filament ring is defective in the mutant. A third possibility derives from evidence that inactive chitin synthetase zymogen is present at numerous sites on the plasma membrane (*29*), and must undergo a localized activation by an activating factor during budding (*4*). The membrane-bound zymogen of the mutant strain may experience a spontaneous and delocalized activation when shifted to the restrictive temperature.

Results obtained with the *cdc24-1* mutant are consistent with the necessity for a reinforcing ring of chitin if cell wall expansion is to result in bud formation, rather than in generalized cell expansion. It is difficult to reconcile this idea with the report of Cabib and Bowers (*12*) that treatment of yeast cells with polyoxin D, an inhibitor of chitin synthetase, can prevent chitin synthesis and formation of the Calcofluor-binding ring, while allowing normal buds to appear. Although their experiment used very high concentrations of polyoxin D (*30*) at a temperature near the upper limit of the normal growth range, it supported the view (*4*) that, although most chitin is synthesized early in the cell cycle, it functions only at the time of cytokinesis. A hypothesis that would explain both results is that the *cdc24-1* mutant is defective in forming an annular structure, such as the microfilament ring (*15*) or the "collar-like circular zone" described by Seichertová *et al.* (*31*), that is necessary both for localized deposition of chitin and for budding.

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25 January 1978

Potent Antidopaminergic Activity of Estradiol at the Pituitary Level on Prolactin Release

Abstract. *Prior incubation of rat anterior pituitary cells with 17β-estradiol led to an almost complete reversal of the inhibitory effect of two dopamine agonists, dihydroergocornine and RU 24213, on both basal prolactin release and thyrotropin releasing hormone–induced prolactin release. These experiments thus demonstrate a direct interference of dopamine action by a peripheral hormone. Prolactin secretion by pituitary cells in primary culture could possibly serve as an easily accessible model of a system under dopaminergic control.*

Recent studies indicate that dopamine may be the main or even the only inhibitory substance of hypothalamic origin controlling prolactin secretion. In fact, the prolactin inhibiting activity contained in hypothalamic extracts could be accounted for by their catecholamine content (*1*), and prior incubation of hypothalamic extracts with aluminum oxide or monoamine oxidase led to complete loss of prolactin release inhibiting activity (*2*).

Estrogens are potent stimulators of prolactin secretion in both man (*3*) and rat (*4*, *5*). Moreover, the increased rate of prolactin secretion in the afternoon of proestrus in the rat is presumably under estrogenic influence (*6*, *7*). These effects of estrogens in vivo could, however, be exerted at the hypothalamic or pituitary level, or both. Our observation that, in

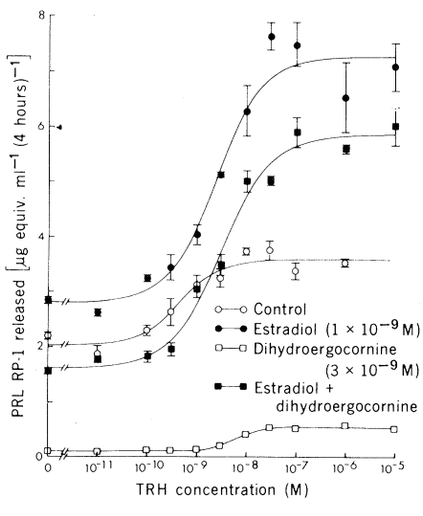

Fig. 1. Effect of 1 n*M* 17β-estradiol (E_2), 3 n*M* dihydroergocornine, or the vehicle alone (control) on the prolactin response to increasing concentrations of TRH in female rat anterior pituitary cells in primary culture. Cells were first incubated for 120 hours in the presence or absence of E_2, and then incubated for 4 hours in the presence or absence of the dopamine agonist DHE and the indicated concentrations of TRH. Data are expressed as means ± standard error of duplicate measurements of triplicate petri dishes. Note that E_2 led to an almost complete reversal of the inhibitory effect of DHE on prolactin release.

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rats, administration of thyrotropin releasing hormone (TRH) leads to a decreased sensitivity to later exposure to the neurohormone (*7*) indicates the difficulty of interpreting experiments in vivo aimed at defining the site of estrogen action.

Since anterior pituitary cells in culture proved to be an excellent system in which to study the specificity of action of sex steroids at the anterior pituitary level (*8*), we used this system, instead of intact pituitaries, to study the interactions of 17β-estradiol (E_2) and dopamine on prolactin release. The present data show that E_2 stimulates basal as well as TRH-induced prolactin release by a direct action at the pituitary level. Moreover, somewhat unexpectedly, the addition of E_2 led to an almost complete reversal of the dopamine-induced inhibition of prolactin release.

Adult female Sprague-Dawley rats (from Canadian Breeding Farms, St. Constant, Quebec) at random stages of the estrous cycle were used for the preparation of primary cultures of anterior pituitary cells (*8, 9*).

