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In Vitro Evidence Against the Anterior Pituitary as a Site of Negative Feedback of Prolactin[1] (38440)

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The decrease in *in situ* pituitary prolactin stores found in rats carrying prolactin-secreting pituitary tumors (1, 2) or normal rat pituitary grafts under the kidney capsule (3) has led to the hypothesis that high levels of circulating prolactin inhibit prolactin secretion. Indirect evidence that the site of feedback of prolactin is the hypothalamus is provided by experiments in which prolactin was implanted into the median eminence of the hypothalamus. Such implants have been reported to decrease prolactin secretion in pseudopregnant (4), pregnant (5), lactating, and proestrous rats (6). In addition, median eminence implants of prolactin into intact or ovariectomized rats increased prolactin-inhibiting factor (PIF) in the hypothalamus (7). However, blood from the median eminence drains directly to the anterior pituitary via the portal vessels, so feedback at the pituitary levels is not ruled out in these experiments. In the present study we studied the effect of prolactin on secretion of prolactin from the pituitary *in vitro*.

Materials and Methods. The pituitaries from male Sprague–Dawley rats (Bioscience, Berkeley, CA) weighing 250–300 g were used in these experiments. The animals were housed in an artificially lighted room with an automatically controlled light cycle (lights on at 0600 hr, lights off at 2000 hr). Rat chow and water were available *ad lib*. All rats were killed by rapid decapitation between 0900 and 1200 hr. The anterior pituitary (AP) was removed, separated into two halves and placed into 25 ml Erlenmeyer flasks containing 3 ml of Medium 199 (Difco Laboratories, Detroit, MI). Each experiment consisted of two sets of five flasks each, containing two AP halves/flask. The two halves of each AP were separated into each set of flasks. The flasks containing AP's were incubated in a Dubnoff metabolic shaker at 37° and gassed constantly with 95% O_2 and 5% CO_2. In the first three experiments, one set of flasks containing AP's had all of the incubation medium removed every 30 min for 4 hr. The flasks were then rinsed and fresh medium was added to replace the medium removed. In the second set of flasks, only 10–20 μl of medium were removed for prolactin assay every 30 min and replaced with an equal volume of fresh medium. This volume represents 0.3–0.6% of the total volume of incubation fluid. In a fourth experiment, ovine S8 prolactin, kindly supplied by NIAMD, was added to flasks containing two AP halves in amounts ranging from 0.1 μg to 100 μg. Every 30 min, 20 μl of medium were removed for prolactin assay. Control flasks were similarly treated except that albumin was substituted for prolactin. The total time of incubation was 120 min. At the end of all incubations, the AP halves were weighed, homogenized in saline and frozen in concentrations of 1 mg/ml.

Prolactin from individual samples of medium and pituitary tissue was assayed at two or three dilutions using the NIAMD radioimmunoassay kit (supplied by A. F. Parlow and NIAMD). All values were expressed relative to NIAMD-RP-1 for rat prolactin, which has a potency of 30 IU/mg. The assay does not detect ovine prolactin.

Results. The results of experiments 1–3 are shown in Fig. 1. There was considerable variation in the amount of prolactin released during different 30 min time intervals, and no consistent difference between the pituitaries incubated without a change in the medium and those in

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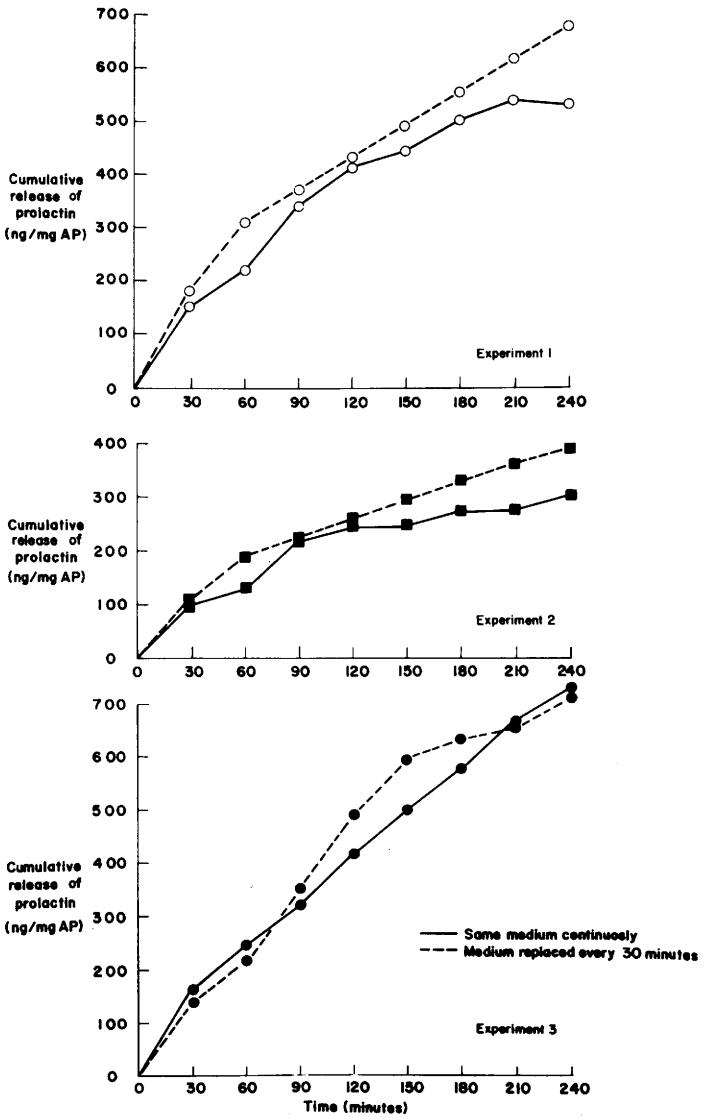

