Effect of Different Steroids on Prolactin Secretion in Pituitary-Hypothalamus Organ Co-Culture.* (29623)

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In addition to the conventional methods for initiation of lactation, several procedures have lately been described which achieve the goal by administration of tranquilizers to man and animals(1,2,3,4). Interruption of the connection between hypothalamus and pituitary also produces prolactin release (5,6). Hypothalamic lesions in rabbits and rats may have the same effect(7,8,9).

Using the *in vitro* organ culture technique, Meites *et al*(10) succeeded in culturing anterior pituitary explants which released considerable quantities of prolactin. Addition of hypothalamic extracts or explants to the hypophysis organ culture, significantly *decreased* the quantity of prolactin released into the medium(11,12). On the other hand, Nicoll *et al*(13) were able to *increase* prolactin secretion by adding estradiol to the pituitary organ culture medium. The same effect was obtained with pituitaries of rats previously treated with estradiol(14,18).

We have tested 30 phenothiazine and other tranquilizers for their ability to release prolactin, and have found perphenazine† to be the strongest agent releasing prolactin *in vivo* (15). With the organ co-culture technique, we have shown that depression of the hypothalamus is solely responsible for this effect (Danon *et al*, 16).

Whereas hypophyseal lactation is easily suppressed by steroids, no substance is yet available which can suppress hypothalamic lactation, known in women as Chiari-Frommel Syndrome. We therefore studied the effect of various steroids on prolactin secretion in co-cultures of pituitary and hypothalamus in order to find potent inhibitors of hypothalamic lactation.

Materials and methods. The rats serving as donors for organ cultures were adult female rats of the Hebrew University "Sabra" strain weighing 220 ±20 g each. The steroids added to the organ cultures were: Estradiol, hydroxydiketopregnane,‡ testosterone, hydrocortisone and progesterone. The methods for running the pituitary culture or the pituitary-hypothalamus co-culture and their evaluation by the intradermal pigeon-crop-gland-test were the same as those previously published (16).

Results. Table I shows that pituitary explants alone secrete 0.05 (±0.01) I.U. prolactin per day, whereas co-cultures of pituitary and hypothalamus secrete only half that amount, 0.025 (±0.01) I.U.

Estradiol (1 μg/ml) and hydrocortisone (10 μg/ml) acted directly on the pituitary explants and increased prolactin secretion to 0.08 (±0.01) I.U. In a pituitary-hypothalamus co-culture the prolactin depressing effect of the hypothalamus disappeared when estradiol or hydrocortisone were added. On the other hand, addition of hydroxydiketopregnane and progesterone caused considerable decrease of prolactin secretion (from 0.05 to 0.025 I.U. respectively). Hydroxydiketopregnane was able to abolish prolactin release altogether from the pituitary in the presence of hypothalamus explants.

Testosterone had no effect on prolactin secretion either in pituitary culture nor in co-culture.

Discussion. By comparing the effect of substances added to pituitary alone and to pituitary-hypothalamus co-culture, the target organ of the added substance can be distinguished.

Estradiol *in vitro* is a strong stimulant of prolactin release, acting directly on the pituitary. This effect was only slightly decreased

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† Perphenazine, 2-chloro-10-{3-[1-(2-hydroxyethyl)-4-piperazinyl] propyl}-phenothiazine (Trilafon-Schering).

‡ 3-alpha-hydroxy-11,20-diketopregnane-3-hemisuccinate =Meket, Organon-Oss, Holland.

TABLE I. Effect of Various Steroids on Prolactin Release in Anterior Pituitary-Hypothalamus Co-cultures.*

	Steroid									
	Saline control	Estradiol		Hydrocortisone		Hydroxydiketopregnane†		Progesterone		Testosterone
	Concentration in μg/ml									
Type of culture	—	1	10	10	100	1	10	10	100	100
Anterior pituitary alone	.05	.08	.65	.08	.05	.025	.025	.025	.025	.05
Anterior pituitary + hypothalamus	.025	.05	.05	.05	.05	<.025	<.025	.025	.025	.025

* The figures show mean prolactin release per day in international units. Standard deviation of the mean is ± .01 I.U.

† 3-Alpha-hydroxy-11,20-diketo-pregnane-3-hemisuccinate = Meket (Organon-Oss, Holland).

by the presence of the hypothalamus. The same conclusion was obtained by Meites *et al* (10). Ratner *et al*(14) have shown this effect of estradiol even when previously administered *in vivo* to the rats and not added in substance to the pituitary culture(18).

