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Metergoline and Other Peipheral Serotonin Antagonists Inhibit Prolactin Secretion through Mechanisms Unrelated to Serotonin (40621)[1]

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A physiological stimulatory role for serotonin on human prolactin secretion has been suggested on the basis of studies in which the metabolic precursors of serotonin, tryptophan, and 5-hydroxytryptophan (5-HTP) were shown to increase serum prolactin concentrations significantly (1–3). In clinical studies involving normal individuals and patients with hyperprolactinemia and acromegaly, serotonin receptor antagonists such as metergoline and methysergide were shown to decrease the secretion of prolactin (4–9). These observations have been interpreted as arguments favoring a positive serotonergic tone in normal and pathological prolactin secretion in man (4, 8). In this study we present evidence that certain serotonin receptor antagonists inhibit prolactin secretion via a direct effect on the dopamine receptor of the rat pituitary lactotroph. Since the regulation of prolactin secretion in the rat is similar to that in man, these observations support the concept that normal and pathological prolactin secretion in man is principally regulated by dopamine and dopamine agonists (10–12), the role of serotonin being unproven.

Methods. Male or female Sprague–Dawley rats weighing 200–220 g obtained from Flow Laboratories in Dublin, Va. were used for most studies. Four flasks each containing three hemipituitary glands were incubated in 1 ml tissue culture medium 199, containing 10 μCi [4,5-^{3}H]leucine (30 Ci/mmole) for 5 hr in an atmosphere at 95% O_2, 5% CO_2 in a Dubnoff shaker at 37°C. Aliquots of pituitary homogenate and incubation medium were subjected to polyacrylamide gel electrophoresis as described in (14). The location of the newly synthesized [^{3}H]prolactin on the gels was ascertained by subjecting NIAMDD reference prolactin RP-1 to the same electrophoretic separation. The segments of the gels containing newly synthesized prolactin were placed in scintillation vials containing 1 ml of NCS (tissue solubilizing agent; Amersham Searle)–water solution (92.8; vol/vol). A toluene-based scintillation fluid was added to each vial at room temperature and the samples were counted in a liquid scintillation spectrometer.

Other studies utilized females rats (Wistar–Furth, 200–220 g, obtained from ARS/Sprague–Dawley, Madison, Wis.). The MtTW15 tumor which secretes prolactin and growth hormone was transplanted in groups of these rats as described previously (13). Six to eight weeks elapsed before the tumor measured approximately 8 cm^2, at which time the animals were sacrificed.

Prolactin in the serum and in the incubation medium was measured by radioimmunoassay using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone distribution program. All results are expressed as mean ± SEM and statistical analysis was performed by analysis of variance.

Results. The injection of male rats with reserpine increased serum prolactin values 300% (Table I). The parenteral administration of 1 mg/kg metergoline to these animals, however, inhibited significantly ($P < 0.01$) the reserpine-mediated stimulation of prolactin secretion.

The *in vitro* effect of metergoline on prolactin synthesis and release was investigated using normal female rats. The data presented in Fig. 1 demonstrate that 1 μM metergoline inhibited the secretion of newly synthesized [^{3}H]prolactin by 44% ($P < 0.01$) and RIA prolactin by 25% ($P < 0.05$). This inhibitory effect of metergoline was completely overcome by the addition of the dopamine receptor antagonist, haloperidol (10 nM). At the

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indicated concentration haloperidol alone was without influence on prolactin secretion.

The injection of methysergide (25 mg/kg i.p. for 3 days) to six rats bearing the prolactin-secreting pituitary tumor MtTW15 caused a decrease in serum prolactin from 10.7 ± 4.2 to 3.97 ± 0.59 μg/ml. The injection of haloperidol (1 mg/kg daily for 3 days) to six tumor-bearing rats injected with methysergide moderated the suppressive effects of methysergide on serum prolactin significantly, 6.25 ± 0.76 μg/ml ($P < 0.01$).

The effect of several other ergot derivatives with known serotonin antagonist activity on prolactin secretion by the pituitary gland *in vitro* is shown in Table II. A significant direct inhibitory effect of methergine, the metabolic product of methysergide, and of LSD on the secretion of both radioimmunoassayable and newly synthesized [^{3}H]prolactin was observed, while 2-Br-LSD had a weaker inhibitory action on prolactin secretion. The *in vitro* inhibitory effect of LSD on the *in vitro* secretion of prolactin was examined and the results are presented in Fig. 2. These results showed that 1 μ*M* LSD caused a 53% inhibition ($P < 0.01$) in RIA prolactin and an 80% decrease ($P < 0.01$) in [^{3}H]prolactin secretion. Complete blockade of the *in vitro* LSD-mediated inhibition of prolactin secretion was observed by coincubation with 100 μ*M* haloperidol (Fig. 2).