As shown in Fig. 1, the addition of TRH led to a maximum 50 percent increase in prolactin release in anterior pituitary cells in culture at an ED_{50} (median effective dose) value of $4.8 \pm 1.8 \times 10^{-9}M$. The addition of the dopamine agonist dihydroergocornine (DHE), at a concentration of $3 \times 10^{-9}M$, led to a 90 to 95 percent inhibition of basal and TRH-induced prolactin release. Prior incubation of cells for 120 hours with $1 \times 10^{-9}M$ E_2 led to a 35 to 45 percent increase in basal prolactin release ($P < .01$) while the maximum hormone response to the neurohormone was increased two- to threefold. However, the most unexpected finding was that prior incubation of the cells with the estrogen led to an almost complete reversal of the inhibitory effect of the dopamine agonist on both basal and TRH-induced prolactin release. In fact, basal prolactin release in the presence of DHE alone was 78 ± 1 ng equivalents of PRL-RP-1 per milliliter per 4 hours, whereas addition of E_2 to DHE increased prolactin release to 1551 ± 14 ng. The maximum prolactin responses to high concentrations of TRH were 519 ± 11 and 5855 ± 100 ng in the corresponding groups.

A more detailed analysis of the antagonism between E_2 and dopaminergic action on prolactin release is presented in Fig. 2. Whereas the potent dopamine agonist RU24213 led to an 80 to 85 percent inhibition of spontaneous prolactin release in the absence of E_2 (Fig. 2A), the maximal inhibition was reduced to 40 to 45 percent in cells previously incubated with E_2 (Fig. 2B). At a concentration of $1 \times 10^{-8}M$, TRH did not significantly affect the RU24213 ED_{50} value of inhibition of prolactin release. Exposure to the estrogen did, however, lead to a significant increase in the RU24213 ED_{50} value ($P < .01$) as reflected by the higher concentrations of the dopamine agonist required to inhibit prolactin release.

Implantation studies had already suggested an action of estrogens at the pituitary level on prolactin secretion (*10*). The present data clearly demonstrate a direct pituitary site of action of estradiol on prolactin secretion and extend previous information obtained with pituitary explants (*11*). These data do not exclude the possibility that the hypothalamus is also an important site of control of prolactin secretion by estrogens (*12*). The observation of only a small stimulatory effect of E_2 alone on basal prolactin secretion in pituitary cells in culture can probably be explained by the already high rate of hormone secretion in these cells which are freed from the strong hypothalamic inhibitory influence which normally exists in vivo (*13*). Since relatively little change of prolactin cell content was observed upon addition of estrogens, the marked stimulation of the prolactin response to TRH indicates a specific effect on the sensitivity of the release mechanisms. The small effect of E_2 on basal hormone secretion is analogous to the effect of the estrogen on the thyroid stimulating hormone (TSH) response to TRH in vivo in the rat; E_2 alone had only a small stimulatory effect on the TSH response but it led to half-maximum and complete reversal of the inhibitory effect of thyroid hormone on the response to E_2 in intact and hypothyroid animals, respectively (*5*).

The present findings of a potent antagonistic effect of E_2 on the action of dopamine agonists may well explain the observation that injections of L-dopa cause a much lower decrease in prolactin release in normal than in stalk-sectioned female monkeys (*14*). Since the stalk had been cut 8 weeks previously, these animals were likely to be under low estrogenic stimulation. The antagonistic effect of E_2 on dopaminergic action may be also responsible, up to an unknown extent, for the observation that apomorphine inhibited prolactin secretion to a greater degree and for a longer period when administered 14 days as opposed to 1 day after destruction of the medial basal hypothalamus (*15*).

The direct stimulatory effect of E_2 on prolactin secretion at the anterior pituitary level could at least partly explain the observation that the serum prolactin response to phenothiazines (*16*), arginine (*17*), and TRH (*18*) is higher in women than in men. Moreover, the stress of major surgery is accompanied by higher

