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Influence of Cholinergic and Anti-Cholinergic Drugs on Plasma Luteinizing Hormone and Prolactin Levels in Male and Female Rats[1] (41257)

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Abstract. The cholinergic agonist, pilocarpine, significantly reduced serum levels of luteinizing hormone (LH) and prolactin (PRL) in gonadectomized male and female rats 15, 30, and 60 min after drug administration. Similar effects on serum LH were observed after administration of another cholinergic agonist, physostigmine. The inhibitory effects of the two cholinergic agonists on serum levels of LH and PRL were counteracted by administering the cholinergic blocker, atropine. No effects on serum hormone levels of LH or PRL were observed 15, 30, and 60 min after administration of atropine alone. Administration of atropine or scopolamine (another anticholinergic drug) every 6 hr significantly elevated serum levels of LH in castrated male rats. These results indicate that the cholinergic system exerts an inhibitory influence on LH and PRL secretion after castration.

Acetylcholine is present in relatively high concentrations in the hypothalamus (1), and has been reported to influence the secretion of gonadotropins (2–6), prolactin (PRL), (3, 5, 7–11), growth hormone (GH), (10–12), and ACTH (13). Sawyer and Everett (14) found that systemic administration of atropine (ATROP), an anticholinergic drug, inhibited ovulation. Libertun and McCann (5) similarly observed that injection of ATROP into the brain inhibited LH release in rats, and that pilocarpine (PILO) or physostigmine (PHYSO), both cholinergic agonists, initially inhibited and later permitted LH release in ovariectomized estrogen-primed rats. These investigators used relatively large doses of ATROP and cholinergic drugs. Fiorindo and Martini (15) and Simonovic *et al.* (16) reported that acethylcholine and PHYSO stimulated LH and FSH release in a hypothalamic–pituitary coincubation system, but again relatively high doses of these drugs were used.

Contradictory results also have been reported on the effects of cholinergic drugs on PRL secretion. Libertun and McCann (3), and McLean and Nikitovitch-Winer (17) found that systemic or central injections of large doses of ATROP inhibited PRL release in male and female rats. However, Grandison *et al.* (8) and Subramanian and Gala (18) reported that systemic or central injection of lower doses of ATROP had no effect on basal serum PRL levels, but counteracted the inhibitory effects of PILO and PHYSO on PRL secretion. Subramanian and Gala (18, 19) observed that the cholinergic agonists, arecoline, nicotine, and carbachol, each significantly inhibited the afternoon surge of PRL in ovariectomized, estrogen-treated rats. Cholinergic drugs reduced basal serum PRL levels in female and male rats, and inhibited the rise of serum PRL after 5-hydroxytryptophan administration, ether, or restraint stress, and the nocturnal rise of PRL during pseudopregnancy (7). These inhibitory effects of cholinergic drugs on PRL secretion were reported to be mediated by increasing the activity of the hypothalamic dopaminergic system (8). Lawson and Gala (20) were unable to find any effect of cholinergic agonists or antagonists on basal secretion of PRL in rats. The present study was undertaken to further evaluate the effects

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of cholinergic and anti-cholinergic drugs on LH and PRL secretion in rats.

Materials and Methods. *Animals.* Male and female rats (250–275 g), from the Sprague–Dawley strain (Harlan Industries, Cumberland, Ind.) were housed in a temperature (23 ± 1°)- and light-controlled room (lights on from 0500 to 1900 hr). Rat food pellets (Ralston Purina Co., St. Louis, Mo.) and tap water were provided *ad libitum.*

Drugs. Atropine sulfate (ATROP, Sigma Chemical Co., St. Louis, Mo.), pilocarpine nitrate (PILO, Nutritional Biochemicals, Cleveland, Ohio), physostigmine sulfate (PHYSO, Merck, Sharp and Dohme, Rahway, N.J.), and scopolamine hydrobromide (SCOP, Sigma) were dissolved in 0.87% NaCl solution (SAL) just before use.

Experiment I: Effects of drugs in castrate rats on plasma LH. After arrival, male and female rats were allowed several days to acclimatize, followed by gonadectomy of both sexes. Three days after orchidectomy, and 7 days after ovariectomy, rats were randomly assigned to one of the following groups: PILO, 10 mg/kg body wt; ATROP, 10 mg/kg body wt; and PILO, 10 mg/kg + ATROP, 10 mg/kg body wt. Control rats received an equivalent volume of saline (SAL) vehicle only. Male rats were used 3 days after castration, since LH secretion rises quickly after orchidectomy, whereas the rise in serum LH after ovariectomy occurs more slowly (21), and hence a 7-day period was permitted to elapse before the female rats were used. All drugs and SAL were administered ip, and blood (0.6 ml) was obtained by orbital sinus puncture under light ether anesthesia 15, 30, and 60 min after injection. Blood samples were similarly collected in subsequent experiments.

