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Received June 30, 1967. P.S.E.B.M., 1967, v126.

Effects of Cortisol and Diethylstilbestrol on Growth Hormone Release by Rat Pituitary *in vitro*.* (32440)

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The secretion of growth hormone in response to hypoglycemia and the administration of arginine in man has been shown to be greatly influenced by certain non-pituitary hormones. Corticosteroids decrease growth hormone response to these stimuli in man (1,2) while estrogens and the synthetic estrogenic compound diethylstilbestrol facilitate the release of growth hormone(3,4). The mechanism of action of these modifying effects has not been established. Corticosteroids have little effect on the concentration of growth hormone in the pituitary of the normal rat(5). If corticosteroids are administered during the induction of hypoglycemia, the expected drop in growth hormone content is inhibited(5). Estrogens on the other hand decrease the concentration of growth hormone even when compared to pair fed control rats(6).

The modifying effects of estrogens and corticosteroids on growth hormone secretion could be exerted either at the level of the hypothalamus by affecting the secretion of the somatotropin releasing factor or these hormones could affect the secretion of growth hormone by acting directly on the somatotropic cells themselves. The observations of Pecile and Müeller(5) have suggested that cortisol may deplete the hypothalamus of somatotropin releasing factor. In their experiments hypothalamic extracts from cortisol treated animals had a much reduced capacity to cause a depletion of bioassayable pituitary growth hormone in recipient animals when compared to hypothalamic extracts from normal rats. Cortisol-treated recipient rats retained their ability to respond with a depletion of bioassayable growth hormone when injected with hypothalamic extracts from

* Supported by Research Grant AM-5105, Nat. Inst. Health.

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normal rats. Although one can not have complete confidence in the specificity of bioassay of crude pituitary extracts, these observations suggest that cortisol affects growth hormone release at the level of the hypothalamus.

Despite these observations we felt that it was important to study possible influences of corticosteroids and estrogens directly on the pituitary in the absence of hypothalamic tissue. These studies have been greatly facilitated by the development of a radioimmunoassay for rat growth hormone(6,7,8).

Methods. Using sterile technique the adenohypophyses of male Sprague-Dawley rats (110 to 150 g) were removed, hemisected, weighed and transferred to a capped glass culture tube (10 × 65 mm) containing 1.0 ml culture media TC 199 in Earle's salt mixture. Each tube was incubated at 37°C during gentle agitation in a Dubnoff shaker and was continuously gassed with a humidified mixture of 95% O_2 and 5% CO_2. An initial incubation period (Period I) of 12 hours was carried out to compare growth hormone release from 2 hemipituitaries. It was found in each case that the 2 anterior pituitary fragments released hormone at similar rates, when expressed as millimicrograms of growth hormone released per milligram of wet pituitary. Therefore, one hemipituitary was able to serve as a control while the effects of additions to the culture medium could be observed on the other.

Following Period I, medium containing either cortisol in the concentration of 2.75×10^{-5}, 5.5×10^{-6}, or 1.28×10^{-6} molar (10, 2, 0.5 μg/ml) or diethylstilbestrol (Stilbestrol) 1.88×10^{-4}, 7.5×10^{-6} or 1.88×10^{-6} molar (50, 2, 0.5 μg/ml) was added for 2 subsequent incubation periods (Period II and III) of 24 hours each. The control hemi-pituitary received medium lacking only the steroid for Periods II and III. Medium was removed from the culture tubes at the end of each period and stored undiluted at −18°C until assayed and for the subsequent incubation period fresh medium was added.

Pituitary explants were homogenized in 0.01 M sodium hydroxide. Growth hormone was assayed by the radioimmunoassay as previously reported by Birge *et al*(6). The growth hormone immunoassay utilized rat growth hormone standards, anti-rat growth hormone antiserum and, for increased stability of labeled antigen, we have employed porcine growth hormone labeled with I^{125}. Rat growth hormone (R86567-2 IU/mg) for standards and for preparation of antibody was kindly supplied by A. E. Wilhelmi, Atlanta, Georgia. Porcine growth hormone (NIH P 482B-1.7 IU/mg) used for iodination was obtained from the Endocrine Study Section of the National Institutes of Health, Bethesda, Md.

