Prolactin-Inhibiting Activity in Hypophysial Stalk Blood and Elevation by Dopamine

Prolactin release by the anterior pituitary is believed to be inhibited by the brain through a neurohumoral mechanism. When this mechanism is impaired by such procedures as pituitary transplantation[1], pituitary stalk transection[2–4] or hypothalamic lesions[5], prolactin secretion increases as indicated by persistent function of the corpus luteum. A hypothalamic factor which inhibited prolactin release was demonstrated by PASTEELS[6] and TALWALKER et al.[7]. They showed that the rate of prolactin release from pituitaries in tissue culture was reduced when hypothalamic tissue or extracts were added to the culture medium. Subsequently, prolactin-inhibiting activity, which is attributed to the so-called prolactin inhibiting factor (PIF), has been demonstrated in hypothalamic tissue of several species[8]. It is assumed that PIF reaches the anterior pituitary via the hypophysial stalk portal vessels. However, such activity has not been observed in the blood of the portal vessels heretofore.

SAWYER et al.[9] first suggested that neurotransmitter substances are involved in the control mechanism of ovulation which is dependent upon the release of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) from the anterior pituitary. Conversely, depletion of the stores of monoamines in the brain by reserpine[10–12] as well as inhibition of catecholamine synthesis[13–15] blocks ovulation in many species. KANEMATSU and SAWYER[16] have shown that reserpine implants in the hypothalamus initiated milk secretion in rabbits which they attributed to the catecholamine-depleting effect of reserpine. Later, VAN MAANEN and SMELIK[17] confirmed these findings and suggested that the effect was due to the deficiency of dopamine in the hypothalamus. They concluded that a dopaminergic system in the hypothalamus, which was demonstrated by FUXE et al.[18–20], inhibited the release of prolactin.

We have observed that dopamine injected into the third ventricle of the brain of rats inhibited the release of prolactin[21] while simultaneously increasing the release of LH[22,23] and FSH[24,25]. However, the infusion of dopamine directly into the anterior pituitary via a cannulated stalk portal vessel had no effect on the release of either prolactin[21], LH[22,23] or FSH[24,25]. Yet, when dopamine was injected into the third ventricle, the LH-[26] and FSH-[27] releasing factor activity in stalk portal blood increased markedly. These observations led us to test the hypothesis that dopamine may also stimulate the release of PIF into the blood of the hypophysial portal vessels.

Materials and methods. Blood was collected simultaneously from the femoral artery and pituitary stalk[28] for 2–4 h from male Sprague-Dawley rats weighing 350–450 g. These animals were treated in one of two ways. One group was given dopamine hydrochloride (equivalent to 2.5 µg of dopamine) in 2.5 µl of 0.15 *M* NaCl which was injected through a microcannula into the third ventricle. The cannula was inserted through the floor of the third ventricle midway between the optic chiasma and the median eminence. In the other group, the ventricular injection was omitted.

Blood plasma from 10–12 similarly-treated donors was combined, and aliquots were assayed in vitro for prolactin-inhibiting activity. Anterior pituitaries from rats killed by decapitation were bisected in the midline. One half of a pituitary was used as the control, and the opposite half was used as the experimental tissue. All incubations were performed under an atmosphere of 95% O_2 and 5% CO_2 in a metabolic shaker set for 45 rev/min and 25 °C. Each pituitary half was kept in a separate flask containing 0.5 ml of a tissue culture fluid (Difco medium 199, pH 7.2). After 1 h, the incubation fluid was decanted; and blood plasma from the femoral artery (peripheral plasma) was added to one half of a pituitary while hypophysial stalk plasma was added to the opposite half of the same gland. The incubation was continued for an additional h. The quantity of prolactin released into the incubation medium by each pituitary half was determined by radioimmunoassay[29].

Results and discussion. Although prolactin in the peripheral plasma was undetectable, stalk plasma from animals treated with dopamine and from the untreated animals contained 1.4 and 1 ng prolactin per ml, equivalent to a reference standard having a potency of 28 IU of prolactin per mg. These quantities were subtracted in computing the net release of prolactin.

