References and Notes

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7. We thank J. B. Hanson for helpful suggestions and C. Pinkham for technical assistance. Supported in part by a grant from the Office of Saline Water, Department of the Interior (14-01-0001-2153).

19 September 1969; revised 5 December 1969

δ-Aminolevulinic Acid Synthetase: Induction in Embryonic Chick Liver by Glucagon

Abstract. *Glucagon elicits a twofold increase in δ-aminolevulinic acid synthetase activity in the livers of 18-day-old chick embryos. This rise occurs when RNA synthesis is inhibited, but is prevented when protein synthesis is blocked. Cyclic adenosine monophosphate appears not to be involved.*

The first step in heme biosynthesis is the condensation of glycine and succinyl coenzyme A to form δ-aminolevulinic acid (ALA). This reaction is mediated by ALA synthetase, the rate-limiting enzyme in the pathway (*1*). This enzyme can be induced in embryonic chick liver by barbiturates, collidines, steroids, and certain other substances (*2*).

Acute intermittent porphyria is an inborn error of metabolism. Biochemically it is characterized by an excessive production of ALA and porphobilinogen. The primary lesion is an elevation in hepatic ALA synthetase (*3*). A high carbohydrate diet leads to a reduction in the formation of porphyrin precursors in patients with acute intermittent porphyria (*4*). Moreover, the induction of ALA synthetase in rat liver is blocked by glucose (*5*). To further our understanding of the relationship between carbohydrate metabolism and the regulation of heme synthesis, I investigated the effect of glucagon on the activity of ALA synthetase in embryonic chick liver.

Fertilized White Rock eggs were obtained from a local hatchery and kept at 38°C in a forced draft incubator. The samples under investigation were glucagon, cycloheximide, adenosine 3′,5′-monophosphate (cyclic AMP), and N^6,$O^{2'}$-dibutyryladenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) (Sigma Chemical Co.), and dactinomycin (Merck Sharp and Dohme).

Each drug, dissolved in 0.5 ml of saline, was injected into the air space through a hole made in the shell of 18-day-old eggs. Glucagon was administered as a suspension. The hole was then closed with cellophane tape. Six hours later the ALA synthetase activity in individual livers was determined (*6*). The ALA synthetase activity in the liver of an 18-day-old chick embryo was around 8 nmole of ALA formed per gram of liver per hour and was relatively uniform from one individual to another (Table 1). The administration of glucagon resulted in an approximately twofold increase in the activity of the enzyme in 6 hours. The response to the hormone varied with only about half of the individuals showing an increase above normal. The increase induced by glucagon was not prevented by dactinomycin while dactinomycin alone was without effect. However, in the presence of cycloheximide this increase was inhibited.

To determine whether glucagon acts by way of cyclic AMP, cyclic AMP was injected into eggs and no effect was seen (Table 1). In the induction of tyrosine aminotransferase by cyclic AMP in fetal rat liver, more pronounced effects are achieved with its analog, dibutyryl cyclic AMP (*7*). However, dibutyryl cyclic AMP, when injected into eggs, was also ineffective in increasing the activity of ALA synthetase.

The inclusion of cyclic AMP in the assay medium at concentrations from 10^{-5} to $10^{-3}M$ had no effect on the activity. Similarly, glucagon ($10^{-7}M$) in the assay medium was without effect.

The induction of ALA synthetase in avian and mammalian liver by a variety of compounds led to a 10- to 40-fold increase in the activity of the enzyme (*5, 8, 9*), whereas the increase induced by glucagon was only twofold. However, this small increase is the result of protein synthesis as implied by its inhibition by cycloheximide.

The massive inductions of ALA synthetase in both chick and rat liver are subject to genetic regulatory mechanisms (*2, 9*). Because the increase induced by glucagon occurs in the presence of dactinomycin, an inhibitor of RNA synthesis, the regulatory mechanism is nongenetic.

