CHORIONIC GONADOTROPHIN, ACTH, AND THE ADRENAL-HYALURONIDASE RELATIONSHIP*

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It has been shown that either adrenocorticotrophic hormone (ACTH) or the 11-oxygenated adrenal cortical steroids inhibit the hyaluronidase-enhanced spreading phenomenon in the rabbit and in the mouse.[7, 8, 9]

In view of the favorable influence of pregnancy on certain of the collagen diseases, it was of interest to determine whether chorionic gonadotrophin had any influence on this phenomenon. This was found to be the case, but in the course of these investigations it became apparent that the inhibition produced by this agent was probably due, not to the gonad-stimulating principle, but to a contaminant which exhibited many of the properties previously associated with ACTH from pituitary tissue.

While this work was in progress, Jailer and Knowlton[4] published a paper in which the presence of ACTH-like activity in extracts of placenta was indicated. The present study is to be regarded as of a preliminary nature since many points concerning the nature of the adrenal cortical stimulating principle remain to be clarified.

Materials and methods

Detailed methods and precautions as to procedures have been described in earlier publications.[6, 7] Male and female mice of the inbred CBA strain, 10 to 12 weeks of age and weighing an average of 25 to 30 gm. were used in these investigations.

The Wyeth standardized hyaluronidase preparations used in these studies were designated W-108-A and W-160-A; the latter enzyme preparation was approximately three times as potent as the former.

The ACTH preparations were from the Armour laboratories. (Earlier experiments, using Lot H-7911 in a variety of experimental conditions, gave anomolous results, presumably due to the high oxytocin and pressor activity present.) The preparation used in most of these studies was Lot H-8412 and contained 0.2 unit oxytocin and 0.14 unit pressor substance. The contents of one vial were noted to be equivalent to 35 mg. of Armour Standard LA-1-A.

The chorionic gonadotrophin (CG) and the heat-inactivated chorionic gonadotrophin (HICG) preparations from human pregnancy urine were supplied by Dr. R. H. Barnes and Dr. R. G. Westfall of Sharp & Dohme, Inc. They were prepared by the method of Gurin[5] and assayed in the 18-day albino rat, the criterion of response being the results of vaginal smears at 100 hours following subcutaneous injection. Preparations of varying gonadotrophic potency were tested: (1) = 400 U/mg.; (2) = 785 U/mg.; (3) = 330 U/mg. The HICG was prepared by heating a water solution of CG for 15 min. at 100° C. in a boiling water bath, followed by cooling and lyophilization. It is

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noteworthy that such a procedure does not inactivate ACTH preparations.* However, ACTH activity can be lost in such a procedure if the degree of acidity is not carefully controlled.

The CG and HICG preparations were dissolved in sterile physiological saline just prior to experimental use. Where administered in large doses, the question of the toxicity of certain other CG preparations tested is of importance. It is quite possible that lack of precautions exercised initially in the collection of urine and in the isolation of the gonadotrophins might result in bacterial contamination; consequently, pyrogenic effects might be noted with their use. This was not the case in the samples tested and here, in part, reported. Although in adrenalectomized mice tested six days postoperatively, dosages of ACTH, CG, and HICG in excess of 3.5 mg. proved to be toxic, this was found not to be true in the normal animal. Dosages ranged from 0.5 to 5 mg. in the various investigations and are cited in detail in the individual experiments. Control groups were injected with equal amounts of saline.

Bilaterally adrenalectomized animals were tested one and six days postoperatively. They were not maintained on saline.

Groups of castrated animals of both sexes were used four and 10 days postoperatively. It is interesting that in these experiments the males and females exhibited a slight sex difference in hyaluronidase-enhanced spreading areas, and that castration alone appeared to inhibit to a slight degree this spreading.

The hypophysectomized mice† were 12 weeks of age, of both sexes, and weighed 25 to 30 grams. They were maintained on 5 per cent glucose and were tested forty-eight hours postoperatively. Autopsies on all animals reported here showed the completeness of hypophysectomy. Only a slight degree of adrenal atrophy occurred in the period prior to the experiment while the weight loss was between 1 and 2 grams.

The previous practice of carrying out comparable bilateral intradermal injections of saline and India ink and of hyaluronidase and India ink has been employed in most of the studies presented here. However, in the assay procedure, since the major effect of the inhibition has been shown to be on the exogenously added hyaluronidase, the tables have been set up uniformly to give information on the hyaluronidase-spreading areas.

