for 3 mice.

DISCUSSION

It has been demonstrated that IPR activates adenyl cyclase in mouse parotid glands. This is consistent with earlier findings that there is a decreased glycogen concentration in mouse salivary glands within 15 minutes after injection of IPR (6).

Theophylline, previously shown to increase salivary gland weight (11), had an additive effect with IPR on stimulated DNA synthesis. It is possible that the full effect of theophylline is not manifest due to an inhibition of protein synthesis. The present study suggests a role for cyclic 3',5' AMP in IPR-stimulated DNA synthesis.

ACKNOWLEDGMENTS

This research was supported in part by grant #P-534 from the American Cancer Society.

REFERENCES

1. Barka, T. Exptl. Cell Res., 39, 355 (1965).
2. Baserga, R. Life Sci., 5, 2033 (1966).
3. Bray, G. A. Anal. Biochem., 1, 279 (1960).
4. Hinrichs, H. R., Peterson, R. O., and Baserga, R. Arch. Pathol., 78, 245 (1964).
5. Krishna, G., Weiss, B., and Brodie, B. B. J. Pharmacol. Exptl. Ther., 163, 379 (1968).
6. Malamud, D., and Baserga, R. Exptl. Cell Res., 50, 581 (1968).
7. Sasaki, T., Litwack, G., and Baserga, R. Manuscript submitted.
8. Streeto, J. M., and Reddy, W. J. Anal. Biochem., 21, 416 (1967).
9. Sutherland, E. W., and Robison, G. A. Pharmacol. Rev., 18, 145 (1966).
10. Weiss, B., and Costa, E. Biochem. Pharmacol., 17, 2107 (1968).
11. Wells, H. Am. J. Physiol., 212, 1293 (1967).