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EFFECT OF DESIALYLATION ON OVULATION-INDUCING ACTIVITY OF OVINE INTERSTITIAL CELL-STIMULATING HORMONE, OVINE FOLLICLE-STIMULATING HORMONE, PREGNANT MARE'S SERUM GONADOTROPIN, AND HUMAN CHORIONIC GONADOTROPIN IN THE HAMSTER*

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It has been demonstrated that following the treatment of gonadotropins with neuraminidase in order to release sialic acid, variable effects on the potency of the gonadotropins are observed, dependent on whether an in vivo or in vitro assay is employed. Neuraminidase-treated interstitial cell-stimulating hormone (ICSH) of ovine,[1, 2] bovine,[3] or porcine origin[3] did not markedly alter the potency of hormones as measured by in vivo assays. However, similar treatment of human ICSH[4, 5] and human follicle-stimulating hormone (FSH)[6] greatly reduced the biologic activity, although Papkoff[7] has reported human ICSH to be unaffected. On the other hand, desialylated preparations of gonadotropins may show slight enhancement in activity in stimulating cultured granulosa cells[8] and some increase in immunoreactivity to the antiserum for the native hormone.[4]

Farmer, Sairam, and Papkoff[9] have shown that desialylated gonadotropins are equally effective as the native hormones in stimulating in vitro the production of lactic acid from prepubertal rat ovaries. In the ovarian ascorbic acid depletion bioassay, human chorionic gonadotropin (HCG) shows a progressive loss of activity when increasing amounts of sialic acid are removed by neuraminidase treatment.[5] It has been suggested that the marked reduction in the in vivo biologic activity of gonadotropins following neuraminidase treatment is probably due to increased hepatic uptake and an increased clearance from the plasma of the desialylated hormone.[10]

This paper reports our studies in which highly purified gonadotropins have been compared with their desialylated derivatives with respect to ovulation-inducing activity in the hamster. In addition, the effect of specific antisera against ICSH and FSH in inhibiting ovulation was studied.

MATERIALS AND METHODS

Golden hamsters (*Mesocricetus auratus*) weighing between 90 and 130 gm. were kept on a 10.5-hr. light and 13.5-hr dark schedule with the light on from 0730 to 1800 hr. Females with the characteristic vaginal discharge that occurs after ovulation[11] were selected each morning, and thereafter segregated into four groups: postlordosis Day 1 (PLD-1), PLD-2, PLD-3, and PLD-4, respectively. Only those females that showed more than three consecutive 4-day cycles were used for this experiment.

Ovine ICSH (2 × NIH-LH-SI) and ovine FSH (50 × NIH-FSH-SI) were prepared according to the methods of Papkoff et al.[1, 12] Pregnant mare's serum gonadotropin (PMSG) (15,000 I.U./mg.) was ob-

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tained by the method of Schams and Papkoff,[13] and HCG (1,000 I.U./mg.), Lot H21B, was a product of Organon, Inc. One aliquot of each preparation was taken for neuraminidase treatment and the second as a control. Neuraminidase (*Clostridium perfringens*, Sigma Co., Type V) in pH 5.5, 0.1 M sodium acetate buffer containing 0.005 M $CaCl_2$ was added to 1 aliquot to give the enzyme-hormone ratio of 1:100 by weight. Incubation was continued for 3 hr. at room temperature, and the neuraminidase-treated aliquot was thereafter frozen until use. The concentration of enzyme used here is in considerable excess of that required for complete desialylation. The second aliquot (control) was treated identically except that the neuraminidase was not added to the solution. Both ICSH A/S and FSH A/S were obtained from male New Zealand albino rabbits according to the procedures described by Moudgal and Li.[14] Characterization studies showed each to be hormone-specific.

Single injections of various doses of ICSH (1.25–5 μg.), FSH (1.25–10 μg.), PMSG (0.5–0.2 μg.), and HCG (0.05–0.5 μg.) in 0.2 ml. aliquot were given to intact females at 0500 hr. on PLD-4 intraperitoneally. Much higher doses of gonadotropins were used for induction of ovulation in the hypophysectomized females between 0400 and 0600 on PLD-4 immediately after the operation. The desialylated preparations of ICSH, FSH, PMSG, and HCG were similarly injected in intact females. In antiserum neutralization experiments, 0.25 or 0.50 ml. of ICSH A/S, or 0.25, 0.50, or 0.60 ml. of FSH A/S was mixed with either 20 μg. of ICSH or 20 μg. of FSH in the aliquot. The mixture was injected in hypophysectomized animals as described above. Hypophysectomy was performed by the parapharyngeal approach after a tracheotomy. Animals were anesthesized by intraperitoneal injection of Nembutal. All treated animals were killed and autopsied 17 hr. after the hormonal injection. For enumeration of newly ovulated tubal eggs, eggs with cumulus in the distal portion of the fallopian tube were located and released by breakage of that part with a pair of needles under transparent light. The ovaries were separated from the fallopian tubes and examined with reflected light for the number of ruptured follicles.