The rats employed in the assay for ACTH activity were males of the Sprague-Dawley strain. Hypophysectomized animals‡ weighing between 180 and 200 grams were used on the third postoperative day. Under hexobarbital or light sodium pentobarbital anesthesia one adrenal gland was removed before and the other one hour after 0.2 ml. of the test material was introduced into the inferior vena cava. Tail blood samples were obtained at the same time intervals. Eosinophile counts and ascorbic acid were determined as described in an earlier paper.[1] Intraperitoneal injections were made on unanesthetized animals.

Experimental

The systemic administration of ACTH, CG, or HICG in dosages ranging between 1 and 5 mg. produced a marked inhibition of the dermal spreading of India ink with hyaluronidase in normal animals. Results of some representative experiments are show in Table 1. The similarity of action between

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* Li, C. H. and Evans, H. M.: *Vitamins and hormones. Advances in research and applications.* New York, Academic Press, Inc., 1947. Vol. V, p. 220.

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‡ Obtained from Hormone Assay Laboratory, Chicago, Illinois.

ACTH and CG, in terms of percentage inhibition, is clearly seen, and while the known impurity of both CG and ACTH preparations makes it impossible to quantitate dosages, the effects noted are qualitatively identical. The inhibition of spreading appeared to be maximal in the dosage ranges used, and it is of interest that preparations of known varied gonadotrophic potency produced this similar and constant degree of inhibition of spreading. Further, that while extremely large doses were purposely tested, no symptoms of toxicity resulted in the normal mice. Control studies using serum albumin in similar dosages were without inhibitory effect.

TABLE 1

COMPARISON OF EFFECTS OF ACTH, CHORIONIC GONADOTROPHIN, AND HEAT-INACTIVATED CHORIONIC GONADOTROPHIN IN MICE

Substance injected	*Dose* mg.*	*Route of administration*	*Time† interval hours*	*Area of spreading with hyaluronidase and India ink*		
				Control (saline) sq. mm.	*Experimental (test substance) sq. mm.*	*% Change from control*
			Normal mice			
ACTH	1.0	i.p.	3	418 (12)	265 (12)	—37
ACTH	3.5	i.p.	3	418 (12)	205 (12)	—51
CG (1)	1.0	i.p.	3	400 (12)	247 (12)	—38
HICG (1)	1.0	i.p.	3	368 (12)	190 (12)	—48
CG (2)	2.0	i.p.	3	390 (15)	211 (12)	—46
CG (3)	2.0	i.p.	3	390 (15)	185 (12)	—53
CG (1)	3.5	i.m.	3	400 (12)	208 (12)	—48
CG (1)	5.0	i.m.	3	354 (12)	207 (12)	—41
HICG (1)	5.0		3	354 (12)	197 (12)	—44
			Adrenalectomized mice			
ACTH	1.0	i.p.	3	645 (12)	638 (12)	0
ACTH	3.5	i.p.	3	645 (12)	694 (12)	+7
CG (1)	3.5	i.p.	3	687 (12)	672 (12)	—2

CG (1) = 400 U/mg.
CG (2) = 785 U/mg.
CG (3) = 330 U/mg.
* Diluent equals 0.5 cc. physiological saline.
† Interval between injection of steroids and hyaluronidase.

The fact that HICG gave entirely similar results in terms of degree of inhibition produced was of importance in that the procedure of heat-inactivation destroyed essentially all gonadotrophic activity, but it has been noted that under similar conditions ACTH activity is not destroyed.*

* Li, C. H. and Evans, H. M.: *Op. cit.*, vol. V, p. 220.

Numerous experiments showed that CG and HICG were completely without effect in the adrenalectomized animal. Their inhibitory action on hyaluronidase appeared to be mediated through the adrenal,† as was found in similar experiments employing ACTH. One to 3 mg. doses of these three substances were well tolerated, but dosages of 5 mg. of HICG, CG, or ACTH proved fatal to adrenalectomized animals. The fact that certain other CG preparations proved toxic to the adrenalectomized animal in smaller dosages led to further investigations.

