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PROLACTIN SECRETION OF RAT ANTERIOR PITUITARY CULTIVATED *IN VITRO**

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It is a well known concept that the secretion of gonadotropin from the anterior pituitary is neurohumorally controlled by the hypothalamus through the portal vascular system (Harris, 1955). This concept was ascertained by our previous *in vitro* experiments, in which the rat anterior pituitary cells cultivated were losing the potency to secrete gonadotropin gradually in the course of cultivation, but they were restored under the addition of the hypothalamic extract (Kobayashi *et al.*, 1961 and 1963).

Concerning luteotropin secretion, Everett (1954 and 1956) and Desclin (1956) reported that the corpora lutea were long maintained in the female rats after the autotransplantation of the pituitary to the renal capsule, indicating that LTH (prolactin), but neither FSH nor LH, was secreted from the pituitary even after removal of the hypothalamic control. However, the question has been left unanswered for the mechanism of LTH secretion. Then, we studied the problem by tissue culture of the rat anterior pituitary itself, in other words, under complete isolation from the hypothalamic control.

MATERIALS AND METHODS

Mature female rats of the Wistar strain, weighing 130 to 160 g, were killed by decapitation under chloroform anesthesia. The pituitary was excised, and the anterior lobe was dissected into 4 to 6 pieces. The dissected pieces were put into test tubes. One tube contained the number of explants equivalent to 4 anterior lobes. After resting in a sloping position, it was charged with the 1 ml of synthetic culture medium '199', to which 2 units of Zn-free insulin, 50 I.U. of penicillin G potassium, and 0.1 mg of streptomycin sulfate were added. The medium was adjusted to pH 7.0 with sodium bicarbonate before use. The tubes were placed in a roller at 20 revolutions per hr. which was set in an incubator at 37°C. The cultivation was continued for 12 days and the medium renewed every other day during the culture period. The same aged culture media were collected and lyophilized for the assay of the gonadotropic potency by the mouse uterine-weight method and the prolactin potency by the pigeon crop sac method of Lyons and Page (1935). For the histological examination the explants were fixed in formaline at the end of cultivation and then stained with hematoxylin-eosin.

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RESULTS AND DISCUSSION

The gonadotropic potency in the medium gradually declined after the beginning of cultivation and reduced to an almost undetectable level in the 12-days-old culture medium (Fig. 1). The results were agreed in our previous experiments on the single-cell culture of the hypophysis (Kobayashi *et al.*, 1961 and 1963).

On the contrary, prolactin secretion was maintained under this condition. The potency of this hormone in the medium increased during first 3 days of cultivation, and thereafter it was maintained at the increased level until the end of cultivation (Fig. 1). The pituitary explants of 12 days in age of cultivation proved to contain prolactin, but no gonadotropin (Tables 1 and 2). Histological examination revealed that there were no degeneration and no central necrosis in the explants throughout the period of cultivation.

Fig. 1. Showing the fluctuation of gonadotropin and prolactin secretion from pituitary explants during cultivation

The solid line connecting open circles and the interrupted line connecting crosses represent, respectively, the changes of gonadotropic and of prolactin potency during cultivation.

Table 1. Prolactin contents of pituitary explants 12 days after cultivation

Extract* tested	Pigeon crop sac reaction	
	After cultivation	Before cultivation
10-pituitary extract	+	+
5-pituitary extract	+	+
1-pituitary extract	—	—

* Each extract was prepared from the corresponding number of pituitaries and tested in a pigeon.

Table 2. Gonadotropin contents of pituitary explants various days after cultivation

Materials tested	No. of mice	Uterine weight (mg)
Extract from pituitaries not cultured	5	17.8±6.0*
Extract from 6-days-old culture	5	15.0±2.0
Extract from 12-days-old culture	5	10.0±0.1
Untreated controls	5	10.4±0.2

In each assay 2 pituitaries were used.

* Mean±standard error

Our results support the findings obtained from the *in vivo* experiments by Everett (1954 and 1956) and Nikitovitch-Winer and Everett (1958) and indicate that the secretion or production of prolactin by the anterior pituitary is always maintained or rather accelerated under the condition of complete deprivation of the hypothalamic control, while gonadotropin is neither secreted nor produced without the hypothalamic regulation.

SUMMARY

The rat anterior pituitary was cultivated by means of organ culture technique. The prolactin secretion in this gland was maintained or rather accelerated during 12 days cultivation. This means that the rat anterior pituitary can secrete prolactin under complete deprivation of hypothalamic control. Gonadotropin secretion was declined under the same culture condition.

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