Stimulation of glycogenolysis by glucagon is mediated by cyclic AMP (*10*). Glucagon activates adenyl cyclase, thus enhancing the production of cyclic AMP, which in turn activates phosphorylase. Because cyclic AMP has no effect on ALA synthetase activity when supplied to the embryo or when added to the homogenate, it is concluded that it is not involved in the induction.

The activity of ALA synthetase in fasted rats ranges from 8 to 24 nmole of ALA per gram per hour while it varies only from 4 to 15 nmole of ALA per gram per hour in fed rats (*6*). Fasting stimulates the release of glucagon by the pancreas. Thus the higher concentration of glucagon after fasting probably induces ALA synthetase in the rat. However, in their study of the induction of ALA synthetase in mature rat liver, Marver *et al.* (*1*) found that glucagon was not an inducer. The difference between their findings and mine may be developmental in origin. Greengard (*7*) has shown that glucagon is an inducer of tyrosine aminotransferase in fetal rat liver but that responsiveness to glucagon in the liver of the adult is lost. Alternatively, the lack of similarity between the observations could arise from a peculiarity of avian liver in view of the demonstration (*9*) that certain steroids are potent inducers of ALA synthetase in embryonic chick liver whereas they are ineffective in mammals.

Table 1. Effect of various compounds on ALA synthetase activity (nanomoles of ALA formed per gram per hour).

Compound	Embryos (No.)	Activity
None	6	8 ± 3
Glucagon (0.25 mg)	12	15 ± 10 ($P < .025$)*
Glucagon (0.25 mg) + dactinomycin (0.25 mg)	8	16 ± 7 ($P = .015$)*
Dactinomycin (0.25 mg)	8	6 ± 4
Glucagon (0.25 mg) + cycloheximide (5 μg)	8	5 ± 5
Cyclic AMP (0.5 mg)	8	6 ± 6
Dibutyryl cyclic AMP (0.5 mg)	4	10 ± 3

* Significantly different from untreated embryos with P calculated according to the Mann-Whitney test.

The ALA synthetase in rat liver has a half-life of about 70 minutes (*1*). It is possible that glucagon achieves its rapid low-amplitude induction by promoting conditions which retard the rapid destruction of already present enzyme.

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21 November 1969; revised 15 January 1970

Polyinosinic Acid • Polycytidylic Acid: Inhibition of DNA Synthesis Stimulated by Isoproterenol

Abstract. *Polyinosinic acid • polycytidylic acid (poly I • poly C) inhibits isoproterenol-stimulated DNA synthesis in salivary glands of mice. A single intraperitoneal injection of 250 micrograms of poly I·poly C inhibits the stimulation of DNA synthesis when given 10 minutes before isoproterenol or at any time during the 20-hour lag period between the injection of isoproterenol and the subsequent DNA synthesis. The inhibition caused by poly I·poly C is not due to a generalized toxic action but seems to be exerted through a relatively selective mechanism. Polyuridylic acid and diethylaminoethyl dextran are less effective in inhibiting isoproterenol-stimulated DNA synthesis.*

The synthetic double-stranded RNA, polyinosinic acid • polycytidylic acid, poly I • poly C induces synthesis of interferon in mammalian cells (*1*) and inhibits the growth of certain virus-induced sarcomas and leukemias in mice, as well as of some other tumors not known to contain infectious oncogenic viruses (*2, 3*). Since these results did not conclusively show that the mechanism of action of poly I • poly C was antiviral, we investigated its effect on the stimulation of DNA synthesis and cell division that is caused in salivary glands of mice by isoproterenol (IPR), a synthetic catecholamine (*4*). Both poly I • poly C and the ammonium salt of polyuridylic acid (poly U) (*5*) were stored at 4°C; they were dissolved shortly before use in distilled-deionized water. Diethylaminoethyl (DEAE) dextran was stored at room temperature. Isoproterenol [2-(3,4-dihydroxyphenyl)2-isopropylaminoethanol hydrochloride] was dissolved in water at a concentration of 4.0 mg/0.2 ml immediately before intraperitoneal injection into male Swiss mice weighing about 30 g. [^{3}H]Thymidine (10 μc) was injected subcutaneously into each mouse 26 hours after the administration of IPR (the peak of DNA synthesis in mice) (*6*), and the animals were killed 30 minutes later by cervical dislocation. The salivary glands were quickly dissected from adhering fat and lymph nodes. The three major salivary glands (submaxillary, sublingual, and parotid) from each mouse were pooled and homogenized in 0.25*M* sucrose containing 5 percent citric acid. The homogenates were extracted by the method of Scott *et al.* as modified by Hinrichs *et al.* (*7*), to obtain the specific activity of salivary gland DNA.