RESULTS

In animals which had been induced to ovulate at 0500 of PLD-4, the lordosis response was noted by 1400 of the same day, which was 9 hr. following the treatment of gonadotropins.[15] The minimal effective doses for induction of ovulation in 100% of treated intact females (MED_{100}) were shown to be 5 μg. of either ICSH or desialylated ICSH (Table 1); 5 μg. of FSH and more than 40 μg. of desialylated FSH (MED_{50} = 40 μg.) (Table 1); 0.2 μg. of PMSG and 80 μg. of desialylated PMSG (Table 2); 0.1 μg. of HCG and 40 μg. of desialylated HCG (Table 2). The potency of the native gonadotropins per weight is therefore in the order of HCG > PMSG > FSH = ICSH, whereas that of desialylated hormones is Des-ICSH > Des-HCG > Des-PMSG > Des-FSH.

In hypophysectomized hamsters, the MED_{100} was 20 μg. for ICSH, 10 μg. for FSH, 1.0 μg. for PMSG, and 0.5 μg. for HCG, respectively (Table 3). In general, two to five times greater amounts of gonadotropins were necessary for induction of ovulation in the hypophysectomized animals than in the intact animals. The potency of the native gonadotropins per weight for induction of ovulation in hypophysectomized animals is therefore in the order of HCG > PMSG > FSH > ICSH, which was almost the same as for the intact females except that FSH appeared to be more potent than ICSH in the hypophysectomized animals (Table 3).

TABLE 1. *Induction of Ovulation in the Intact Hamster by a Single Intraperitoneal Injection of Ovine ICSH, Ovine FSH, or Desialylated Preparations of Either ICSH or FSH at Proestrous Stage at 0500 on Postlordosis Day 4**

Preparation	Dose	No. of animals			No. of ruptured follicles mean ± S.E.	No. of tubal eggs mean ± S.E.
		Treated	With ruptured follicles	With tubal eggs		
	μg.					
ICSH	1.25	9	0	0	0	0
	2.5	10	6	5	3.4 ± 1.0	3.4 ± 1.0
	5.0	10	10	10	8.5 ± 0.6	8.5 ± 0.6
Desialylated ICSH	1.25	9	0	0	0	0
	2.5	10	6	6	2.6 ± 1.3	1.8 ± 0.9
	5.0	10	10	10	9.0 ± 0.3	8.2 ± 0.4
	10.0	8	8	8	9.4 ± 0.5	9.1 ± 0.7
FSH	1.25	9	0	0	0	0
	2.5	8	5	5	5.9 ± 2.1	5.0 ± 2.0
	5.0	8	8	8	11.2 ± 0.5	10.2 ± 0.7
	10.0	10	10	10	11.9 ± 0.8	11.5 ± 0.7
Desialylated FSH	10.0	8	0	0	0	0
	40.0	6	3	3	3.8 ± 2.4	2.3 ± 1.3

* Animals were examined for the number of ruptured follicles and newly ovulated tubal eggs 17 hr. later.

TABLE 2. *Induction of Ovulation in the Intact Hamster by a Single Intraperitoneal Injection of PMSG, HCG, or Desialylated Preparations of Either HCG or PMSG at 0500 on Postlordosis Day 4**

Preparation	Dose	No. of animals			No. of ruptured follicles mean ± S.E.	No. of tubal eggs mean ± S.E.
		Treated	With ruptured follicles	With tubal eggs		
	μg.					
PMSG	0.05	6	1	1	1.3 ± 1.3	1.2 ± 1.2
	0.1	8	4	4	3.3 ± 1.1	2.9 ± 1.1
	0.2	8	8	8	8.5 ± 0.3	7.6 ± 0.5
Desialylated PMSG	20.0	6	0	0	0	0
	40.0	6	4	4	8.4 ± 2.5	8.0 ± 2.4
	80.0	6	6	6	9.3 ± 1.4	8.8 ± 1.2
HCG	0.05	10	2	2	1.4 ± 1.4	0.8 ± 0.8
	0.1	9	9	9	9.4 ± 1.3	7.8 ± 1.5
	0.25	9	9	9	9.2 ± 0.7	8.3 ± 0.4
	0.50	9	9	9	10.2 ± 0.6	10.0 ± 0.7
Desialylated HCG	5.0	6	0	0	0	0
	10.0	5	2	1	2.6 ± 2.1	1.8 ± 1.8
	20.0	5	4	4	9.0 ± 3.0	8.8 ± 2.9
	40.0	5	5	5	12.6 ± 0.5	12.2 ± 0.9

* Animals were examined for the number of ruptured follicles and newly ovulated tubal eggs 17 hr. later.