Blood samples were first placed in a refrigerator for 4 hr before centrifuging at 5000 rpm for 10 min at 4°. The sera were kept frozen at −20° until assayed for LH.

Experiment II: Effect of PILO and PHYSO on serum LH levels in gonadectomized male and female rats. Gonadectomized male and female rats were maintained under the same conditions as in Experiment I. Rats from both sexes were randomly divided as follows: controls (SAL); PILO, 10 mg/kg body wt; and PHYSO, 0.4 mg/kg body wt. Drugs and SAL were administered ip and blood samples (0.4 ml) were obtained 15, 30, and 60 min later. The sera were kept frozen until assayed for LH.

Experiment III: Effect of multiple injections of cholinergic and anti-cholinergic drugs on serum levels of LH in castrated male rats. Male rats were castrated and immediately thereafter, injected with SAL, PILO, ATROP, or PILO + ATROP. Drug and SAL injections were repeated at 6-hr intervals and blood samples were collected 6, 24, and 48 hr after gonadectomy. The sera were kept at −20° until assayed for LH.

Experiment IV: Effect of multiple injections of cholinergic and anti-cholinergic drugs on serum levels of LH in castrated male rats. Male rats were castrated and immediately thereafter, injected with SAL, PILO, PHYSO, ATROP, or SCOP (2 mg/kg). Drug and SAL injections were repeated as in Experiment III. Blood samples were collected 6, 24, and 48 hr after gonadectomy. The sera were kept at −20° until assayed for LH.

Experiment V: Effect of single injections of cholinergic or anti-cholinergic drugs on serum PRL levels in male and female gonadectomized rats. Gonadectomized male or female rats were maintained under the same conditions as in Experiment I and used 3 days after orchidectomy or 7 days after ovariectomy. Rats of both sexes were randomly assigned to one of the following groups: SAL, PILO, ATROP, or PILO + ATROP. SAL or drugs were administered ip and blood samples were collected 15, 30, and 60 min later.

Experiment VI: Effect of drugs on PRL surge in rats on afternoon of proestrus. Female rats showing two consecutive 4-day cycles were used. On the day of proestrus, intact rats were injected at 1200 hr with SAL, PILO, ATROP, or PILO + ATROP. SAL and drugs were administered immediately after initial blood collection, except that a second PILO group was injected at 1400 hr. Blood was collected again at 1700 hr and serum was stored for PRL assay.

Hormone assays and statistical procedure. Serum hormone levels were assayed

by radioimmunoassay (RIA). LH was assayed by the method described in the NIAMDD kit. Plasma PRL was measured by the double-antibody method of Niswender *et al.* (22) with the NIAMDD kit provided by Dr. A. F. Parlow (Harbor General Hospital, Torrance, Calif.). Levels of LH and PRL were expressed as nanograms per milliliter in terms of the reference preparations NIAMDD-LH-RP-1 and NIAMDD-PRL-RP-1.

Analysis of variance and Student–Newman–Keul's test for multiple comparisons among groups were used to analyze the data. The level of significance chosen was $P < 0.05$.

Results. Data from Experiment I are summarized in Table I. SAL administration had no effect on serum LH levels. PILO significantly decreased serum levels of LH in both male and female gonadectomized animals, and LH remained low for the entire 60 min after drug administration. ATROP alone had no effect on serum LH values, but effectively counteracted PILO inhibition of LH release.

The results of Experiment II are summarized in Table II. PILO and PHYSO each significantly reduced serum LH levels 15, 30, and 60 min after drug administration in gonadectomized male and female rats when compared with SAL-injected controls. PILO administration immediately after castration of male rats (Experiment III) was able to inhibit the rise of LH for the first 6 hr after castration, but continued administration of the drug failed to prevent a further rise of LH (Table III). On the other hand, repeated injections of ATROP produced a twofold greater increase in serum LH levels 24 hr after castration than in SAL-injected controls, and partially counteracted PILO inhibition of LH secretion 6 hr after gonadectomy.