Discussion. Ergot alkaloids, such as ergotamine, ergocryptine, and ergonovine, are nat-

TABLE I. THE INHIBITORY EFFECT OF METERGOLINE ON THE RESERPINE-MEDIATED INCREASE IN SERUM PROLACTIN[a]

	Serum prolactin (ng/ml)
Control	8.9 ± 2.5
Reserpine treated	27.5 ± 6.0*
Reserpine + metergoline treated	7.4 ± 1.7*

[a] Male rats were injected i.p. with reserpine 2 mg each at 10:00 PM and metergoline (1 mg/kg) the next morning at 9:00 AM. Blood was collected at 10:00 AM. All values are mean ± SEM ($n = 6$).

* $P < 0.01$ versus controls.

** $P < 0.01$ versus reserpine group.

TABLE II. THE EFFECT OF ERGOT DERIVATIVES ON PROLACTIN SECRETION BY NORMAL FEMALE PITUITARY GLANDS *in Vitro*[a]

	Incorporation of [^{3}H]leucine into prolactin (cpm/mg pituitary gland)	Prolactin measured by radioimmunoassay (μg/mg pituitary gland)
Control	10,825 ± 540	13.81 ± 0.54
Methergine 1 μ*M*	600 ± 150*	3.97 ± 0.54*
LSD 500 n*M*	4,650 ± 750*	9.14 ± 0.82*
2-Br-LSD 1 n*M*	7,630 ± 190*	13.03 ± 0.83

[a] Four flasks per group each containing three hemipituitary glands; mean ± SEM.

*$P < 0.01$ versus controls.

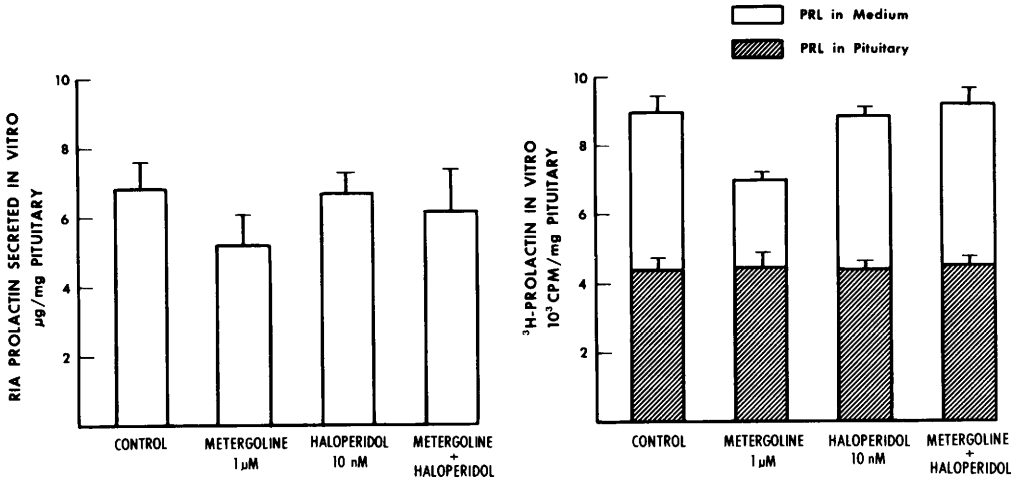

FIG. 1. Effect of haloperidol on metergoline-mediated inhibition of prolactin secretion *in vitro*. Each group contained four flasks containing three hemipituitaries incubated in medium 199 containing the indicated amounts of metergoline and haloperidol. The amount of newly synthesized or radioimmunoassayable prolactin in the pituitary and incubation medium was measured

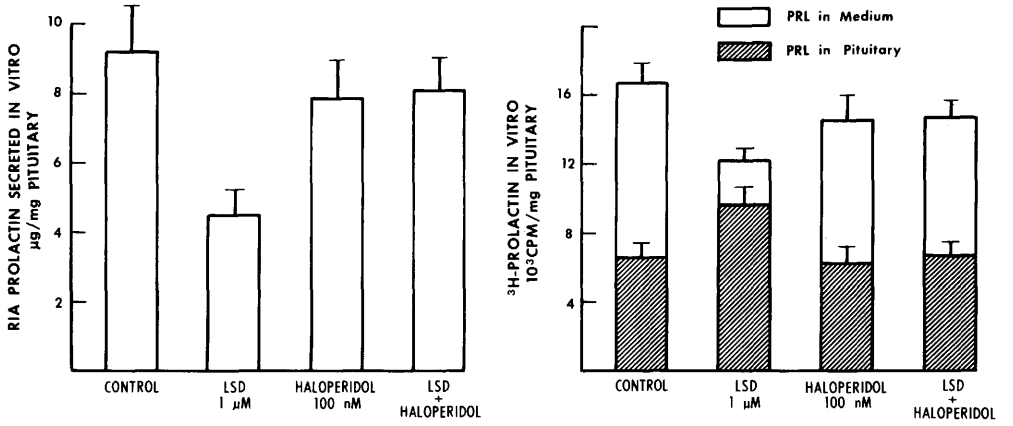

FIG. 2. Effect of haloperidol on LSD-mediated inhibition of prolactin secretion *in vitro*. Each group contained four flasks containing three hemipituitaries incubated in medium 199 containing the indicated amounts of LSD and haloperidol. The amount of newly synthesized or radioimmunoassayable prolactin in the pituitary and incubation medium was measured.