In the neutralization experiments (Table 4), when 20 μg. of ICSH were mixed with 0.25 or 0.50 ml. of antiserum to ICSH, the complete inhibition of the ovulation-inducing activity of ICSH was obtained. Nevertheless, similar amounts of ICSH antiserum did not block the action of 20 μg. of FSH in inducing ovulation. In the reverse

TABLE 3. *Induction of Ovulation in the Hypophysectomized Hamster by a Single Intraperitoneal Injection of Ovine ICSH, Ovine FSH, PMSG, or HCG Immediately after the Hypophysectomy at Proestrous Stage between 0400 and 0600 on Postlordosis Day 4**

Preparation	Dose	No. of animals			No. of ruptured follicles mean ± S.E.	No. of tubal eggs mean ± S.E.
		Treated	With ruptured follicles	With tubal eggs		
	μg.					
ICSH	10.0	10	5	5	4.5 ± 1.4	4.3 ± 1.0
	20.0	10	10	10	10.3 ± 0.9	9.5 ± 0.9
FSH	1.25	6	1	1	0.6 ± 0.6	0.6 ± 0.6
	5.0	6	5	5	6.2 ± 1.6	5.4 ± 1.4
	10.0	6	6	6	10.6 ± 0.5	10.0 ± 1.1
PMSG	0.25	5	0	0	0	0
	0.5	5	1	1	1.5 ± 1.5	1.3 ± 1.3
	1.0	5	5	5	9.3 ± 1.9	9.0 ± 1.7
HCG	0.25	6	0	0	0	0
	0.5	6	6	6	7.7 ± 0.8	6.8 ± 1.2
	1.0	6	6	6	8.0 ± 1.0	8.0 ± 1.0

* Animals were examined for the number of ruptured follicles and newly ovulated tubal eggs 17 hr. later.

TABLE 4. *Neutralization of Ovulation-Inducing Activity of 20 μg. of Ovine ICSH or Ovine FSH with Antiserum to ICSH or FSH in the Hypophysectomized Hamsters**

Gonadotropins	Antiserum		No. of animals			No. of ruptured follicles mean ± S.E.	No. of tubal eggs mean ± S.E.
			Treated	With ruptured follicles	With tubal eggs		
μg.		ml.					
ICSH 20	ICSH A/S	0.20	4	4	4	8.5 ± 1.3	8.0 ± 1.0
ICSH 20	ICSH A/S	0.25	5	2	2	4.4 ± 2.7	4.4 ± 2.7
ICSH 20	ICSH A/S	0.50	4	0	0	0	0
ICSH 20	FSH A/S	0.50	3	3	3	9.3 ± 0.9	8.6 ± 1.1
ICSH 20	FSH A/S	0.60	3	3	3	10.7 ± 0.9	8.3 ± 0.5
FSH 20	FSH A/S	0.25	4	4	4	10.0 ± 1.3	9.6 ± 1.5
FSH 20	FSH A/S	0.50	4	0	0	0	0
FSH 20	FSH A/S	0.60	4	0	0	0	0
FSH 20	ICSH A/S	0.25	4	4	4	10.3 ± 0.9	10.3 ± 0.9
FSH 20	ICSH A/S	0.50	4	4	4	9.6 ± 1.2	9.2 ± 0.7

* The mixtures of gonadotropin with antiserum were intraperitoneally injected into females immediately after hypophysectomy at proestrous stage between 0400 and 0600 on postlordosis Day 4. Animals were examined for the number of ruptured follicles and newly ovulated tubal eggs 17 hr. later.

of this experiment, when 20 μg. of ICSH were mixed with 0.50 or 0.60 ml. of antiserum to FSH, no inhibitory effect of the antiserum on ovulation-inducing activity of ICSH was seen. By contrast, similar amounts of antiserum to FSH completely neutralized the ovulation-inducing activity of 20 μg. of FSH (Table 4).

DISCUSSION

The results obtained in these experiments are in agreement with earlier studies showing that 5 μg. of ovine ICSH are the MED_{100} for the induction of ovulation in pentobarbital-blocked or intact proestrous hamsters.[16, 17] Although ovine FSH appeared to be much less potent than ICSH for induction of ovulation in previous investigations,[16, 18] the present study shows that FSH is equal to or even slightly more potent than ICSH in the induction of ovulation (Tables 1 and 3). It should be pointed out that the FSH preparation used in these experiments is about 50 times more active than NIH-FSH-SI and had an ICSH activity of 0.02 × NIH-LH-SI. The ICSH preparation (2 × NIH-LH-SI) contained less than 1% of FSH contamination.[1] Furthermore, the neutralization experiments in this study demonstrate that there is no marked contamination or intrinsic immunocross-reactivity of ICSH with FSH or vice versa (Table 4). Therefore, it is very clear that antigenetically different species of gonadotropins, ICSH and FSH, independently exerted their effect for induction of ovulation (i.e., both ICSH and FSH intrinsically have this property in this system.