Hydrocortisone also promotes the release of prolactin *in vitro,* but less effectively than estradiol. The mechanism of its action is probably directly through the hypophysis. This may be deduced from the fact that the presence of the hypothalamus did not substantially diminish prolactin secretion as it did in the control.

Hydroxydiketopregnane and progesterone *in vitro* are depressants of prolactin release, acting directly on the hypophysis. On the other hand, hydroxydiketopregnane was shown by us to prevent *in vivo* the mammotropic effect of perphenazine(15). It should be borne in mind that the mammotropic effect of perphenazine is no doubt due to the suppression of a prolactin-inhibiting factor present in the hypothalamus(16).

Thus it seems that we must distinguish between 2 groups of steroids: those stimulating prolactin release at low dosage and depressing it at high dosage (estradiol and hydrocortisone); and those depressing prolactin release at any dosage (progesterone and its pregnane derivatives).

None of the steroids studied so far appear to stimulate prolactin release through hypothalamic inhibition as do the tranquilizers studied by us (Khazan *et al* 17). The difference between the prolactin-releasing effects of hypothalamic tranquilizers and of steroids is being studied further.

Summary. Various steroids were added to pituitary culture or pituitary-hypothalamus co-culture *in vitro,* and their effect on prolactin release was studied. 1. Estradiol and hydrocortisone are strong stimulants of prolactin release, apparently acting directly through the pituitary. 2. 3-Alpha-hydroxy-11, 20-diketo-pregnane-3-hemisuccinate and, to a lesser degree, progesterone are depressants of prolactin release, acting directly on the hypophysis. 3. Testosterone does not affect prolactin release from the pituitary.

Estradiol, 3-alpha-hydroxy-11, 20-diketo-pregnane-3-hemisuccinate = Meket, testosterone, hydrocortisone and progesterone were generously supplied by Dr. G. A. Overbeek of Organon-Oss, Holland, and perphenazine (Perphenan) by "Taro," Pharmaceutical Ind., Haifa, Israel.

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Physical Heterogeneity of Bovine Gamma Globulins: Gamma-1M Globulin Electrophoretic Heterogeneity.* (29624)

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The distributions of 7S gamma globulins mediating particular primary and secondary antibody functions, such as binding of antigen(1), fixation of complement(2) and fixation to skin(3) may be heterogeneous within the electrical charge continuum of this protein taken as a whole. The length of the immunoelectrophoretic arc of gamma-1 macroglobulin (gamma-1M) suggests the presence of a continuum of molecules differing in net electrical charge in a manner analogous to the spectrum of 7S gamma globulin molecules. In addition to electrical charge heterogeneity, the identification in macroglobulin preparations of very large molecules by ultracentrifugal analysis (26S, 32S)(4) provides evidence of a polymer type heterogeneity perhaps analogous to the 7S gamma-1A globulin polymer heterogeneity. However, no experimental definition of the extent of gamma-1M globulin electrophoretic heterogeneity is available, nor has there been any reported success in attempting to associate particular antibody activities with gamma-1M globulins having particular mobility properties. Data are presented here to contend that in the bovine species at least one biological activity, the ability to fix complement in the presence of an appropriate antigen, is associated with gamma-1M globulin molecules whose mean net electrical charge differs from that of the protein as a whole.

Materials and methods. Sera. Serum samples were taken from adult cattle, 1243 and 1244, experimentally infected with *Anaplasma marginale*. Samples were pooled from those days early in the disease where DEAE Sephadex chromatography of whole serum and subsequent serological assay of fractions indicated strong, complement-fixing antibody response exclusively in the macroglobulin. This was manifest by high antibody titer in DEAE peak III fractions together with failure to obtain any fixation with DEAE peak II protein—the fast 7S gamma globulin containing antibody activity in later response(5).

Gel filtration. Bovine sera were fractionated on Sephadex G200 (Pharmacia Fine Chemicals, Inc., New York) in a column 7 × 70 cm containing approximately 150 g (dry weight) of the cross-linked dextran, according to the technique of Flodin for large columns(6). Elution buffer consisted of 0.1M Tris + 1M NaCl at pH 8.0. Protein appeared in the eluent in 3 main peaks as has been shown with human serum. Analysis of the distribution of proteins in these

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