PROLACTIN CONTENT OF THE PITUITARY
AFTER INJURY TO THE MEDIAN EMINENCE
AND THE MEDIAL PORTION OF THE HYPOTHALAMUS

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It has been found that the formation of all its hormones except prolactin is depressed (or even totally suppressed) in the anterior pituitary after division of its connections with the hypothalamus [4-6,15]. Investigations have shown that the secretion of prolactin may actually be stimulated after division of the pituitary stalk or after transplantation of the gland [2,8,12,13,15]. The pituitary can also form prolactin in vitro [11,14], and this synthesis is inhibited by extract of the hypothalamus. For that reason, the view is widely held in the literature that the hypothalamus inhibits the secretaion of prolactin. However, in the experiments cited above (apart from those carried out in vitro), the secretion of prolactin was judged from one of the secondary reactions (dependent to a large extent on other conditions also) — by the ability of the uterus to form a deciduoma.

The present paper describes the results of the direct determination of the prolactin content in the pituitary of rats in which extensive foci of destruction were produced by tissue electrolysis in the median eminence and in the neighboring region of the hypothalamus.

EXPERIMENTAL METHOD

Electrolytic destruction of the medial portion of the hypothalamus was carried out on female rats weighing 205-215 g by means of a stereotaxic apparatus. The results of trial operations (on 25 rats, in which the hypothalamus was studied after decapitation in serial histological sections) showed that the coordinates for obtaining the necessary focus of destruction in the rats of the population used in this investigation were as follows (in accordance with DeGroot's map [3]): A = 5.0, V = −4.0, L = ±0.6. A current of 5 mA was passed for 30 sec through an electrode, consisting of an entomological needle, 0.3 mm in diameter, covered with insulating varnish except for a distance of 0.6 mm at its point.

Observing the conditions described above, operations were performed on 38 rats, 7 of which died during the first 2 days after the operation; the remaining rats (31) were sacrificed by decapitation 35-40 days after the operation. The hypothalamus of each rat was carefully studied macro- and microscopically (in serial histological sections stained by Nissl's method). As a result, 14 rats were selected in which the foci of destruction occupied the whole medial portion of the hypothalamus, and in particular, the ventro- and dorso-medial nuclei, the nucleus arcuatus, and the median eminence (see figure). The results described in this paper relate to these 14 experimental and to 12 control rats which were kept throughout the period of the investigation in identical environmental conditions and on the same diet.

After decapitation of the experimental and control animals, the endocrine glands and the uterus were extracted, weighed, and treated histologically (with the exception of the pituitary); in the anterior pituitary, the content of prolactin was determined separately for each animal by the method of Grosvenor and Turner [7], based on the local reaction of the crop glands in pigeons. For this purpose, a homogenized (in 0.6 ml of distilled water) suspension of the anterior pituitary was injected intradermally into a pigeon (so far as possible into the same place above the right crop gland) in a dose of 0.1 ml daily for 4 days (altogether 0.4 ml or the equivalent of $\frac{2}{3}$ of a pituitary). The pigeon was sacrificed 24 h after the last injection.

This method was modified with regard to the interpretation of the reaction. The pigeon's crop was dissected, an incision made into it on the dorsal aspect in the mid line, and it was stretched out on a wax slab and allowed to dry at room temperature. As a result, a constant preparation was obtained, consisting of a transparent dry film in

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Mean Weight of Endocrine Organs and Uterus, and Prolactin Content of Pituitary in Control and Experimental Rats*

Animals	No. of animals	Body weight (in g)		Weight of organ (in mg)				Sex cycle	Prolactin content in pituitary (in i. u.)		
		at beginning of experiment	postmortem	pituitary	adrenals	ovaries	uterus		per mg	per mm³	per whole pituitary
Control	12	198 ±5.1	223 ±6.0	10.9 ±0.5	57.6 ±2.4	66.0 ±5.1	580.0 ±25.2	Normal	0.026	0.060	0.288
Experimental	14	213 ±4.3	340 ±10.6	5.2 ±0.3	29.5 ±3.1	36.6 ±2.5	209.5 ±27.2	Anestrus	0.014	0.045	0.074

*The postmortem examination was carried out 35-40 days after electrolytic destruction of the region of the median eminence and the medial portion of the hypothalamus.
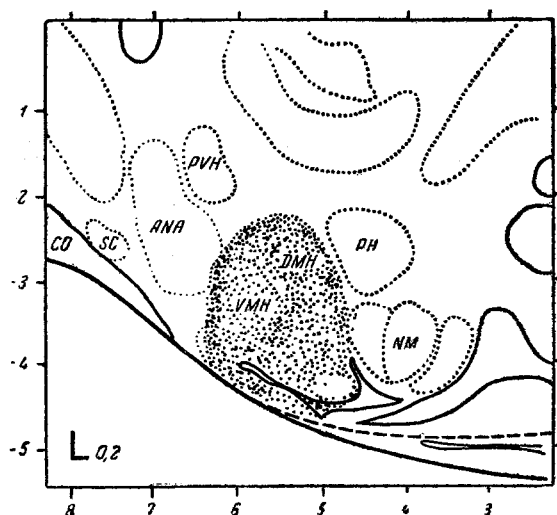
Schematic drawing of the extensive focus of destruction in the medial portion of the hypothalamus and the median eminence in a rat. The region of destruction is marked by dots. CO) optic chiasma; SC) suprachiasmatic nucleus; ANA) anterior nucleus of hypothalamus; PVH) paraventricular nucleus; VMH) ventromedial nucleus; DMH) dorsomedial nucleus; PH) posterior hypothalamic nucleus; ARC) nucleus arcuatus; NM) mammillary nuclei.

which the reacting area was clearly visible, and its size could be measured much more accurately than in the recently excised crop.

