PUROMYCIN: A POTENT METABOLIC EFFECT INDEPENDENT OF PROTEIN SYNTHESIS‡

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The investigation of hormone action frequently requires a decision as to whether or not protein synthesis is involved in the mechanism. This decision is often made on the basis of results with puromycin, an inhibitor of the incorporation of amino acids into protein (Yarmolinsky and de la Haba, 1959). The suitability of this antibiotic for such studies has been questioned due to the fact that its potent effects on glycogen metabolism can be mimicked by puromycin analogues which are ineffective as blocking agents in protein synthesis (Hofert and Boutwell, 1963; Garren et al., 1966; Søvik, 1966). The present work demonstrates that puromycin can produce elevated levels of cyclic 3',5' adenylic acid (cyclic AMP) in rat hemidiaphragms in vitro and that this effect is due partially, if not completely, to the inhibition of cyclic AMP phosphodiesterase. These results are discussed in relation to the apparent involvement of the cyclic nucleotide in a number of hormonal control systems.

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Effect of Puromycin on Cyclic AMP Levels in Rat Hemidiaphragms

Male Wistar rats were killed with an overdose of Nembutal and exsanguinated following decapitation. Diaphragms were quickly removed and placed in 150 mM Tris HCl, pH 7.3, at room temperature. Paired hemidiaphragms were transferred to polyethylene vials containing 5 ml of the same buffer previously gassed with 95°/o O_2 - 5°/o CO_2 to pH 7.0 (Baker and Rutter, 1964). Additions were made and, after being re-gassed, the vials were incubated at 37° with shaking. At the times indicated the tissues were removed, rapidly frozen on an aluminum block cooled in dry ice, weighed, and powdered in a cooled steel mortar. The preparation of the boiled extract from the powdered muscle was carried out by the procedure of Posner et al., (1964) as modified by Hammermeister et al., (1965). An additional treatment involved destruction of nucleotides other than cyclic AMP by the addition of 0.2 mg of Crotalus atrox venom to each sample 20 minutes before it was passed through the Dowex-1 column used in the purification. The lyophilized nucleotide fraction from the columns was taken up in 50 mM glycerophosphate buffer, pH 7.2, equivalent to four times the weight of the hemidiaphragm and adjusted to pH 7.0 with 1 N NaOH. Cyclic AMP was assayed by the activation of muscle phosphorylase b kinase using the method of Posner et al., (1964) modified so that the activation of the kinase and the kinase reaction were carried out simultaneously. Results are reported in micromoles per kilogram of tissue (wet weight).

A typical series of experiments is shown in Table I. Puromycin at a concentration of 0.5 mM in the incubation media produced an increase in the tissue level of cyclic AMP in all experiments although the control varied from animal to animal. These data would explain the increased glycogenolysis in liver and decreased glycogen synthesis in diaphragm which have been observed with this antibiotic (Hofert and Boutwell, 1963; Søvik, 1966), and it may also provide a mechanism for the insulin antagonism of puromycin (Søvik and Walaas, 1964). Butcher et al., (1966) and Exton et al., (1966) have reported that insulin, under appropriate conditions, reduced the level of cyclic AMP in liver

TABLE I

Effect of Puromycin on Cyclic AMP Levels

Experiment	Time of Incubation min	Puromycin	Amount of Cyclic AMP μmoles/10^3g	o/o Increase
1	15	-	1.7	50
		+	2.5	
2	15	-	1.9	75
		+	3.3	
3	30	-	2.8	10
		+	3.0	
4	30	-	1.0	80
		+	1.8	

and adipose tissue. It should be noted that the values obtained for cyclic AMP in these experiments are considerably higher than those required to activate phosphorylase b kinase or to "inactivate" glycogen synthetase in vitro. (Posner et al., 1964; Appleman et al., 1966).

Effect of Puromycin on Cyclic AMP Phosphodiesterase in Diaphragm Extracts

Freshly removed rat diaphragms were freed of fat and homogenized for 30 seconds in a conical glass homogenizer containing 2.5 volumes of cold 50 mM Tris, 3 mM EDTA, pH 7.3. Cell debris was removed by centrifugation at 15,000 x g for 20 minutes, and fat particles were filtered out by passing the supernatant solution through glass wool. 3',5'-cyclic AMP phosphodiesterase was assayed at 30° and pH 7.3 in the presence of 5 mM $MgCl_2$ by measuring the release of adenosine-^{3}H from the tritiated cyclic nucleotide through the combined action of the enzyme and an excess of Crotalus atrox venom (Kemp and Krebs, 1966). At a cyclic AMP concentration of 0.1 mM, one milliliter of the diaphragm extract, containing 6 mg of protein, hydrolyzed 7 mμ moles of the nucleotide per minute.

Figure 1 indicates competitive inhibition of 3',5'-cyclic AMP phosphodiesterase by 1.6 mM puromycin. A K_I of 0.8 mM was obtained from these data. A similar degree of inhibition was observed with the puromycin analogue,

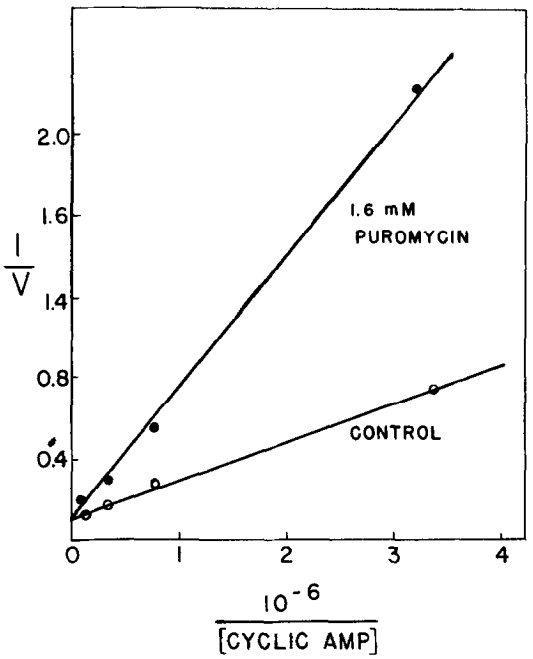

Figure 1

Inhibition of 3',5'-cyclic AMP phosphodiesterase by puromycin

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dimethylaminopurine, which, as shown by Hofert and Boutwell (1963), elicits glycogenolysis in rat liver while not significantly decreasing amino acid incorporation into liver protein.

Cyclic AMP phosphodiesterase activities in extracts of rabbit skeletal muscle and rat adipose tissue also were inhibited by 1.5 mM puromycin. The methyl xanthines, caffeine and theophylline, which have previously been shown to be competitive inhibitors of the phosphodiesterase of beef heart (Butcher and Sutherland, 1962), similarly affect the activity in extracts prepared from rat diaphragm and the above mentioned tissues.

At least seven hormones interact with tissues to produce changes in the concentration of cyclic AMP (Sutherland et al., 1965). The results described

above indicate that puromycin inhibits cyclic AMP phosphodiesterase leading to increased cyclic nucleotide levels in rat diaphragm. Therefore, it is apparent that caution must be used in interpretation of data obtained when puromycin is employed as an inhibitor of protein synthesis, particularly in hormone studies.

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