Data were analyzed by the t test for paired data(9).

Results. The isolated rat adenophyophysis released growth hormone for the 60-hour period of incubation (Table I). The total growth hormone recovered from the medium and that contained in the incubated hemipituitary was greater than in the fresh non-incubated anterior pituitary hemi-sections from the same gland (Table I). This is evidence that net synthesis of hormone had occurred during the several periods of incubation. In fact, the amount of growth hormone released into the medium during the three periods of incubation was nearly equal to that recovered from the non-incubated hemi-pituitaries. Despite the release of this amount of growth hormone into the medium, the incubated hemi-pituitaries contained up to 41% as much growth hormone as their non-incubated counterparts when the experiment was terminated. The rate of release of growth hormone into the culture medium was initially rapid, but it progressively declined

TABLE I. Comparison of Incubated and Non-Incubated Rat Anterior Hemipituitary. Mean growth hormone ± SEM released from or remaining in pituitary. (μg/mg wet anterior pituitary weight.)

	Incubated hemisection (8 pituitaries)	Non-incubated hemisection
Period I (12 hr)	7.2 ± 2.1	
Period II (24 hr)	19.4 ± 1.0	
Period III (24 hr)	13.5 ± 1.1	
Tissue	17.0 ± 2.2	41.7 ± 4.0
Total	57.1 ± 4.0	41.7 ± 4.0

$t = 6.04$
$p = <.01$

TABLE II. Rate of Release of Growth Hormone into Medium During Various Incubation Periods.

	No.‡	GH mμg/mg wet AP†/hr
Period I (0 to 12 hr)	108	1,001 ± 48*
Period II (12 to 36 hr)	81	617 ± 29
Period III (36 to 60 hr)	89	373 ± 13

* All values expressed as mean growth hormone released into medium ± SEM.
† AP = anterior pituitary.
‡ No. = number hemipituitary incubations analyzed.

TABLE III. Effect of Cortisol Added to Incubation Medium on Growth Hormone Release (Period II).

	No.†	Control	Steroids	p
Cortisol (10 μg/ml)	10	695 ± 58*	475 ± 32	<.01
Cortisol (2 μg/ml)	17	660 ± 40	553 ± 32	<.05
Cortisol (.5 μg/ml)	16	757 ± 48	605 ± 26	<.01

* Mean growth hormone released into medium, mμg/mg wet anterior pituitary/hour ± SEM.
† Number hemipituitary culture pairs.

TABLE IV. Effect of Cortisol and Stilbestrol on Pituitary Growth Hormone Content *in vitro*.

	No.‡	Pituitary GH, μg/mg wet weight Control	Steroid	
Cortisol (10 μg/ml)	13	18.6 ± 1.9*	19.1 ± 1.8	N.S.†
Cortisol (2 μg/ml)	17	20.9 ± 2.4	19.6 ± 1.4	N.S.
Cortisol (.5 μg/ml)	16	20.6 ± 1.9	21.4 ± 1.1	N.S.
Stilbestrol (50 μg/ml)	5	27.3 ± 1.9	29.9 ± 2.2	N.S.
Stilbestrol (2 μg/ml)	12	24.3 ± 2.3	24.1 ± 1.6	N.S.
Stilbestrol (.5 μg/ml)	13	22.9 ± 1.5	24.2 ± 1.6	N.S.

* Mean value ± SEM.
† N.S. = not significant.
‡ No. of explants assayed.

during the successive periods of incubation (Table II).

Cortisol suppressed the release of growth hormone from the hemipituitary (Table III). During the first 24 hours of addition (Period II) cortisol in a concentration of 10 μg/ml caused a significant suppression ($p<.01$) of growth hormone release. Cortisol had no effect on the amount of growth hormone recovered from the hemi-pituitaries following incubation (Table IV). Growth hormone released into the medium during Period III was not significantly different in the cortisol *vs* control incubations.