TABLE 2

INFLUENCE OF CHORIONIC GONADOTROPHIN AND HEAT-INACTIVATED CHORIONIC GONADOTROPHIN ON CASTRATED MICE AND NORMAL MICE

Substance injected	*Dose† mg.*	*Route of administration*	*Time interval hours*	*Area of spreading with hyaluronidase and India ink**		
				Control (saline) sq. mm.	*Experimental (test substance) sq. mm.*	*% Change from control*
Normal female mice						
CG (1)	3.5	i.p.	3	642 (12)	262 (12)	—59
Castrated female mice						
CG (1)	3.5	i.p.	3	502 (12)	210 (12)	—58
Normal male mice						
HICG (1)	3.5	i.p.	3	580 (12)	203 (12)	—65
Castrated male mice						
HICG (1)	3.5	i.p.	3	543 (12)	217 (12)	—60

* Enzyme preparation W-160-a. Potency approximately 3x that of W-108-A. Spreading time of 6 hours.
† Diluent 0.5 cc. saline.

Experiments in normal animals wherein hyaluronidase spreading was allowed to continue over time intervals of one to six hours showed that the inhibition appeared almost maximal at one hour, thus indicating a rapid and marked stimulation of the adrenal with subsequent release of C-11 adrenal steroids resulting in an almost total inhibition of enzyme activity.

The fact that ACTH, HICG, and CG preparations of different gonadotrophic potency produced similar inhibitory effects on hyaluronidase activity, and that none of these effects was seen in the adrenalectomized animal, showed similarity of action between CG, HICG, and ACTH.

† When administered locally at the intradermal site of injected hyaluronidase, these preparations showed no inhibitory effect in either normal or adrenalectomized mice. Similarly, in *in vitro* studies, CG, HICG, and ACTH did not inhibit hyaluronidase.

Earlier work has shown that the sex steroids, progesterone, pregnenolone, estradiol benzoate, and testosterone are without effect in the normal and adrenalectomized animal.[8,9] However, the possibility remained, as pointed out by Greep,[3] that CG may stimulate the secretion of substances other than estrogens and androgens. The role of the gonads in this relationship was then investigated.

In Table 2 are seen the results of representative experiments showing the very marked inhibitory effects produced by the administration of CG and HICG to groups of castrated animals of both sexes. Control groups of castrated animals were injected with physiological saline. In the results here summarized, a high-potency enzyme preparation and increased

TABLE 3

EFFECT OF CHORIONIC GONADOTROPHIN IN HYPOPHYSECTOMIZED MICE

				Area of spreading with hyaluronidase and India ink		
Substance injected	*Dose mg.*	*Route of administration*	*Time interval hours*	*Control (saline) sq. mm.*	*Experimental (test substance) sq. mm.*	*% Change from control*
CG (2) males	1	i.p.	3	414 (6)	215 (5)	—48
CG (2) males	3	i.p.	3	414 (6)	190 (5)	—54
CG (1) females	3	i.p.	3	538 (6)	232 (5)	—57
HICG (1) females	3	i.p.	3	538 (6)	135 (5)	—75

hyaluronidase-spreading time were employed to determine whether there would be equal effectiveness in inhibitory capacity with respect to enzyme potency. Results show that potency of enzyme or time interval of spreading was not a limiting factor in CG inhibitory capacity. The presence or absence of the gonads exerted no effect in this investigation; in fact, the absence of the gonads appeared slightly to inhibit hyaluronidase spreading in control castrated as compared with normal animals. It is interesting that the absolute areas of spreading and degree of percentage inhibition produced were nearly constant in animals receiving CG and HICG.

The obviously critical point in evaluating the ACTH-like activity of CG and HICG was the establishment of what effects would be shown by the administration of these preparations in the absence of the pituitary. The results of experiments with 48-hour hypophysectomized mice are given in Table 3. It is clearly seen that CG and HICG inhibit the hyaluronidase-enhanced spreading phenomenon even in the absence of the pituitary.

It appears from the data presented in the foregoing tables that the area of spread resulting from the inhibition of hyaluronidase by ACTH, CG, or HICG is relatively constant. Earlier investigations[8] and, more recently,

studies with the pure steroids F, E, and A, employing *in vitro* as well as *in vivo* methods yielded a similar degree of constancy.[9] It has been concluded that the area of spreading under these conditions is minimal and represents essentially complete inhibition of the enzyme hyaluronidase. The percentage inhibition presented in the tables is a relative figure and because of the method of calculation (in which there is always a certain degree of spreading when saline and India ink intradermal injections alone are employed) it is apparent that in these terms, 100 per cent inhibition would be impossible to obtain. However, on a theoretical basis it is likely that these substances have brought about approximately complete inhibition. This same marked inhibition of hyaluronidase has been evident in experiments with the pure steroid Compounds E and F. One may assume that the

