previously described inhibitors of anaphylactic reaction. The compound does not destroy penicillin, does not affect its antibiotic action, is devoid of antihistaminic and anti-inflammatory activity, and does not act by a generalized depression of the reactivity of the organism. Incubation of penicillin antibody with chlorphenesin decreases the antibody titer; thus, chlorphenesin may act by blocking certain antibody sites and in this manner interfere with specific antigen-antibody reactions.

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Effects of Epinephrine and Acetylcholine on Hypothalamic Content Of Prolactin Inhibiting Factor.*† (31729)

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Virgin female rats injected subcutaneously for 10 days with 10 μg estradiol daily, followed by twice daily injections of 25 mg acetylcholine iodide/kg body weight or 0.25 mg epinephrine in oil for 5 days, showed extensive lobulo-alveolar mammary growth and lactation(1,2). Similar results were obtained when epinephrine, acetycholine iodide or serotonin were injected into estrogen-primed female rabbits(3). Epinephrine, acetylcholine and serotonin can also induce pseudopregnancy in rats under certain experimental conditions(4). The results are interpreted as indicating that these neurohumors stimulate release of prolactin from the pituitary.

The presence of a prolactin-inhibiting factor (PIF) in acid extracts of rat hypothalami has been demonstrated(5). The *in vitro* assay method developed for PIF indicated that the suckling stimulus during lactation(6) or injections of estrogen(6) or reserpine(7) significantly decreased the PIF content of the hypothalamus. Since it has been found that epinephrine and acetylcholine have no direct effect on the pituitary *in vitro* (5), it was of interest to determine whether their lactational effects were exerted by depressing the PIF content of the hypothalamus.

Materials and methods. The work reported here follows in detail the methods described previously(5,6) except where otherwise noted. For each of 2 experiments designed to investigate the effects of epinephrine, experimental hypothalamic donors were 12 Carworth CFN female rats injected subcutaneously with 0.25 mg epinephrine in oil (Parke-Davis Co.) twice daily for 5 days and killed

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TABLE I. Effects of Epinephrine and Acetylcholine on Hypothalamic PIF Content.

Exp	No. of pigeons	Mean prolactin responses (RTU)*	P
		Epinephrine:	
1	11	†C = .500 ‡E = .955	<.05
2	12	C = 1.000 E = 1.708	<.05
Combined data	23	C = .761 E = 1.308	<.0025
		Acetylcholine bromide:	
1	12	C = .480 E = .750	<.06
2	12	C = .833 E = 1.083	<.10
Combined data	24	C = .656 E = .917	<.006

* RTU = Reece-Turner units.
† C = control prolactin content.
‡ E = experimental prolactin content.

one hour after the last injection. Twelve CFN female control animals received injections of 0.1 ml corn oil twice daily.

For each of 2 experiments designed to study the effects of acetylcholine, 12 CFN female rats comprising the experimental group were injected with 25 mg acetylcholine bromide (Eastman) per kilogram body weight twice daily. Control animals received saline injections.

The incubation procedures involved use of neutralized acid extract equivalent to 2 hypothalami for each pituitary equivalent incubated. In each incubation, 12 adult female rat (Spartan Research, Haslett, Mich.) pituitaries were divided among 3 flask pairs, *i.e.*, there were 2 pituitary equivalents per flask. The medium from each incubation was assayed for prolactin content in 11-12 White King pigeons by the local crop sac method (8), the control medium being injected over one side of the crop sac and the experimental medium over the other side. The responses were rated by the Reece-Turner method(9).

Results and discussion. The results are presented in Table I. Epinephrine treatment produced a definite reduction in hypothalamic PIF content. Each experiment was significant at the .05 level. Acetylcholine bromide produced results which achieved acceptable levels of statistical significance when the 2 experiments were combined. The differences in prolactin release between experiments are due primarily to use of donor pituitaries for incubation from rats in different stages of the estrous cycle. Such variations are not seen between experiments when male rat pituitaries are employed (Kragt and Meites, in press).

This work indicates that epinephrine and acetylcholine can reduce the hypothalamic PIF content and presumably its rate of release, thereby permitting increased amounts of prolactin to be released by the pituitary. It appears, therefore, that the lactogenic effects of these neurohormones are mediated through the hypothalamus. It is probable that other drugs and environmental agents which promote prolactin release by the pituitary (4) also act by depressing synthesis and release of PIF in the hypothalamus.

Summary. The effects of epinephrine or acetylcholine bromide on the hypothalamic content of prolactin inhibiting factor (PIF) were determined in female rats. The drugs were injected twice daily for 5 days, while control rats were given the injection vehicle only. Each drug caused a significant decrease in hypothalamic PIF content, suggesting that this is the mechanism by which they induce enhanced release of prolactin from the anterior pituitary.

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