The effects of PHYSO or SCOP on the postcastration LH rise in male rats (Experiment IV) can be seen in Table IV. Both PILO and PHYSO prevented the postcastration rise in LH 6 hr after gonadectomy, and PHYSO continued to depress serum LH levels 24 and 48 hr after castration. ATROP and SCOP each significantly raised serum LH levels above those produced by castration alone.

Table V shows the effects of PILO and ATROP on PRL release. It can be seen that PILO reduced plasma PRL values both in male and female rats, whereas ATROP alone had no effect on serum PRL levels. The dose of ATROP used was able to counteract the inhibitory action of PILO on PRL release.

The results from Experiment VI are summarized in Table VI. PILO significantly reduced the PRL surge on the afternoon of proestrus when administered at 1200 or

TABLE I. EFFECTS OF SINGLE INJECTIONS OF PILOCARPINE, ATROPINE, OR BOTH ON SERUM LH LEVELS IN GONADECTOMIZED MALE AND FEMALE RATS

Group	*N*	15 min	30 min	60 min
		Males[a] (ng/ml)		
Saline (SAL)[b]	10	370.5 ± 37.5[c]	430.2 ± 57.1	398.3 ± 44.7
PILO (10 mg/kg)[b]	9	115.8 ± 20.7*	132.7 ± 31.4*	130.8 ± 27.4*
ATROP (10 mg/kg)[b]	9	325.6 ± 36.7	380.4 ± 44.3	400.7 ± 40.4
PILO + ATROP[b]	9	406.7 ± 30.4	410.0 ± 56.7	395.0 ± 39.4
		Females[d] (ng/ml)		
Saline (SAL)[b]	7	486.1 ± 44.5	393.1 ± 45.6	397.1 ± 28.1
PILO (10 mg/kg)[b]	10	227.2 ± 23.2*	219.6 ± 32.4*	250.6 ± 21.3*
ATROP (10 mg/kg)[b]	9	437.0 ± 44.5	307.1 ± 54.2	394.0 ± 34.8
PILO + ATROP[b]	8	413.3 ± 58.5	372.4 ± 61.5	377.0 ± 31.9

[a] Male rats were castrated 3 days before experimentation.
[b] Drugs or SAL were administered ip and blood samples were obtained by orbital sinus puncture 15, 30, and 60 min later.
[c] Results are mean ± SE. *N* = number of animals per group.
[d] Female rats were ovariectomized 7 days before experimentation.
* Statistically different ($P < 0.05$) from SAL-injected controls.

TABLE II. EFFECTS OF SINGLE INJECTIONS OF PILOCARPINE AND PHYSOSTIGMINE ON SERUM LH LEVELS IN GONADECTOMIZED MALE AND FEMALE RATS

Group	N	15 min	30 min	60 min
		Males[a] (ng/ml)		
Saline (SAL)[b]	8	355.6 ± 42.2[c]	458.2 ± 68.6	400.3 ± 51.6
PILO (10 mg/kg)[b]	8	139.9 ± 23.8*	123.4 ± 23.3*	140.6 ± 24.7*
PHYSO (0.4 mg/Kg)[b]	8	234.3 ± 30.6*	86.0 ± 10.0*	110.3 ± 18.7*
		Females[d] (ng/ml)		
Saline (SAL)[b]	8	448.9 ± 59.4	400.1 ± 15.9	390.5 ± 26.1
PILO (10 mg/kg)[b]	8	290.8 ± 59.0*	81.8 ± 13.1*	137.5 ± 21.1*
PHYSO (0.4 mg/kg)[b]	8	155.4 ± 24.2*	90.0 ± 13.9*	140.6 ± 10.7*

[a] Male rats were castrated 3 days before experimentation.
[b] Drugs or SAL were administered ip and blood samples obtained by orbital sinus puncture 15, 30, and 60 min later.
[c] Results are mean ± SE. N = number of animals per group.
[d] Female rats were ovariectomized 7 days before experimentation.
* Statistically different ($P < 0.05$) from SAL-injected controls.

1400 hr. ATROP had no significant effect on the PRL surge. When administered together, ATROP counteracted PILO inhibition of PRL release.

Discussion. The results of the present experiments show that the two cholinergic drugs, PILO and PHYSO, in the doses used, each significantly inhibited the LH rise after castration of male and female rats. The inhibition by PILO was completely counteracted by concomitant administration of ATROP. Furthermore, when either ATROP or SCOP alone was injected into castrated male rats, serum LH was significantly elevated above that in castrated controls. These latter observations suggest that the cholinergic system may exert a dampening effect on the stimulation to LH secretion by castration.