TABLE 4

ASSAY OF ACTH ACTIVITY OF CHORIONIC GONADOTROPHIN IN THE HYPOPHYSECTOMIZED RAT

Rats	*Chorionic gonadotrophin* mg./100 g. body wt.*	*Adrenal % change in ascorbic acid mg./100 g.*	*total mg.*	*Blood % change in eosinophiles*
29 Hypox. 3 days	.....	—2 ± 1.4	+ 3 ± 1.7	....
8 Hypox. 3 days	2, intraven.	—7 ± 1.9	—13 ± 1.6	—74
2 Hypox. 4 days	1, intraper.	.....	.....	—45

* Preparation #1 (400 U/mg.).

C-11 oxygenated steroids that are released as a result of ACTH or CG administration are quantitatively similar in their effects to a dosage of Compounds E and F that have been found to bring about the same marked inhibition of the enzyme.

Additional tests of the ACTH activity of CG 1 were done in rats, where a 60 per cent reduction of the adrenal ascorbic acid and circulating eosinophiles was observed in normal and castrated male animals injected with 4 mg./100 g. of CG intraperitoneally. The action of CG in rats whose hypophyses had been removed provided a somewhat better measure of the adrenocorticotrophic action of this preparation; results of these experiments compared with a series of operated controls are recorded in Table 4. Hypophysectomized rats responded to intraperitoneal injections of CG, 1 mg./100 g., with marked eosinopenia in one hour. With intravenous administration the decrease in blood eosinophiles far exceeded the per cent fall in adrenal ascorbic acid. Since there is no evidence that eosinopenia can be produced in one hour in the hypophysectomized rat either by surgical trauma or agents other than ACTH,[5] the reduction in eosinophiles and the small but significant decline of the ascorbic acid in the adrenal,

effected by CG, support the conclusions obtained from the studies of its inhibition of the hyaluronidase action in the mouse.

Discussion

Several similarities in the biologic activity of ACTH and CG from human pregnancy urine have been shown. Furthermore, it has been demonstrated that CG exhibits ACTH-like effects that cannot be associated with its gonadotrophic activity.

The following evidence establishes this similarity in action: both CG and HICG cause a marked inhibition of hyaluronidase-enhanced spreading in normal and castrated animals in the presence of the adrenals but are without effect in the adrenalectomized animal. The possibility that this effect was due to non-specific toxicity or mediation through the gonads has been ruled out insofar as it was possible. This effect was qualitatively identical to that found with ACTH. In the hypophysectomized animals, both CG and HICG were found to exhibit the same marked degree of inhibition of hyaluronidase activity.

There are two possibilities as to the source of the ACTH-like activity found in these preparations from human pregnancy urine. The first is that the process of extraction includes such quantities of pituitary ACTH as may have been excreted in the urine; the second is that the placenta may normally form an ACTH-like agent, a portion of which is also excreted in the urine. These experiments provide no means of discriminating between the two possibilities; however, the recent report of Jailer and Knowlton[4] that ACTH-like activity can be demonstrated in placental tissue indicates that the urinary factor demonstrated by these experiments might have a similar origin.

These investigations suggest the possibility that ACTH or ACTH-like material of human origin may be obtained for clinical use, thus providing another source to supplement the limited quantities of this agent now available only from animal sources.

Summary

1. Chorionic gonadotrophins (CG) and heat-inactivated chorionic gonadotrophins (HICG) isolated from human pregnancy urine caused a marked inhibition of hyaluronidase-enhanced spreading in normal and castrated mice in the presence of the adrenals and were without effect in the adrenalectomized animal. Furthermore, this effect was qualitatively similar to that found with ACTH.

2. The possibility that this inhibitory effect on hyaluronidase-spreading was due to non-specific toxicity, or mediation through the gonads, was ruled out insofar as possible.

3. In hypophysectomized mice, both CG and HICG were found to

exhibit the same marked degree of inhibition in hyaluronidase-enhanced spreading.

4. The presence of ACTH activity in the CG preparation was indicated by the eosinopenia and reduction of adrenal ascorbic acid observed in the hypophysectomized rat.

5. Evidence is presented showing similarity of action of ACTH, CG, and HICG with respect to the specific biological effects investigated.

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