produced by the *Myrothecium* species. We have evidence that satratoxins C and F are also members of the 12,13-epoxy-Δ^9-trichothecene series. Because so many members of the roridin-verrucarin series of sesquiterpenoid fungal metabolites are highly toxic and produce biological effects in experimental animals similar to those observed in stachybotryotoxicosis, it is reasonable to speculate that the members of this class of compounds which have been described in this report are, indeed, the chemical agents responsible for this disease.

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15. This paper is part of a dissertation to be submitted to the Graduate School, University of Maryland, by R. M. Eppley in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry.

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Prostaglandin Involvement in Hypothalamic Control of Gonadotropin and Prolactin Release

Abstract. *Prostaglandin E_2 (PGE_2) injected into the third ventricle of ovariectomized rats increased plasma luteinizing hormone dramatically and follicle stimulating hormone slightly. PGE_1 elevated prolactin; $PGF_{1\alpha}$ or $PGF_{2\alpha}$ had no effect. PGE_2 or PGE_1 injected directly into the anterior pituitary were ineffective. These results suggest that specific prostaglandins act at the hypothalamus to control pituitary hormone release.*

Prostaglandins (PG) are being implicated as intermediates in an ever-increasing number of physiologic systems, including several aspects of reproduction (*1*). Although participation of PG's in the regulation of ovarian function has been extensively studied, little is known about their role in relation to the hypothalamic pituitary axis. It was recently postulated that PG's may be involved in the process of ovulation because ovulation was blocked by inhibitors of PG synthesis (*2–4*). The site (or sites) of action of PG's in ovulation remains undetermined. Some data (*4, 5*) suggest that they have an effect directly on the ovaries. Other indirect evidence (*2, 3*) implies an effect on pituitary release of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) which bring about ovulation.

Until now there has been no direct evidence for the involvement of PG's in the hypothalamic control of pituitary gonadotropin secretion. The hypothalamus regulates secretion of pituitary gonadotropins and prolactin by releasing specific neurohormones into the hypophyseal portal vessels. The neurohormones are then transported down the hypophyseal stalk via the portal vessels to the pituitary where they stimulate or inhibit release of particular anterior pituitary hormones (*6*). We now report that prostaglandins can affect pituitary secretion of LH, FSH, and prolactin and that this effect is probably mediated by the hypothalamus.

Female Sprague-Dawley rats (250 to 300 g at the time of treatment) were ovariectomized and housed in a room maintained at 24°C with a light-dark cycle of 14 hours of light and 10 hours of darkness. The rats were allowed free access to Purina rat chow and tap water. Two weeks after castration, each animal was implanted with a permanent cannula (23-gauge stainless steel), which was placed in the third ventricle or in each lobe of the anterior pituitary by means of a stereotaxic instrument (*7*). Proof of placement of the cannulas in the third ventricle was verified by leakage of cerebrospinal fluid upon removal of the mandril; intrapituitary implants were verified by observation of cannula tips within the pituitary tissue when the animal came to autopsy. Cannulas were considered properly placed when the tips extended into the tissue at least 0.5 mm from any lateral edge of the gland. Three to five days after surgery, 5 μg of PGE_1, PGE_2, or $PGF_{1\alpha}$ in a 5-μl volume [95 percent ethanol and 0.02 percent Na_2CO_3 (1:9)] were injected into the third ventricle. Five micrograms of $PGF_{2\alpha}$ (tromethamine salt) were injected in 5 μl of 0.9 percent NaCl. PGE_1 or PGE_2 (2.5 μg in 2.5 μl) were injected into each lobe of the anterior pituitary (5 μg in 5 μl total dose). Injections were made with a 10-μl Hamilton microsyringe connected by polyethylene tubing (PE 10) to a 30-gauge stainless steel cannula which fit snugly within and extended to the tip of the cannula. In all cases, fluid was injected at the rate of 2 μl/min. Heparinized blood samples (0.9 to 1 ml) were obtained by jugular puncture from lightly etherized animals before injection and 15, 30, and 60 minutes afterward. Concentrations of LH in the plasma were measured by radioimmunoassay by the method of Niswender *et al.* (*8*); concentrations of FSH and prolactin were measured by radioimmunoassay, with the kits provided by National Institute of Arthritis and Metabolic Diseases. The results were expressed in terms of the RP-1 rat pituitary reference preparations for FSH and prolactin and the NIH-LH-S1 standard for LH. Data were statistically analyzed by the paired *t*-test.

PGE_2 injected into the third ventricle

produced a four- to fivefold ($P < .01$) rise in plasma LH 15 minutes after treatment (Fig. 1). Plasma FSH was slightly increased by PGE_2 ($P < .02$, $P < .01$, and $P < .05$ at 15, 30, and 60 minutes, respectively). The rise in FSH was maintained throughout the period studied, whereas LH tended to decline. Plasma prolactin, in contrast to LH and FSH, was not affected by PGE_2. Prolactin, however, was significantly increased at 15 ($P < .01$) and 30 ($P < .05$) minutes by PGE_1 (Fig. 1). At 60 minutes prolactin had declined and was not significantly different from that of the control before treatment. $PGF_{1\alpha}$ and $PGF_{2\alpha}$ did not significantly alter the concentrations of LH, FSH, or prolactin in the plasma at any of the periods studied.