Our results contrast sharply with those reported by most earlier workers (3, 5, 6), although the conditions of their experiments and particularly the doses of drugs used, differed greatly from ours. PILO at a dose of 50 mg/kg body wt was reported to initially depress serum LH levels 30 and 90 min after injection into ovariectomized estrogen-primed rats, but 6 hr later this resulted in an increase in serum LH values (5). By contrast, we used a dose of 10 mg/kg body wt of PILO in castrated male and female rats, and observed only inhibition of LH release up to 6 hr after injection. The *in vitro* results reported (16) on the stimulatory effects of cholinergic drugs on LH release in hypothalamic–pituitary coincubations also may be due to the large doses of acetylcholine and PHYSO used (23). The doses of ATROP used by Everett and Sawyer (14) to inhibit LH release also were relatively large (700 mg/kg body wt), as compared to the dose employed by us (10 mg/kg body wt). It is well established that large doses of ATROP produce many cen-

TABLE III. EFFECTS OF MULTIPLE INJECTIONS OF PILOCARPINE, ATROPINE, OR BOTH, ADMINISTERED IMMEDIATELY AFTER CASTRATION AND EVERY 6 hr THEREAFTER, ON SERUM LEVELS OF LH (ng/ml) IN MALE RATS

Group	N	6 hr	N	24 hr	N	48 hr
Saline (SAL)[a]	8	87.9 ± 11.0[b]	7	604.9 ± 48.7	7	630.0 ± 101.0
PILO (10 mg/kg)[a]	8	32.6 ± 6.3*	7	510.8 ± 63.5	7	520.7 ± 74.4
ATROP (10 mg/kg)[a]	8	88.2 ± 19.8	7	1213.0 ± 161.0*	7	877.0 ± 127.8
PILO + ATROP	8	65.1 ± 10.3	8	550.0 ± 37.8	7	694.1 ± 34.4

[a] Male rats were castrated and immediately injected with SAL or drugs. Injections were repeated every 6 hr and blood samples were taken 6, 24, and 48 hr later.
[b] Results are mean ± SE. N = number of animals per group.
* Statistically different ($P < 0.05$) from SAL-injected controls.

TABLE IV. EFFECTS OF MULTIPLE INJECTIONS OF PILOCARPINE, PHYSOSTIGMINE, ATROPINE, AND SCOPOLAMINE, ADMINISTERED IMMEDIATELY AFTER CASTRATION AND EVERY 6 hr THEREAFTER ON SERUM LEVELS OF LH (ng/ml) IN MALE RATS

Group	N	6 hr	24 hr	48 hr
Saline (SAL)[a]	10	92.4 ± 18.3[b]	531.6 ± 61.1	484.6 ± 43.6
PILO (10 mg/kg)[a]	10	38.1 ± 7.2*	401.2 ± 39.5	420.1 ± 35.4
PHYSO (0.4 mg/kg)[a]	10	43.2 ± 5.3*	382.1 ± 33.1*	389.7 ± 32.6*
ATROP (10 mg/kg)[a]	10	87.2 ± 20.1	750.1 ± 39.3*	832.6 ± 47.3*
SCOP (2 mg/kg)[a]	10	79.4 ± 22.2	642.3 ± 57.5	725.2 ± 46.2

[a] Male rats were castrated and immediately injected with SAL or drugs. Injections were repeated every 6 hr and blood samples were taken 6, 24, and 48 hr later.

[b] Results are mean ± SE. N = number of animals per group.

* Statistically different ($P < 0.05$) from SAL-injected controls.

tral and peripheral effects in the body unrelated to inhibition of the cholinergic system (24). The explanation of why PHYSO inhibited postcastration LH release for 48 hr, whereas PILO inhibited LH release for only 6 hr, is not clear. It is possible that this relates to the fact that PHYSO is a cholinesterase inhibitor, whereas PILO is a muscarinic agonist (25).

The effects of PILO and ATROP on serum PRL levels observed here confirm those reported by us previously in intact male and female rats, but are in disagreement with results reported by other investigators who used much higher doses of these drugs (3, 14, 16, 17). More recently, McCann *et al.*'s laboratory (26) published results showing that cholinergic drugs can inhibit *in vitro* PRL secretion, in agreement with the present observations. In the present study, PILO given to castrated male and female rats also reduced serum PRL levels, and ATROP counteracted the inhibitory action of PILO on PRL release. ATROP given alone, at a dose of 10 mg/kg body wt had no effect on serum PRL values. PILO partially inhibited the PRL surge that normally occurs on the afternoon of proestrus in rats, in agreement with earlier results from this and other laboratories (7, 18, 27).

