MECHANISM OF PARATHYROID HORMONE ACTION: EFFECTS OF ACTINOMYCIN D ON HORMONE-STIMULATED ION MOVEMENTS IN VIVO AND IN VITRO[1,2]

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Activation or stimulation of the biochemical expression of genetic information has been shown to be an important action common to several, but not to all, hormones (Edelman, et al., 1963, Greengard and Acs, 1962, Steiner and King, in press, and Ui and Mueller, 1963). As DNA-directed synthesis of RNA is necessary for the normal expression of genetic information, specific inhibition of this RNA synthesis by the antibiotic, Actinomycin D (Reich, et al., 1962), has been used to investigate the proposed "genotropic" action of certain hormones. It should be recognized that antagonism of the action of a hormone by Actinomycin D (actinomycin) does not prove that the hormone acts primarily on the gene. Such antagonism indicates that genetic activity must be intact at the time the hormone is acting, or that a short-lived product of gene activity is an essential mediator of hormone action.

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The calcium-mobilizing and phosphaturic actions of parathyroid hormone are mediated by unknown biochemical mechanisms. The experiments reported here were performed to investigate whether these classical acute actions of parathyroid hormone could be blocked by actinomycin, indicating a dependence of hormone action upon DNA-directed RNA synthesis. A model system now being used to examine the biochemical effects of the hormone is its stimulation of the uptake of inorganic phosphate (P_i) by isolated liver mitochondria (Rasmussen and DeLuca, 1963). The effects of actinomycin upon this *in vitro* system were also studied.

MATERIALS AND METHODS

The calcium-mobilizing and phosphaturic effects of parathyroid hormone were tested *in vivo* in acutely parathyroidectomized, male, Holtzman rats by modifications, described in Table 1, of the methods of Munson (1961) and Kenny and Munson (1959), respectively. Rats were given a low calcium diet 3 days before the experiments were performed. Plasma and urine creatinines were determined by the method of Bonsnes and Taussky (1945).

The parathyroid hormone preparations were either highly purified parathyroid hormone (PTH) or crude U.S.P. Parathyroid Extract (PTE) (Eli Lilly Co.). The preparation of PTH (20,000 $\overset{\times}{\div}$ 1.27 units/mg N) involved phenol extraction of an acetone powder of bovine parathyroid glands (Aurbach, 1959) followed by final purification by the gel filtration method of Rasmussen and Craig (1962), except that Sephadex G-100 was used in place of G-50.

Actinomycin, a gift from Merck, Sharpe and Dohme, freshly dissolved in 100% ethanol, was diluted (1:20) to a final concentration of 100 µg/ml in saline (0.15 M) and administered intravenously in a 1.0 ml dose.

TABLE 1

Treatment	No. of rats	Plasma calcium[a] (mg/100 ml)	Urinary P_i excretion[a] (μg/rat/hr)
		Experiment No. 1	
Ethanol + HCl	7	6.0 ± 0.39	48 ± 10
+ PTH	6	8.9 ± 0.42	418 ± 73
Actinomycin + HCl	6	5.9 ± 0.42	55 ± 11
+ PTH	7	6.6 ± 0.39	527 ± 68
		Experiment No. 2	
Ethanol + HCl	5	5.9 ± 0.31	70 ± 29
+ PTH	5	9.9 ± 0.31	486 ± 80
+ PTE	5	9.1 ± 0.31	432 ± 87
Actinomycin + HCl	5	6.0 ± 0.31	82 ± 29
+ PTH	4	6.5 ± 0.34	215 ± 90
+ PTE	5	6.6 ± 0.31	308 ± 87

[a]Mean ± standard error

Groups of rats (90-130gm) were injected with 5% ethanol in saline (Ethanol) or with 100 μg actinomycin (Actinomycin). Three hours later all rats were parathyroidectomized (Munson, 1961) and injected subcutaneously either with HCl (0.001 N), PTH (19 μg), or PTE (60 units). Each rat was then placed in a separate metabolism cage. After 4 hours of urine collection (7 hours after actinomycin) all animals were sacrificed and blood obtained by cardiac puncture.

Rat liver mitochondria were prepared and incubated using the techniques described by Sallis et al. (1963) except that the incubation medium contained 10 mM $MgCl_2$.

Statistical treatment of the data was by analysis of variance.

RESULTS

Rats receiving actinomycin 3 hours before parathyroidectomy showed the same rapid post-operative fall in plasma calcium and urinary P_i excretion as control animals receiving ethanol (Table 1). Both PTH and PTE were found to produce a phosphaturic response in actinomycin treated animals, as well as in ethanol treated controls.