Contrary to their effect when injected intraventricularly, PGE_1 or PGE_2 (at the same dose) failed to alter LH, FSH, or prolactin when injected directly into the anterior pituitary (Fig. 2).

Since exogenous LH was incapable of overcoming the antiovulatory effect of indomethacin, an inhibitor of PG synthesis, it was concluded that the drug exerts its effect directly on the ovaries (*4*, *5*). These studies, however, do not exclude the possibility of additional sites of action of PG inhibitors in blocking the ovulatory process, such as on the hypothalamus or the pituitary. In support of the former site of action, either LH or synthetic LH releasing hormone (*3*) was capable of overcoming the blockade of ovulation produced by another inhibitor of PG synthesis, namely, aspirin. Our results indicate that PG's are capable of acting at the hypothalamic level to release LH, FSH, and prolactin. A pituitary site of action is ruled out by the absence of an effect when the PG's were injected directly into the anterior pituitary. In support of the last finding is the observation that PG's incubated with anterior pituitaries in vitro failed to influence basal release of LH and FSH (*9*).

Presumably PGE_2 acts to induce a release of gonadotropin releasing hormone or hormones which then pass down the portal vessels to stimulate the release of LH and FSH. On the other hand PGE_1 may stimulate the release of prolactin either by inhibiting the discharge of the inhibitory hypothalamic hormone for prolactin, prolactin inhibiting factor (PIF), or by stimulating a release of the postulated prolactin releasing factor (*6*). The results suggest that particular PG's may be

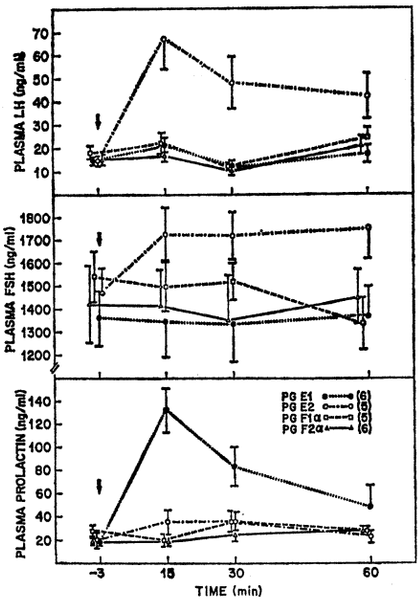

Fig. 1. Effects of third ventricular injections of PGE_1, PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ in ovariectomized rats on plasma concentrations of LH, FSH, and prolactin. Numerals in parentheses show the number of animals used and vertical lines indicate standard error of the mean. Arrows indicate the time of injection.

involved in the release of particular neurohormones from the hypothalamus, and it may well be that PG's are an intermediate in the release of all hypothalamic hypophyseal stimulating and inhibiting hormones. In support of this is the recent evidence that PG's injected into the median eminence can

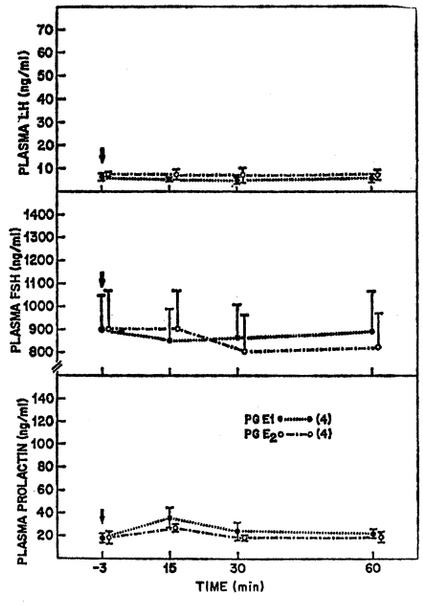

Fig. 2. Effects of intrapituitary injections of PGE_1 and PGE_2 on the amounts of LH, FSH, and prolactin in plasma. Arrows indicate the time of injection.

release corticotropin releasing factor (*10*). Since PG's have been implicated in transmission across adrenergic synapses in the superior cervical ganglion and in the cerebellum (*11*), we suggest that PG's may act by mediating or modulating the action of synaptic transmitters such as norepinephrine and dopamine, which have already been shown to be capable of releasing gonadotropin releasing factors and PIF (*12*).

Ovariectomized rats were used in our study to avoid possible variation in response to PG's due to ovarian cyclicity. Further studies are warranted in intact animals. Our results emphasize the possibility of fertility control by the use of inhibitors of PG action or synthesis.

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