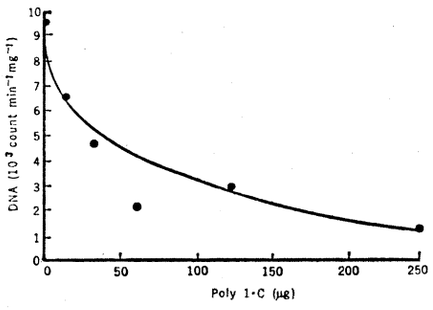

Fig. 1. The effect of increasing amounts of poly I • poly C on isoproterenol-stimulated DNA synthesis in salivary glands of mice. Poly I • poly C was given 10 minutes before isoproterenol, and the specific activity of salivary gland DNA was determined 26.5 hours after injection of isoproterenol and 30 minutes after an injection of tritiated thymidine.

The effect of a single intraperitoneal injection of 250 μg of poly I • poly C, poly U, or DEAE dextran 10 minutes before the injection of IPR on DNA synthesis in mouse salivary glands is shown in Table 1. Whereas DEAE dextran and poly U have only a moderate effect, poly I • poly C almost completely inhibits the stimulation of DNA synthesis caused by IPR in the salivary glands of mice.

The inhibition of IPR-stimulated DNA synthesis by poly I · poly C is dependent on the dose used (Fig. 1). As little as 15 μg of poly I · poly C produced a 30-percent inhibition of DNA synthesis.

When poly I · poly C (250 μg) was injected at the intervals, before or after IPR as indicated in Fig. 2, and DNA synthesis was tested 26 hours after IPR was administered, the stimulation of DNA synthesis was inhibited, under these conditions, by a single intraperitoneal injection of poly I · poly C from 10 minutes before until 18 hours after injection of IPR. After DNA synthesis began (about 20 hours after IPR), the injection of poly I · poly C was without effect. Neither poly U nor DEAE dextran caused an inhibition of DNA synthesis at any time after IPR (not shown).

It has been reported that poly I · poly C has some toxic effects on rabbits (*8*). The fact that poly I · poly C does not interfere with DNA synthesis once synthesis has begun seems to rule out a generalized toxic effect. This hypothesis was confirmed by the following experiment. A single injection of IPR causes a marked decrease in the α-amylase activity of salivary glands of rats

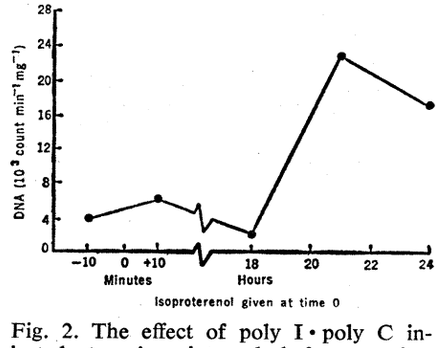

Fig. 2. The effect of poly I • poly C injected at various intervals before or after isoproterenol, on isoproterenol-stimulated DNA synthesis in mouse salivary glands. On the abscissa is the time before or after isoproterenol at which poly I • poly C was injected. On the ordinate is the specific activity of salivary gland DNA as determined 26.5 hours after injection of isoproterenol and 30 minutes after an injection of tritiated thymidine.