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Inhibition of Prolactin Secretion by Cholinergic Drugs (37988)

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There is considerable evidence that adrenergic and serotonergic components are involved in regulation of LH, FSH and prolactin release from the pituitary (1–4). In general, catecholamines have been reported to stimulate release of gonadotropins but to inhibit release of prolactin, whereas serotonin and its precursors inhibit release of gonadotropins but stimulate release of prolactin. Anti-cholinergic drugs were observed to inhibit ovulation (5). Little has been reported on the relation of the cholinergic system to prolactin release, but recently Libertun and McCann (6) observed that subcutaneous or intraventricular injection of atropine, a cholinergic blocking drug, into male and female rats, inhibited the release of LH, FSH and prolactin, suggesting that acetylcholine stimulated release of these three hormones. The present study was undertaken to explore more fully the relation of acetylcholine and other cholinergic drugs to release of prolactin from the pituitary of the rat.

Materials and Methods. Sprague-Dawley rats were obtained from Spartan Research Animals (Haslett, Michigan) and maintained in a temperature controlled room (75°F ± 1) with 14 hr of light (5am—7pm) and 10 hr of dark. Wayne Lab Blox pellets and water were given *ad lib.*

Female rats were implanted with polyethylene tubing for lateral intraventricular injections according to the description of Verster *et al.* (7). After at least two regular four or five day estrous cycles as determined by observing daily vaginal smears, the rats were injected at 11am on the morning of proestrus with 50 μg of acetylcholine bromide (K and K Laboratories, Inc., Plainsview, NY) in a volume of 8 μl of 0.85% NaCl or with 0.85% NaCl only. Blood was collected under light ether anesthesia by cardiac puncture 15, 30 and 60 min after injection.

In the second experiment female rats with at least two regular four or five day estrous cycles were injected ip with pilocarpine nitrate (Nutritional Biochemical Corporation, Cleveland, OH) 9 mg/kg at noon on the day of proestrus. Blood samples were collected by cardiac puncture under light ether anesthesia 15, 30 and 60 min after injection. In the third experiment male rats were injected with pilocarpine nitrate at doses of 5, 10 and 18 mg/kg, and blood was collected by cardiac puncture under light ether anesthesia 15, 30 and 60 min after injection.

In the fourth experiment male rats were injected ip with physostigmine sulfate (Merck and Co., Rahway, NJ), an inhibitor of acetylcholine metabolism, in doses of 0.3, 0.4 and 0.5 mg/kg. Blood samples were taken under light ether anesthesia 30, 60 and 90 min after injection by cardiac puncture. Student's *t* test was used to test differences between any two groups. Dunnett's multiple range test (8) was used for determining differences between control and

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TABLE I. Serum Prolactin in ng/ml of NIAMD-RP-1 after Intraventricular Injection of Acetylcholine Bromide (50 μg) into Proestrous Female Rats.

Treatment and No. of rats	15 min	30 min	60 min
Controls (15)	30 ± 3	43 ± 7	40 ± 7
Acetylcholine Bromide (12)	16 ± 2[a]	22 ± 3[b]	22 ± 5

[a] $P < 0.001$, controls vs drug, Student's t test.
[b] $P < 0.02$, controls vs drug, Student's t test.

treatment values in the physostigmine and pilocarpine experiments in male rats.

Results. Injection of acetylcholine directly into the lateral ventricles of the brain produced a 47% decrease in serum prolactin by the end of 15 min (Table I). This reduction in serum prolactin was also observed at 30 and 60 min, although the 60 min values were not different statistically from control values. When pilocarpine was administered systemically into proestrous females, significant reductions in serum prolactin were observed after 15 and 30 min but not 60 min after injection (Table II). Pilocarpine at the 5 mg/kg dose significantly reduced serum prolactin in male rats (Table III). A dose of 10 mg/kg also reduced serum prolactin values although these differences were not significant. The highest dose of pilocarpine, 18 mg/kg, apparently had no effect on serum prolactin. This may be due to the stressful effects of this dose since piloerection and diarrhea were observed in these rats.

Male rats showed reductions in serum prolactin after physostigmine injection (Table IV). This effect did not appear to be dose dependent since the highest dose produced no depression in serum prolactin 30 or 60 min after injection of the drug. However, the depression in serum prolactin was sustained for a longer period of time (90 min).

Discussion. The observations in this study indicate that cholinergic drugs act to depress prolactin release from the pituitary of male and female rats. Injection of acetylcholine directly into the lateral ventricles of the brain was used since acetylcholine does not cross the blood brain barrier when given systemically (9). Pilocarpine stimulates cholinergic receptors, and physostigmine prevents acetylcholine metabolism by inhibiting choline esterase action (10, 11). Both drugs cross the blood brain barrier when administered systemically (10, 12).