The results of diethylstilbestrol addition on release of growth hormone from the isolated rat anterior pituitary *in vitro* are recorded (Table V). Only when a concentration of 50 μg/ml of the steroid was employed was there any significant effect. When the concentrations of 2.0 and 0.5 μg/ml were added to the incubation medium no significant effect was noted on the amount of growth hormone released into the medium or recovered from the tissue folowing incubation. The very large dose of diethylstilbestrol caused significant suppression ($p<.01$) of growth hormone release from pituitary hemi-sections during Periods II and III but did not decrease the amount of hormone recovered from the hemipituitary following incubation (Table IV).

Discussion. Until recently, the measurement of rat growth hormone has been hampered by the limited sensitivity and difficulty of bioassay procedures. Radioimmunoassay has distinct advantages over techniques that have previously been employed to measure rat growth hormone. It is much more sensitive than the tibial test bioassay and permits accurate measurement of hormone released *in*

TABLE V. Effect of Diethylstilbestrol Added to Incubation Medium on Growth Hormone Release.

	No.	GH release mμg/mg wet ant. pit/hr Control	Steroid	
		Period II		
Diethylstilbestrol (50 μg/ml)	5	939 ± 88*	747 ± 28	<.01
Diethylstilbestrol (2 μg/ml)	12	333 ± 30	332 ± 22	NS
Diethylstilbestrol (.5 μg/ml)	13	356 ± 31	347 ± 19	NS
		Period III		
Diethylstilbestrol (50 μg/ml)	5	419 ± 27	130 ± 13	<.01
Diethylstilbestrol (2 μg/ml)	12	429 ± 27	418 ± 23	NS
Diethylstilbestrol (.5 μg/ml)	13	348 ± 23	333 ± 24	NS

No. = No. of hemipituitaries.
* Mean ± SEM.

vitro from a single or even a portion of a pituitary. The ease with which a large number of measurements can be carried out has permitted evaluation of the significance of relatively small experimental variations.

Under control incubation conditions the pituitary released growth hormone at a decreasing rate for 60 hours into the incubation medium. The pituitary explants lose some of their stored growth hormone to the medium over the period of incubation. However, over the 60-hour period of incubation, the amount released into the medium is equivalent to the total amount of growth hormone contained in the non-incubated hemipituitary. This result would indicate that *de novo* synthesis of growth hormone is necessary to maintain the concentration of hormone in the explant while an amount of growth hormone equivalent to that in the non-incubated hemipituitary is released into the medium.

Cortisol significantly decreased the release of growth hormone from the isolated rat adenohypophysis *in vitro* in Period II. This decrease was observed with a concentration of cortisol as low as 0.5 μg/ml which was the lowest concentration tested. Since the amount of growth hormone remaining in the pituitary is the same after control and cortisol incubations and only the amount of growth hormone appearing in the medium is decreased by the presence of steroid incubations it must be assumed that the synthesis of growth hormone was affected by the presence of cortisol in the incubation medium. Whether the effects of cortisol noted here are on the basis of a decreased synthesis of growth hormone with a resultant decrease in hormonal release into the medium, or whether the effect of cortisol is on the release of growth hormone which then results in a decreased synthesis of hormone is not clarified by these studies.

The failure to demonstrate a significant effect of diethylstilbestrol on growth hormone synthesis and release by the isolated rat adenohypophysis *in vitro* suggests that the effects of estrogens on growth hormone secretion that have been observed *in vivo* are probably mediated by some other mechanism than a direct effect on the anterior pituitary. Presumably the influence of estrogens is mediated by the central nervous system by way of the hypothalamic-hypophysial axis.

Although greater than physiological amounts of cortisol were employed in these studies the observations made suggest that some of the actions of concentrations of corticosteroids on growth hormone release *in vivo* may be the result of a direct effect on the pituitary.

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Received July 14, 1967. P.S.E.B.M., 1967, v126.