

AN EFFECT OF ANTERIOR PITUITARY HORMONES ON BOVINE CORPUS LUTEUM PHOSPHORYLASE

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Stimulation of adrenal phosphorylase by adrenocorticotrophic hormone (ACTH) has been reported by Haynes and Berthet (1957) as a possible biochemical site of action of this hormone. Haynes suggested that this effect of ACTH made more TPNH available for steroid hormone synthesis. Mason *et al.* (1961) have shown that chorionic gonadotrophin *in vitro* increased the output of progesterone from bovine corpus luteum slices. The observation that TPNH also stimulated progesterone synthesis suggested a system similar to that proposed by Haynes for the adrenal. The present study was undertaken to examine the effect of anterior pituitary hormones on bovine corpus luteum phosphorylase.

MATERIALS AND METHODS

Bovine ovaries were obtained from the abbatoir and kept on ice until use. Slices of corpora lutea weighing approximately 100 mgm. were made with a Stadie-Riggs microtome and incubated on a Dubnoff metabolic shaker for 30 minutes at 37° in 1 ml. of Krebs-Ringer bicarbonate buffer (pH 7.4) in an atmosphere of 95% O₂ and 5% CO₂. Following the incubation each hormone dissolved in 1 ml. of buffer was added to the appropriate flask and incubation continued for another 30 minutes. One ml. of

buffer was added to flasks containing control slices. At the end of incubation the slices were homogenized in 1 ml. of 0.1M sodium fluoride and phosphorylase was then determined in the homogenate as previously reported (Williams and Field, 1961).

Human chorionic gonadotrophin (2000 units per ml.) was obtained from Ayerst Laboratories, Inc. and ACTH (0.5 units per mg.) from the Upjohn Company. Follicle stimulating hormone, luteinizing hormone, growth hormone and prolactin were gifts of the Endocrinology Study Section of the National Institutes of Health. Thyroid stimulating hormone (8.7 units per mg.) was the gift of Dr. Peter Condliffe of the National Institutes of Health. The anterior pituitary extract was made by homogenization of a whole bovine anterior pituitary weighing 450 mgm. in 10 ml. of buffer followed by centrifugation at 1600 x g for 15 minutes. Glucagon was obtained from Eli Lilly and Company.

RESULTS

The effects of anterior pituitary extract and the various hormones on bovine corpus luteum phosphorylase are outlined in Table I. Pituitary extract, chorionic gonadotrophin, and growth hormone all had a highly significant stimulatory effect on the level of active phosphorylase, while follicle stimulating hormone, thyroid stimulating hormone, luteinizing hormone, prolactin, and ACTH had no significant effect. Glucagon significantly decreased the phosphorylase in the corpus luteum.

The wide variation in control levels of phosphorylase may be related to differences in ages of the various corpora lutea used. The control levels of phosphorylase are comparable to those found in the liver, and much higher than that found in the remainder of the ovary or other endocrine glands (Williams, unpublished data). Neither chorionic gonadotrophin nor the

TABLE I

Effect of Various Hormones on Bovine Corpus Luteum Phosphorylase

Hormone	Amount	Phosphorylase in mgm P liberated/gm tissue		P value
		Control	After Hormone	
Pituitary Extract (3)	1 ml	1.74 \pm .10*	2.42 \pm .07	<0.01
Chorionic Gonadotrophin (8)	2000 units	2.08 \pm .38	2.79 \pm .33	<0.01
Follicle stimulating hormone (6)	1 mg	5.38 \pm .89	5.57 \pm .47	n.s.
Thyroid stimulating hormone (6)	0.1 mg	5.38 \pm .89	5.96 \pm .55	n.s.
Luteipizing hormone (6)	1 mg	3.01 \pm .34	2.98 \pm .66	n.s.
Prolactin (3)	1 mg	0.79 \pm .20	0.73 \pm .23	n.s.
Adrenocorticotrophin hormone (3)	1 unit	0.79 \pm .20	0.54 \pm .32	n.s.
Growth hormone (6)	1 mg	1.54 \pm .16	3.21 \pm .67	<0.01
Glucagon (6)	1 mg	1.54 \pm .15	1.03 \pm .15	<0.01

Figures in parentheses represent number of flasks in each experiment.

* Standard error of the mean.

anterior pituitary hormones had an effect on whole ovary phosphorylase.

DISCUSSION

The stimulatory effect of chorionic gonadotrophin on corpus luteum phosphorylase may represent a mechanism of action of this hormone on increasing progesterone synthesis; a mechanism similar to that proposed by Haynes (1958) for the effect of ACTH on adrenal

phosphorylase. TPNH has been shown to be involved in progesterone synthesis (Constantopoulos and Tchen, 1961) and in vitro addition of TPNH to bovine corpus luteum increased progesterone release into the medium (Mason, et al. 1961). The crude pituitary extract used here had a significant stimulatory effect on corpus luteum phosphorylase, which may be due to its gonadotrophin content, as most of the other anterior pituitary hormones except growth hormone had no effect on phosphorylase activity. The stimulation of corpus luteum phosphorylase by growth hormone is unusual as growth hormone is not known to have an effect on hormone synthesis in the corpus luteum. On the other hand, luteinizing hormone has been shown to stimulate progesterone output from corpus luteum slices (Mason et al. 1961), yet this hormone has no effect on phosphorylase activity. This suggests a different site of action of luteinizing hormone on progesterone synthesis.

SUMMARY

In vitro addition of a crude anterior pituitary extract, chorionic gonadotrophin, and growth hormone to bovine corpus luteum slices increases the amount of active phosphorylase in the tissue. Prolactin, luteinizing hormone, ACTH, follicle stimulating hormone and thyroid stimulating hormone had no significant effect on corpus luteum phosphorylase. Glucagon decreased phosphorylase activity in the corpus luteum.

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