The mechanisms by which acetylcholine inhibits prolactin release is not clear at present. Acetylcholine may act directly on the pituitary to inhibit prolactin release although a previous study indicated no direct effect of acetylcholine on pituitary prolactin release *in vitro* (13). It may act by stimulating release of dopamine from its terminal nerve endings in the median eminence of the hypothalamus and thereby promote release of prolactin inhibiting factor (PIF) into the hypothalamo-pituitary portal vessels. There is also the possibility that acetylcholine may act by reducing secre-

TABLE II. Serum Prolactin in ng/ml of NIAMD-RP-1 after Intraperitoneal Injection of Pilocarpine Nitrate (9 mg/kg) into Proestrous Female Rats.

Treatment and No. of rats	Body wt. (g)	15 min	30 min	60 min
Controls (11)	276.4 ± 6.9	24.7 ± 2.7	25.6 ± 4.1	15.8 ± 2.6
Pilocarpine Nitrate (14)	272.0 ± 3.7	11.7 ± 1.4[a]	11.7 ± 1.8[b]	10.1 ± 2.0

[a] $P < 0.005$, controls vs drug, Student's t test.
[b] $P < 0.001$, controls vs drug, Student's t test.

TABLE III. Serum Prolactin in ng/ml of NIAMD-RP-1 after Intraperitoneal Injection of Pilocarpine Nitrate in Male Rats.

Treatment and No. of rats	Body wt. (g)	15 min	30 min	60 min
Controls (6)	448 ± 12	26.9 ± 4.5	25.9 ± 4.3	11.1 ± 3.0
5 mg/kg (7)	443 ± 10	9.9 ± 2.5 (1) [a,b]	8.4 ± 3 (3) [a,c]	11 ± 4.8 (3) [a]
10 mg/kg (5)	449 ± 12	17.1 ± 3.3	18.6 ± 1.9 (1) [a]	—
18 mg/kg (6)	453 ± 8	30.8 ± 5.2	18.2 ± 4.1	15.0 ± 3.6

[a] Rats with serum levels of prolactin too low to be detected. No. of rats indicated in ( ).
[b] $P < 0.01$, controls vs drug, Dunnett's multiple range test.
[c] $P < 0.02$, controls vs drug, Student's t test.

tion of prolactin releasing factor (PRF) from the hypothalamus or by modifying the action of serotonin. The dose of acetylcholine bromide used here may be higher than the acetylcholine concentrations normally present in the rat brain, and high doses may alter brain monoamines. However, the action of pilocarpine does not appear to be mediated through catecholamines, since pretreatment with methyl dopa, precursor analogue, did not prevent inhibition of prolactin release (unpublished observations). The effects of pilocarpine and physostigmine are not believed to be mediated through peripheral stress, since stresses have been reported to stimulate rather than to inhibit prolactin release (14). On the other hand, the lack of effect of the higher doses of pilocarpine and physostigmine on prolactin release may be due to the stressful actions of the drugs.

Our results are in apparent disagreement with the work of Libertun and McCann (6) who found that atropine injected systemically or into the 3rd ventricle of rats inhibited prolactin release. Since atropine blocks cholinergic receptors, the opposite results might have been expected. However, the doses used by these workers were very high, and large doses of atropine are known to exert central excitatory as well as toxic effects (17). In a recent abstract, Libertun (18) reported that systemic injections of pilocarpine or physostigmine initially inhibited and later increased release of LH and prolactin.

On the basis of present and previous reports, it appears that at least three different kinds of biogenic amines participate in the control of prolactin release. Catecholamines and acetylcholine exert an inhibitory effect on prolactin release and serotonin a stimulatory action. Catecholamines have been reported to promote PIF release from the hypothalamus (14), but also have been observed to act directly on the anterior pituitary to inhibit prolactin release (15, 16).

Summary. The effects of cholinergic drugs on serum prolactin concentration

TABLE IV. Serum Prolactin in ng/ml of NIAMD-RP-1 after Intraperitoneal Injection of Physostigmine Sulfate in Male Rats.

Treatment and No. of rats	Body wt. (g)	30 min	60 min	90 min
Controls (8)	447 ± 9.6	39 ± 8.6	21.8 ± 2.0	31.1 ± 6.1
0.3 mg/kg (8)	439 ± 11	16.8 ± 2.6[b]	8.2 ± 1.7 (2) [a,b]	11.1 ± 3.0[c]
0.4 mg/kg (7)	447 ± 8.3	17.9 ± 2.9[b]	12.8 ± 1.9[c]	10.2 ± 1.7 (1) [a,b]
0.5 mg/kg (8)	438 ± 10.7	36.8 ± 6.4	13.3 ± 3.3	11.8 ± 4.0 (3) [a,c]

[a] Rats with serum levels of prolactin too low to be detected. No. of rats indicated in ( ).
[b] $P < 0.01$ controls vs drug, Dunnett's multiple range test.
[c] $P < 0.05$ controls vs drug, Dunnett's multiple range test.

were determined in male and female rats. Acetylcholine bromide was injected once into the lateral ventricles of the brain, and pilocarpine nitrate and physostigmine sulfate were injected once ip. All drugs reduced serum prolactin values as compared to controls, although pilocarpine and physostigmine were more effective at lower than at higher dose levels. These results suggest an inhibitory role for the cholinergic system in the brain on prolactin release.

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