As a standard sample of prolactin, the "Panlitar"* preparation was used, containing 15 i.u./mg. In experiments on 110 pigeons, receiving daily intradermal injections of different doses of this preparation (from 0.01 to 1.08 i.u. over 4 days), data were obtained showing the relationship between the extent of the reaction and the logarithm of the dose, from which a calibration curve was plotted [1].

EXPERIMENTAL RESULTS AND DISCUSSION

Injury to the hypothalamus in the region of the ventromedial nuclei is known to produce hyperphagia and obesity of animals. The 14 experimental rats also developed considerable obesity. During the 35-40 days after the operation, their mean weight rose by 127 g, five times greater than the mean gain in weight of the control animals (see table).

The weight of the pituitary in the experimental rats fell by about 50% compared with its weight in the controls. A similar decrease in weight took place in the adrenals, the ovaries, and the uterus of the experimental animals. Histologically, marked atrophy of the adrenal cortex, the thyroid and the ovaries was discovered. In the ovaries, neither ripe follicles nor fresh corpora lutea were found, in full agreement with the prolonged state of anestrus detected as a result of intravital investigations of vaginal smears, and also from the considerable decrease in weight of the uterus.

Taken as a whole, these results demonstrate that destruction of the medial hypothalamus and the median eminence leads to a very severe disturbance of the thyrotropic, gonadotropic, and corticotropic functions of the anterior pituitary, i.e. (at least, judging by these functions) to a state of panhypopituitarism.

The results of the prolactin estimation are given in the table. They show that prolactin was present in the pituitary of the experimental animals, but in a much smaller amount than in the control animals (on the average about 50% of the control level, expressed per mg weight of the pituitary, but only 22% if expressed in terms of the whole gland).

The procedures described above (especially in the region of the median eminence) in rats may be accompanied by focal (usually central) necrosis of the tissue of the anterior pituitary. In this case, using the data obtained by Laszlo and co-workers [9], the quantity of prolactin can be calculated approximately per mm^3 of uninjured anterior pituitary tissue after destruction of its connections with the hypothalamus. Even if this correction was introduced, the quantity of prolactin in the anterior pituitary of the experimental animals was less than in the controls.

Taking into account the long time elapsing between the operation and sacrifice, the protein nature of prolactin, and also the fact that the other hormones of the anterior pituitary do not remain in the gland for a long time (as a reserve), the assumption can be dismissed as improbable that after the operation the production of prolactin completely or almost completely ceased, and that only the remains of its preformed reserve could be found in the gland.

In these conditions the content of hormone in the gland was dependent on the relationship between the rate of its formation and the rate of its delivery into the blood. For this reason, the fact discovered in this investigation may be the result either of a slowing of the first process (production) or of a quickening of the second (secretion). The available information is not sufficient to allow this problem to be solved.

Prolactin remained in the blood stream only for a very short time. It was established conclusively that the amount of prolactin in the volume of blood serum which can be injected intradermally into a pigeon is much below the threshold of sensitivity of the reaction used. For this reason, the prolactin content in the blood could not be determined directly.

The widely held view that the pituitary, if transplanted, produces an increased amount of prolactin is based on indirect evidence obtained by the study of the ability of the uterus to form a placenta.

As a result of experiments on a large number of rats, the authors showed that if the median eminence or the stalk of the pituitary is destroyed during the period of estrus, the uterus of these rats is capable of reacting for several weeks to trauma by the formation of a deciduoma [1]. McCann and Friedmann [10] described the same reaction of the uterus in rats 4-11 days after injury to the hypothalamus.

The ability of the uterus to form a deciduoma, however, depends not only on progesterone, the secretion of which is stimulated by prolactin, but also on other factors and, in particular, on the content of estrogens in the blood. We know, for example, that injection of even small doses of estrogens into rats during pseudopregnancy may completely prevent the formation of a deciduoma [17].

Those authors who have observed a reaction of deciduoma formation in rats with an autotransplanted pituitary have concluded that the pituitary, freed from the influence of the hypothalamus, secretes an increased amount of prolactin, from which it followed that the hypothalamus in normal conditions inhibits the secretion of this hormone.

The present authors consider that this is not the only possible explanation. Another interpretation of these facts and of the results of the present investigations seems more probable. Undoubtedly after injury to the medial hypothalamus and the median eminence (or, as the results of the authors' other investigations show, although only for the median eminence), besides considerable atrophy, necrosis and connective-tissue degeneration of the anterior pituitary, the production and secretaion of follicle-stimulating and luteinizing hormones falls sharply (if it does not cease altogether). Although the secretion of prolactin continues, evidently it also is depressed, although to a much lesser degree. As a result, in the anterior pituitary, the relative proportions of these hormones change sharply, with an increase in the proportion of prolactin, creating an apparent or a relative increase in the secretion of prolactin. This evidently may explain the state of pseudopregnancy after disturbance of the connections between the hypothalamus and pituitary.

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