urally occurring substances originating in the fungus Claviceps (Purpurea) (15). Semisynthetic halogenated alkaloids have been developed (e.g., 2-bromo-α-ergocryptine) which suppress normal and pathological prolactin secretion via the drugs' dopamine agonist activity localized at the pituitary level (16, 17). Several ergot alkaloids and related compounds were early recognized as having peripheral tissue antiserotonin activity, particularly on smooth muscle. In this group, lysergic acid derivatives such as diethylamide (LSD), 2-bromo-LSD, 1-methyl-D-lsyergic acid butanolamide (methysergide), and 1-D-lysergic-acid butandamide (Methergine) are especially potent (18). The parenteral administration of LSD and methergine were reported earlier to suppress prolactin secretion in the rat (19, 20). In addition, a new class of compounds, the ergoline derivatives, lisuride, lergotrile, and metergoline, has been developed. Metergoline was originally proposed as a highly potent, long-lasting, and selective antagonist of both central and peripheral serotonin receptors (21, 22). It has inhibitory effects on prolactin secretion in normal individuals (4), it blocks prolactin secretion induced by breast stimulation during the first postpartum week (5), and when administered within 24 hr after delivery and continued for 5 days, metergoline was reported to have inhibitory effects on puerperal lactation in a manner similar to that elicited by bromocriptine (23). Metergoline inhibited both growth hormone and prolactin release in acromegalic patients (6), but this effect could be prevented by pimozide (7). Methysergide has been reported to suppress the sleep related prolactin rise in normal individuals (24) and to inhibit prolactin levels in normal individuals in and amenorrheic women with hyperprolactinemia (8, 9). This prolactin-lowering effect of methysergide was abolished by sulpiride administration (9). These observations were interpreted by some (4–6, 8) as evidence for a direct antiserotonin-related lowering of prolactin secretion, although others (7, 9) suggested the possibility of a dopaminergic activity of both metergoline and methysergide on prolactin secretion.

A direct, dopamine-agonistic action of all tested ergot derivatives with so-called antiserotonin activity (LSD, 2 Br-LSD, methergine, metergoline) was shown in this study on prolactin secretion by the pituitary gland *in vitro*. In this respect the action of these drugs does not differ from that of dopamine (10). Serotonin itself has been shown to be without direct effect on prolactin secretion by the pituitary gland (25). The mechanism of action of methysergide on prolactin secretion turned out to be more complex. We showed that the compound itself has antidopaminergic activity on prolactin secretion by the pituitary gland *in vitro*, while its prolactin-suppressing effect is probably mediated by a dopamine-agonistic action of its main metabolite, methergine (25). These observations are supported

by the blockade caused by administration of haloperidol on the methysergide-mediated suppression of plasma prolactin in the pituitary tumor-bearing rats.

It is becoming more clear that the generally established stimulatory role of serotonin on prolactin secretion in man is not as firmly founded as originally thought. The earlier reports in which intravenous or oral administration of L-tryptophan and 5-HTP were shown to stimulate prolactin secretion (1–3) have not been confirmed despite a thorough investigation of dosage and time of administration (26–29). As shown in this and our earlier studies (25), the so-called specific serotonin antagonists metergoline, methergine, LSD, and methysergide exert their prolactin secretion suppressive effect via a direct agonistic action on dopamine receptors in the pituitary gland. The results of our studies do not support the suggested role of serotonin in prolactin secretion, but rather support the hypothesis that prolactin secretion is principally regulated by dopamine (10–12).

Summary. The mechanism through which metergoline and other peripheral serotonin receptor antagonists inhibit prolactin secretion was studied. Reserpine, an agent well known to deplete brain catecholamines and serotonin, increased the serum prolactin concentration in male rats. The concomitant injection metergoline completely prevented this increase in hormone level, suggesting that metergoline has a direct effect to inhibit secretion of the hormone. Direct addition of metergoline to pituitary glands incubated *in vitro* decrease the amount of prolactin secreted, confirming this suspicion. Haloperidol at the concentration used was without effect on prolactin secretion, but it completely blocked the metergoline-mediated inhibition of prolactin secretion. Similar effects were observed with methergine, a metabolite of methysergide, and with LSD. These data directly related to the finding that methysergide significantly decreased the *in vivo* prolactin secretion by a transplanted pituitary tumor. Simultaneous injection of haloperidol blocked the inhibitory action of methysergide. Similarly, the LSD-mediated inhibition of prolactin secretion *in vitro* was blocked by haloperidol. We conclude that the inhibiting action of metergoline and methysergide to inhibit prolactin secretion is exerted directly at the pituitary through the stimulation of a dopamine receptor.

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