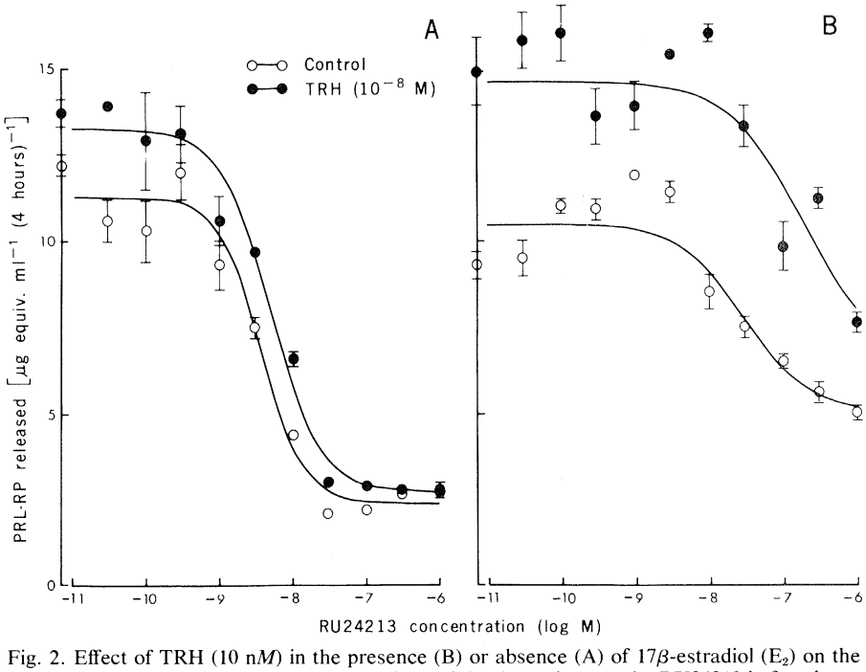

Fig. 2. Effect of TRH (10 nM) in the presence (B) or absence (A) of 17β-estradiol (E_2) on the prolactin response to increasing concentrations of the dopamine agonist RU24213 in female rat anterior pituitary cells in primary culture. Cells were first incubated for 120 hours in the presence or absence of 1 nM E_2, and then incubated for 4 hours in the presence or absence of TRH and the indicated concentrations of RU24213. Note that the inhibitory effect of the dopamine agonist RU24213 on prolactin release was more than 50 percent reversed by E_2 pretreatment.

increases in serum prolactin concentrations in women than in men (*19*).

Our recent observation of a close correlation between the prolactin response to TRH and the concentration of pituitary TRH receptors (*5*) can probably offer a partial explanation for the results obtained. However, the almost complete reversal of the inhibitory effect of dopamine by E_2 clearly shows an important effect of E_2 on dopamine receptor action in the anterior pituitary gland. Since dopamine receptors are not only involved with prolactin secretion but appear to have a role in the modulation of mood and behavior (*20*), and in some diseases (*21*), the present data indicate that the control of prolactin secretion in anterior pituitary cells in culture could well be used as a model system for studies of the effects of peripheral hormones on dopaminergic action.

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Striatal Nondopaminergic Neurons: Possible Involvement in Feeding and Drinking Behavior

Abstract. *Intracaudate injections of kainic acid destroy striatal neurons containing acetylcholine and γ-aminobutyric acid but leave dopaminergic nerve terminals in this brain region intact. Rats injected with the drug are aphagic and adipsic, and have other behavioral abnormalities strikingly similar to those seen in animals with lesions in the dopaminergic nigrostriatal bundle.*

Brain dopaminergic neurons whose cell bodies originate in the pars compacta of the substantia nigra and whose terminals form synapses with cells in the neostriatum (*1*) play an important role in feeding and drinking behavior (*2*). However, little is known about the roles of other neurons in the caudate nucleus that may form synapses with this dopaminergic afferent system. The striatum contains relatively large concentrations of many putative neurotransmitter compounds—acetylcholine, γ-aminobutyric acid (GABA), serotonin, and substance P—in addition to dopamine (*3*); however, it is difficult to study these other types of striatal neurons separate from the contribution of the dopaminergic afferent system, because of their relatively close proximity to one another.

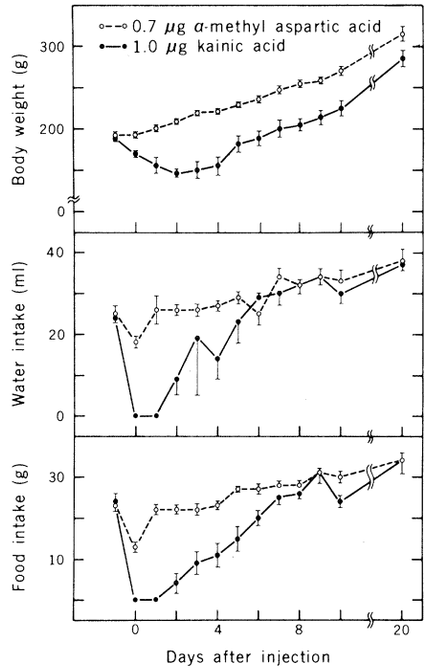

Fig. 1. Daily body weight changes and food and water intake after bilateral intracaudate injections of 1.0 μg of kainic acid or 0.7 μg of an iso-osmotic control solution of α-methylaspartic acid. All values are the means ± standard error of mean (S.E.M.) (N = 14).

Kainic acid (2-carboxyl-3-carboxymethyl-4-isopropenylpyrrolidine), a rigid cyclic analog of glutamic acid, is a powerful neuroexcitant when it is iontophoretically applied to brain or spinal cord neurons (*4*), and has neurotoxic effects on cells in the striatum after intracaudate injections of larger doses (*5*). Although these latter injections destroy striatal cholinergic or GABAergic nerves, nigrostriatal dopaminergic nerve terminals projecting into the caudate nucleus are apparently left intact.

Using intracaudate injections of kainic acid, we now report that rats become aphagic and adipsic after the treatment, and also have other behavioral abnormalities and permanent feeding and drinking regulatory impairments previously seen only in animals with electrolytic (*6*) or chemical (*2*) lesions of the nigrostriatal bundle.

Male albino rats (175 to 200 g; Charles River Laboratories, Wilmington, Mass.) had free access to food (Purina rat chow) and water, and were housed under a constant light-dark cycle (12:12 hours; lights on at 0800). Under Nembutal anesthesia, different groups of rats were stereotaxically injected with various doses of kainic acid or an iso-osmolar control solution (α-methylaspartic acid, 0.7 μg/μl

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