MED_{100} for induction of ovulation with highly purified PMSG and HCG was 0.2 μg. (3 I.U.) and 0.1 μg. (1 I.U.) in the current experiment (Table 2). These potencies of purified PMSG and HCG may be compared with previously reported figures: 20 I.U. of PMS for rats[18] and 2.5–5 I.U. of HCG for hamsters.[15] In comparison with the ovulation-inducing activity of ICSH on a weight basis, PMSG and HCG appear to be 25 times and 50 times more active, respectively (Tables 1 and 2).

The mode of action of gonadotropins in the induction of ovulation is apparently through their direct effect on the ovary, since ICSH, FSH, PMSG, and HCG are able to induce ovulation in both intact or hypophysectomized animals (Tables 1 and 3). Binding of ICSH, FSH, or HCG to cultured porcine granulosa cells and stimulation in the increase of cyclic adenosine monophosphate and ovarian steroids by these gonadotropins have been demonstrated.[8, 19] The necessity of 2- to 4-fold increases in the amount of gonadotropin required for induction of ovualtion in the hypophysectomized animal over that for the intact animal may suggest the importance of synergistic effects between ICSH and FSH or between gonadtropins and other pituitary hormones. It is also possible that a continual small amount of gonadotropin secretion is necessary if the process of ovulation is to be maintained at a high degree of efficiency.

In this study, the ovulation-inducing activity of neuraminidase-treated HCG, PMSG, and ovine FSH was radically reduced. Neuraminidase-treated ovine ICSH was unaffected. Thus, the activity of treated HCG and PMSG was reduced to 1/400 and that of ovine FSH to 1/40 of the native hormones. These results are consistent with the results of others who have evaluated neuraminidase-treated hormones by standard in vivo assays.[1, 3, 5, 6, 12, 13, 20, 21] Desialylation of ovine ICSH does not appear to result in loss of activity in the ascorbic acid depletion test, but does show a significant loss when measured in a nonmammalian assay.[22] It should be reemphasized here that while desialylated preparations of HCG, PMSG, and FSH show losses of activity in several in vivo systems, they are still fully active in a variety of in vitro assays.[4, 8, 9]

The sialic acid and total carbohydrate

content of the hormones used in this study are of interest: ovine ICSH, 0.37% and 16%;[1] ovine FSH, 3.5% and 20%;[23] PMSG, 10.2% and 45.1%;[13] and HCG, 9.0% and 31.2%.[24] It appears, then, that the loss of ovulation-inducing activity of the hormones is more closely related to the sialic acid content than to the total carbohydrate in the molecule. The highly reduced potency of the desialylated gonadotropins in these in vivo experiments is most likely the result of the increased clearance rate of these compounds from the plasma as has been suggested by Van Hall, Vaitukaitis, and Ross.[5] Recent studies[10] indicate that the increased clearance and inactivation of desialylated glycoprotein hormones are due to increased hepatic uptake and degradation of the hormones in the liver.

The ovulation-inducing activity of the gonadotropins is in the order of HCG > PMSG > FSH > ICSH, which shows some parallelism with the sialic acid content of the molecules. This potency order is reversed following desialylation of the preparations.

SUMMARY

Highly purified ovine ICSH, ovine FSH, PMSG, HCG, and desialylated preparations of the same gonadotropins were intraperitoneally injected into either intact or hypophysectomized hamsters between 0400 and 0600 on the day of proestrus and examined for ovulation 17 hr. later. MED_{100} (100% minimum effective dose) for induction of ovulation by the native hormones in the intact hamster was found to be 5 μg. for ICSH, 5 μg. for FSH, 0.2 μg. for PMSG, and 0.1 μg. for HCG. For induction of ovulation in the hypophysectomized animal, however, 2- to 4-fold increases in the amount of hormones were necessary. Desialylation of the gonadotropins with neuraminidase did not affect the ovulation-inducing activity of ovine ICSH, but dramatically reduced the activity of FSH, PMSG, and HCG to less than ⅛, 1/40, and 1/40 of the native hormones, respectively.

Rabbit antisera against ICSH were shown to block the ovulating capacity of ICSH, but not FSH. Similarly, antisera against FSH neutralized ovulation by FSH, but not by ICSH.

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