During the first hour, when medium 199 was the incubation fluid, the spontaneous release of prolactin was 22.2 ± 1.2 (mean and S.E.; N 10) and 22.5 ± 1.5 ng/h

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by one set of pituitary halves (Figure 1) and 24.2 ± 1.2 and 24.7 ± 1.5 ng/h by another set (Figure 2). It is apparent that when the conditions are identical, the amount of prolactin released by one set of pituitary halves is essentially the same as that released by the set of opposite halves. During the second hour, when plasma was substituted for medium 199, the rates of release of prolactin by pituitary halves incubated in peripheral plasma were 21.2 ± 1.2 (Figure 1) and 23.6 ± 1.2 ng/h (Figure 2), which is essentially the same as that released in the first hour when medium 199 was the incubation fluid. The pituitary halves incubated in stalk plasma from untreated animals released 18.7 ± 1.3 ng/h (Figure 1). The halves incubated in stalk plasma from dopamine-treated rats released 5.9 ± 0.6 ng/h or $^1/_4$ as much prolactin as their opposite halves which were incubated in peripheral plasma (Figure 2).

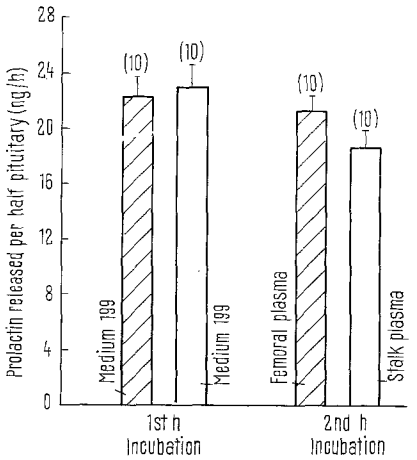

Fig. 1. The effect of hypophysial stalk plasma from untreated rats on prolactin release by rat hemipituitaries in vitro. The number of hemipituitaries is shown in parentheses. The vertical bar represents the standard error.

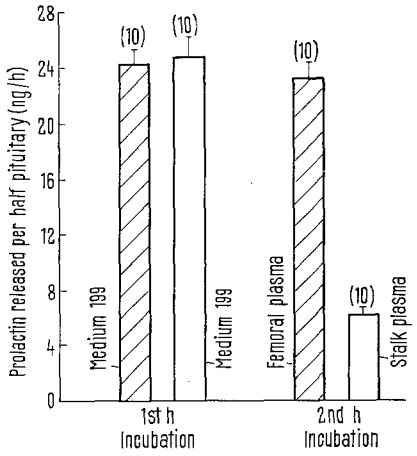

Fig. 2. The effect of hypophysial stalk plasma from dopamine-treated rats on prolactin release by rat hemipituitaries in vitro. The number of hemipituitaries is shown in parentheses. The vertical bar represents the standard error.

An analysis of these data using the *t*-test for paired samples[30] revealed that the pituitary halves incubated in stalk plasma from untreated animals and from dopamine-treated animals released significantly less prolactin than their opposite halves which were incubated in peripheral plasma ($P < 0.001$). In addition, pituitary halves incubated in stalk plasma from dopamine-treated rats released significantly less prolactin than pituitary halves incubated in stalk plasma from untreated rats ($P < 0.001$).

These observations show that pituitary stalk plasma in the rat contains prolactin-inhibiting activity and the activity in stalk plasma is increased when dopamine is injected into the third ventricle of the brain. The prolactin-inhibiting activity is attributed to PIF, the release of which may be regulated by a dopaminergic mechanism[31].

Résumé. Des moitiés d'hypophyse incubées dans du plasma de la tige hypophysaire sécrètent moins de prolactine que les moitiés complémentaires incubées dans du plasma de sang périphérique. Les glandes incubées dans du plasma de la tige hypophysaire de rats traités à la dopamine sécrètent moins de prolactine que celles incubées dans le plasma des rats témoins. L'activité inhibitrice de la prolactine dans le plasma de la tige hypophysaire peut être le résultat d'un facteur particulier, et la sécrétion de ce facteur serait gouvernée par un mécanisme «dopaminergique».

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Indication of an 'Insulin Like' Factor in the Pancreatic Tissue of the River Lamprey *Lampetra fluviatilis* (L.)

The homology between the pancreatic tissue of larval and adult lampreys and the islet tissue of the higher vertebrates first suggested by Cotronei[1], Keibel[2], and Boenig[3] has since been confirmed by both histological and experimental studies[4–15]. In the ammocoete larva of *Lampetra planeri*, a non-parasitic species which does not feed after metamorphosis, Barrington[4] showed that the secretory activity of the pancreatic tissue increased after glucose loading and that removal of the follicles by cautery increased blood sugar levels. Boenig[3] and Sterba[6] suggested that in this species the pancreas of the adult is degenerate and functionless. However, experimental studies by Ermisch[7–8] in which he showed an extract of both the cordal and cranial cords of adult