Recent reports indicate that cholinergic receptors are present on the anterior pituitary (26, 28, 29), suggesting a possible direct effect by cholinergic and anti-cholinergic drugs on PRL and LH release. How-

TABLE V. EFFECTS OF SINGLE INJECTIONS OF PILOCARPINE, ATROPINE, OR BOTH ON SERUM PROLACTIN LEVELS IN GONADECTOMIZED MALE AND FEMALE RATS

Group	N	15 min	N	30 min	N	60 min
		Males[a] (ng/ml)				
Saline (SAL)[b]	10	26.5 ± 0.9[c]	10	26.4 ± 1.7	10	26.0 ± 1.8
PILO (10 mg/kg)[b]	10	12.6 ± 0.8*	10	13.1 ± 1.0*	10	11.2 ± 1.0*
ATROP (10 mg/kg)[b]	10	28.6 ± 1.2	10	28.1 ± 1.2	10	27.3 ± 2.4
PILO + ATROP[b]	10	23.2 ± 1.3	10	22.9 ± 0.7	10	21.7 ± 1.4
		Females[d] (ng/ml)				
Saline (SAL)[b]	10	43.0 ± 5.7	10	38.1 ± 5.0	8	44.3 ± 7.4
PILO (10 mg/kg)[b]	10	26.1 ± 2.7*	10	25.4 ± 2.1*	10	20.0 ± 1.3*
ATROP (10 mg/kg)[b]	9	35.4 ± 3.5	9	33.6 ± 2.0	9	36.0 ± 4.0
PILO + ATROP[b]	8	39.6 ± 1.8	8	31.2 ± 6.6	8	33.3 ± 2.0

[a] Male rats were castrated 3 days, before experimentation.

[b] Drugs or SAL were administered ip and blood samples were obtained by orbital sinus puncture 15, 30, and 60 min later.

[c] Results are mean ± SE. N = number of animals per group.

[d] Female rats were ovariectomized 7 days before experimentation.

* Statistically different ($P < 0.05$) from SAL-injected controls.

TABLE VI. EFFECTS OF SINGLE INJECTION AT 1200 hr OF PILOCARPINE, ATROPINE, OR BOTH ON PROLACTIN SURGE ON AFTERNOON (1700 hr) OF PROESTRUS IN INTACT RATS

Blood collecting time (hr)	SAL[a] (8)	PILO, 10 mg/kg (8)[b]	PILO, 10 mg/kg (8)[c]	ATROP, 10 mg/kg (8)	ATROP + PILO (10)
1200	44.7 ± 11.8[d]	46.0 ± 18.4	45.4 ± 11.8	49.9 ± 11.6	49.4 ± 13.3
1700	179.3 ± 11.2	136.0 ± 13.5*	122.5 ± 23.9*	153.0 ± 13.7	188.2 ± 32.7

[a] Rats were injected with SAL, PILO, ATROP, or both PILO and ATROP immediately after the first blood sampling (1200 hr).
[b] Number of animals per group in parentheses.
[c] One group of rats was injected with PILO at 1400 hrs.
[d] Results are mean ± SE and are expressed in terms of ng/ml of P-NIAMDD-PRL-RP-1.
* Statistically different ($P < 0.05$) from SAL-injected controls.

ever, Campbell *et al.* (30) reported no effect of a number of cholinergic agonists on PRL release using pituitary explants. In addition, PILO *in vivo* and carbachol *in vitro* failed to increase mitotic activity of the lactotrophic cells but increased mitotic activity of basophils and chromophobes, suggesting that the direct effects of cholinergic agonists on the pituitary are exerted on LH rather than on PRL cells (31).

It can be concluded that the contradictory effects reported in earlier investigations on the effects of cholinergic and anticholinergic drugs on LH and PRL release were mainly due to use of high doses of these drugs (7, 9, 18). It is clear from the present and previous results that low doses of cholinergic drugs inhibit LH and PRL release in rats under a variety of endocrine states (9) and low doses of ATROP can counteract these inhibitory actions of the cholinergic drugs (7–9). Further, it is shown here that administration of low doses of ATROP or SCOP, although showing no effects on basal serum LH values, greatly enhanced the rise of serum LH levels produced by castration alone, suggesting that the cholinergic system may exert a braking action on LH release under some conditions.

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