The actinomycin treated animals, however, failed to respond to the calcium-mobilizing action of either hormone preparation.

In another experiment, one group of five rats was injected with actinomycin while a similar control group received ethanol. Both groups were bled 7 hours later. Neither group was subjected to parathyroidectomy. There was no evidence for a calcium lowering effect of actinomycin, the mean plasma calcium being 9.2 $\pm$ 0.13 mg/100 ml in the actinomycin treated group and 9.4 $\pm$ 0.13 mg/100 ml in the control group.

Plasma creatinines from animals in Experiment No. 1 (Table 1) receiving actinomycin and ethanol, but no hormone, were 0.48 $\pm$ 0.02 and 0.54 $\pm$ 0.02 mg/100 ml, respectively, while urine creatinines from the same two groups were 0.10 $\pm$ 0.01 and 0.13 $\pm$ 0.03 mg/hr. Plasma and urine values from the same groups in Experiment No. 2 were similar. Since only the plasma creatinines at the end of urine collections were known, proper creatinine clearances could not be calculated. It would seem unlikely from these data, however, that actinomycin injection produced a large change in the clearance of creatinine.

The results in Table 1 indicate that PTH, administered subcutaneously to an actinomycin treated animal, was able to reach at least one of its target organs (kidney) in significant amounts. In a separate experiment designed to test possible effects of actinomycin on the absorption and transport of another protein hormone, a group of 5 rats was bled from the tail 3 hours after receiving actinomycin and was then given crystalline insulin (0.5 U/rat) subcutaneously. Subsequent blood samples at 20, 40 and 60 minutes gave glucose levels respectively of 59, 57 and 36% of control. These results indicate, in agreement with those of Steiner and King (1964), that actinomycin does not block the hypoglycemic effect of insulin.

The *in vitro* stimulation by PTH of P_i uptake by isolated liver mitochondria was not diminished by pretreatment of the donor rats with actinomycin (Table 2). Likewise, preincubation of mitochondria with actinomycin (0.1 to 1.0 μg/flask) for 5 minutes before addition of PTH caused no inhibition of the effect of the hormone on P_i uptake.

TABLE 2

In vitro treatment	Mitochondrial P_i uptake[a]	
	Control	Actinomycin
	(μM P_i/mg N)	
None	1.6 ± 0.25	1.5 ± 0.25
PTH	3.7 ± 0.25	3.6 ± 0.25

[a]Mean ± standard error

Two groups of 4 rats (90-130 gm) each were injected either with 100 μg of actinomycin or with 5% ethanol in saline. Seven hours later the animals were sacrificed and liver mitochondria isolated from each of the 8 animals. Three aliquots from each of the mitochondrial samples were incubated separately in a P_i containing medium, both with and without added highly purified PTH (100 μg/flask) for 25 minutes and P_i uptake determined (Sallis *et al.*, 1963).

CONCLUSIONS

The data presented show that in the actinomycin treated, parathyroidectomized rat, bovine parathyroid hormone fails to exert its usual calcium-mobilzing action. The dose of actinomycin used in these experiments was found to be lethal in 10 to 20% of animals, but not until 96 hours after administration. As the only known direct effects of actinomycin are on the DNA-directed synthesis of RNA and DNA, it is likely that the calcium-mobilizing action of exogenous parathyroid hormone requires intact genetic mechanisms.

Actinomycin was not observed to interfere with the ability of endogenous hormone to maintain normal plasma calcium levels during the 7-hour time interval of these experiments. At present this apparent difference between exogenous and endogenous hormone cannot be explained.

The production by exogenous hormone of a phosphaturic response in the absence of any calcium-mobilizing response suggests that the two cardinal actions of the hormone may depend upon different mechanisms. It is possible that longer time intervals or larger actinomycin doses may make animals unresponsive to both actions. From the present data, however, it appears that calcium mobilization may require the concurrent synthesis of RNA while increased P_i excretion does not. The rapid (within 5 minutes) production of phosphaturia by infusion of parathyroid hormone into the renal artery of the dog (Hirsch and Munson, in press) is consistent with the idea that the enzymic machinery for this response is immediately available.

The equal responsiveness *in vitro* of liver mitochondria from actinomycin treated and control animals is consistent with the hypothesis (Rasmussen and DeLuca, 1963) that the hormone induced uptake of P_i by such mitochondria represents a valid model for the action of the hormone on the kidney tubule.

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