FIG. 1. Cumulative release of prolactin *in vitro*. Results of three experiments are shown individually. Each point represents the mean of determinations in five flasks.

which the medium was changed. In two of the three experiments, there was a slight decline in prolactin release near the end of the incubation when the medium was not changed but none of the differences between the two groups in the three experiments were statistically significant. The amount of prolactin remaining in the AP halves after incubation was similar in the two groups in each experiment, and varied from 0.3 to 0.46 μg/mg AP tissue at the end of a 4 hr incubation.

Under the conditions of the experiment, addition of ovine prolactin in amounts ranging from 0.1 to 100 μg did not alter the amount of rat prolactin released from the incubated AP during a 2 hr incubation (Fig. 2). There was considerably less prolactin release during the second hour of incubation compared to the first hour in all groups, including controls. There also was no difference in the amount of prolactin remaining in the glands in any of the groups after 2 hr of incubation, with concentrations ranging from 0.49 to 0.65 μg/mg AP tissue. This is somewhat more than the amount after 4 hr of incubation.

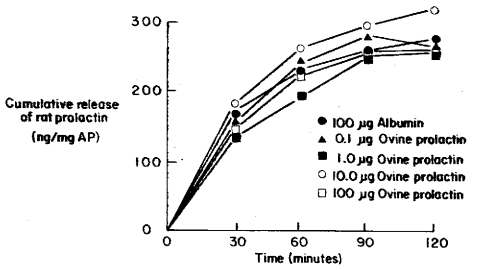

FIG. 2. Cumulative release of prolactin from rat anterior pituitary halves *in vitro* when incubated with albumin or with varying amounts of ovine prolactin. The volume of medium in each flask was 3 ml. Each point represents the mean of volumes obtained in five flasks.

To determine whether the incubation of the medium itself had any effect on prolactin, flasks containing rat prolactin but no AP were incubated for 4 hr. There was no difference in the amount of prolactin in the medium at the beginning and the end of this incubation time.

Discussion. Release of prolactin from rat AP halves incubated *in vitro* was not inhibited by prolactin which accumulated in the vessel. Furthermore, addition of various quantities of ovine prolactin did not inhibit prolactin release over a 2 hr incubation. Nicoll (8) reported that ovine prolactin inhibited the release of prolactin by about 20% the first hour of *in vitro* incubation with rat AP, but over a 4 hr incubation period there was no significant difference from controls. The slight difference between his results and ours may be due to differences in incubation procedure, assay for prolactin or sex of the rat from which the AP was obtained. We used male rats and a rat radioimmunoassay. Nicoll used a disc electrophoretic–densitometric assay method and did not state the sex of the pituitary donors.

Thus, it would appear that prolactin does not act directly on the pituitary to inhibit its own release. In this regard, it appears to differ from melanocyte–stimulating hormone (MSH). Kastin (9) has reported that removal of incubation medium every 5 min increased the release of MSH from whole rat pituitaries. Furthermore, addition of purified MSH significantly reduced release of MSH from the incubated pituitaries.

The evidence presented in this paper plus the reports that implants of prolactin in the median eminence of the hypothalamus inhibit the release of prolactin from the pituitary *in situ* (4–7) suggest that prolactin inhibits its own secretion via the hypothalamus. However, the physiological role, if any, of this short-loop feedback regulation of prolactin remains uncertain.

Summary. Anterior pituitaries of male rats were incubated for 4 hr *in vitro* and the amount of prolactin released when the medium was changed every 30 min was compared to the amount released when the prolactin was permitted to accumulate in the incubating medium. There were no consistent differences. Addition of various doses of ovine prolactin did not affect the release of rat prolactin *in vitro*. These results suggest that the anterior pituitary is not the site at which prolactin feeds back to inhibit its own secretion.

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