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Action of a Gonadotropin Inhibitor from the Bovine Pineal Gland (38358)

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Recent studies in this laboratory demonstrated that an extract of bovine pineal glands is capable of blocking ovulation induced in mice with exogenous gonadotropin (1); *i.e.*, the administration of the pineal substance resulted in a blocking of the action of the Human Chorionic Gonadotropin (HCG). The present paper investigates the action of this substance on the gonadotropin and assesses its inhibitory properties.

Materials and Methods. Animals. Thirty-day-old female mice of the C57Br strain, weighing 21–24 g, from Simenson vendors were housed in air conditioned quarters, illuminated between 6:00 AM and 6:00 PM. Pelletted food (Berkeley diet from Feedstuffs, Inc., San Francisco, CA) and water were offered without restriction.

Bio-assays. 1. Gonadotropin-induced ovulation. The control animals received 1.0 IU Pregnant Mare Serum (PMS) administered ip, followed by 1.0 IU HCG 48 hr later. Ovulation occurred within the next 10 hr, each mouse yielding between 10 and 15 ova. To test the pineal compound, 0.1 ml of extract [prepared as previously described (1)] was administered simultaneously with the HCG. Inhibition of ovulation was evidenced by the absence or reduced number of ova in the animals.

2. Stimulation of mouse uterus with HCG. The control animals received 0.5 IU of HCG ip for 3 consecutive days. This dose produced approximately a fourfold increase in uterine weight as compared with saline-treated animals. The test animals received the treated pineal extract simultaneously with the HCG. All groups were sacrificed on the 4th day, the uteri removed and weighed, and the weights compared.

Effects of pineal fraction on induced ovulation. Extract (0.1 ml) which represents the material from approximately 0.6 g of defatted tissue was administered to each mouse simultaneously with the HCG. In one group both the pineal fraction and the HCG were administered ip, and in the other the HCG was given ip and the pineal fraction by sc injection.

Mouse uterine studies. Two different doses of pineal extract were administered simultaneously with the HCG and the effects on the mouse uterus observed. As in the experiments on induced ovulation, in one group both the pineal material and the hormone were administered ip, and in the other group the HCG was given by ip injection and the pineal fraction given sc.

Examination of the pineal fraction for the presence of a neuraminidase-type enzyme. Pineal extract (0.05 ml) and HCG (5,000 IU) were incubated for 1 hr at 37° at pH 7.0 in 0.01 *M* phosphate buffer. The incubate was extracted for sialic acid, and the sensitive thiobarbituric assay developed by Warren (2) was used to determine the concentration. As a control, a solution containing 5,000 IU HCG that had been incubated at 37° for 1 hr without any pineal extract was assayed also. By comparison with the 0.1 normal sulfuric acid hydrolysate of 5,000 IU HCG at 80° for 1 hr, the percentage of sialic acid released from the hormone by the pineal fraction was determined.

Results and Discussion. The effects of the pineal fraction on induced ovulation are recorded in Table I. Simultaneous administration of both the HCG and the pineal substance by ip injection resulted in the complete absence of ova in all the test animals, whereas in the control group an average of 13.2 ova per mouse was produced. However, when the pineal fraction and the HCG were administered at different sites, no inhibitory effect was observed.

TABLE I. Effects of Pineal Fraction on Gonadotropin-Induced Ovulation.

Number of animals[a]	Treatment	% Ovulation	Ova/mouse (Mean ± SE)
Control			
10	0.1 ml saline	100	13.2 ± 0.8
Experimental			
10	0.1 ml pineal extract ip[b]	0	0
9	0.1 ml pineal extract sc	100	12.8 ± 1.1

[a] Each animal received 1 IU PMS ip followed by 1 IU HCG ip 48 hr later.
[b] 0.1 ml represents the material obtained from approximately 0.6 g of defatted tissue.

The same phenomenon occurred in the mouse uterine assay: Table II shows that HCG administered ip was inactivated when the pineal fraction was also given ip at doses of 0.1 ml and 0.05 ml, but when the pineal fraction was administered sc the uterine stimulation was the same as that produced by HCG alone.

It is well known that the removal of sialic acid from follicle-stimulating hormone by neuraminidase destroys most of the activity of the hormone *in vivo* (3–5). In contrast, luteinizing hormone of ovine, bovine, or porcine origin is unaffected by neuraminidase since it lacks sialic acid (6). Although HCG possesses predominantly luteinizing activity, it has been shown that its inactivation by neuraminidase is extremely rapid (7, 8). Accordingly, we incubated the pineal fraction with HCG and determined that the deactivation could be attributed to the presence of a neuraminidase-type enzyme.

Table III shows the levels of sialic acid released from 5,000 IU HCG as measured in the thiobarbituric acid method. The sulfuric acid hydrolysate yielded 0.06 μmole (considered to be the total *N*-acetylneuraminic acid present), and the incubate of the pineal fraction with the hormone gave 0.055 μmole. As a control, 5,000 IU HCG was incubated under the same conditions in the absence of the pineal extract, and the yield of *N*-acetylneuraminic acid was less than 0.005 μmole.

TABLE II. Effect of Pineal Fraction on the Gonadotropin-Stimulated Mouse Uterus.

Number of animals	Treatment	Mean uterine weight (mg ± SE)
Control		
10	0.1 ml saline	9.2 ± 0.8
Experimental[a]		
10	0.1 ml Tris buffer (0.01 *M*; pH = 7)	28.7 ± 1.8
9	0.1 ml pineal fraction ip	9.4 ± 0.8
10	0.05 ml pineal fraction ip	9.7 ± 0.7
10	0.1 ml pineal fraction sc	26.7 ± 1.6
9	0.05 ml pineal fraction sc	28.2 ± 2.1

[a] Each animal received 0.5 IU HCG per day for 3 consecutive days.

Conclusion. This work demonstrates that the inhibition of gonadotropin-induced ovulation by an extract of the bovine pineal gland is caused by the presence of a neuraminidase-type enzyme that rapidly deactivates the HCG. It is of interest to note that a gonadotropin-inhibiting substance isolated from bovine pituitary extracts, which is capable of inhibiting the ovarian weight increase caused by follicle-stimulating hormone and HCG in rats, has been found to contain a neuraminidase enzyme (9).

Thus the present paper may help to elucidate the reported gonadotropin-inhibiting properties of several pineal extracts. Where previous studies (10–12) have demonstrated that the extracts suppress the stimulatory effect of HCG, human menopausal gonadotropin, or PMS on the rodent gonad or uterus (an effect not caused by melatonin (10)), the presence of this neuraminidase-type enzyme may now explain this inhibition of the action of gonadotropin.

TABLE III. Thiobarbituric Acid Assay of 5,000 IU HCG Subjected to Different Treatment.

Treatment	*N*-acetylneuraminic acid released (μmole)
HCG incubate	<0.005
0.1 *N* H_2SO_4 hydrolysate of HCG	0.06
HCG and pineal fraction incubate	0.055

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