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# A Chronically Hypoprolactinemic Rat Model: Administration of Lergotrile Mesylate by Osmotic Minipump (41087)

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*Abstract.* The feasibility of developing a hypoprolactinemic animal model by inducing prolonged suppression of endogenous prolactin (PRL) secretion by lergotrile mesylate (LM) administered via an ip implanted osmotic minipump was investigated. The response of plasma PRL to a 3-min ether challenge was examined over 7 days in animals receiving either physiological saline or LM at rates of 2.5, 5.0, and 10.0 μg/hr via osmotic minipumps. Control animals demonstrated an increased PRL response to ether, in comparison to Day 1, upon subsequent exposures. The increased response to subsequent ether exposure was prevented by LM administration at rates of 2.5 and 5.0 μg/hr. LM at a rate of 10.0 μg/hr significantly suppressed the PRL response to ether on Days 4, 6 ($P < 0.001$), and 7 ($P < 0.002$) following pump implantation. In addition, spontaneous PRL levels were examined over a 24-hr period in chronically cannulated male rats receiving either saline ($n = 4$) or LM at a rate of 10.0 μg/hr ($n = 4$), on Day 7 following pump implantation. LM administration prevented the diurnal surge which appeared in saline-treated animals from 1400 to 1600 hr ($P < 0.001$), and lowered the mean 24-hr plasma PRL concentration from 19.7 ± 1.7 ng/ml in controls to 13.2 ± 0.4 ng/ml ($P < 0.005$). These data indicate that constant LM administration by osmotic minipump at a rate of 10.0 μg/hr (0.69 mg/kg/day) is sufficient to suppress both spontaneous and ether-stimulated PRL secretion in adult male rats. This route of LM administration is preferable to ip injection for suppression of PRL secretion over a prolonged period of time, as it reduces the amount of drug required for suppression, possibly decreasing side effects, and also reduces the amount of handling of animals during the course of an experiment.

Studies of the physiologic and metabolic effects of prolactin (PRL) are hampered by the lack of a suitable hypoprolactinemic animal model. Rabbit mammary gland PRL receptors have been found to be 50% saturated at circulating PRL levels of 8 ng/ml (1). At normal physiological concentrations (20 ng/ml) PRL receptors may be nearly saturated so that PRL administration may have little, if any biological effect. Hypophysectomy leads to multiple pituitary hormonal deficiencies in addition to diminished prolactin secretion, and due to the difficulties of hormonal replacement in these animals, only thyroxine and corticosteroids are usually given to the animals. Another approach has been pharmacologic suppression of prolactin secretion. This has usually been done with ergot alkaloid derivatives which have dopamine receptor-stimulating activity (2–6). However, the utility of these dopaminergic compounds in the study of the effects of chronic hypoprolactinemia is limited by their toxicity and by their effects on pathways other than those involving PRL secretion (7, 8). A synthetic ergoline derivative, lergotrile mesylate (LM) (Lilly), has been found to possess very potent PRL-inhibiting activities, while being less toxic than several commonly used ergot alkaloids, such as the naturally occurring ergocornine (9), and synthetic bromocryptine (8). In addition, LM possesses very little of the potent vasoconstrictor activity found in naturally occurring ergot compounds (9).

Studies conducted on ovariectomized 6-month and 2-year-old rats demonstrated that daily ip injection of LM at a dose of 3.0 mg/kg over 28 days significantly suppressed serum PRL levels measured following decapitation on Day 28 (10). Another study found that a single ip injection of 10 μg LM into male rats, pretreated with reserpine to elevate PRL levels, reduced serum PRL to basal levels at 1 hr (11). The dose of LM



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required for the suppression of reserpine-stimulated PRL secretion by hourly ip injection suggested by the latter study was equivalent to 1.0 mg/kg over 24 hr, in comparison to the dose of 3.0 mg/kg required for similar suppression by single daily ip injection.

The lower dose of LM required by hourly administration is preferable to single daily injection as it may reduce both the toxic effects of the compound, and the severity of side effects on the animal. However, the use of multiple injections for chronic studies exposes animals to the additional stresses of handling and pain during the experimental period. A method of drug administration which chronically suppresses PRL secretion at the lower dose, yet reduces the amount of handling and stress on the experimental animals is desirable.

The present study sought to evaluate the effectiveness of inducing chronic PRL suppression by continuous administration of LM via osmotic pump implanted ip for 7 days in adult male rats. This allowed the constant ip administration of LM over a 7-day period, without the necessity of handling after the initial pump implantation. The effects of this treatment on spontaneous fluctuations in basal PRL levels over a 24-hr period, and on ether-stimulated PRL secretion over the 7-day period were examined.

**Materials and Methods.** *Experimental animals.* Forty-three adult male Sprague–Dawley rats weighing 225–250 g were purchased from Harlan Sprague–Dawley, Madison, Wisconsin. Animals were housed in a temperature- (21 ± 1°) and light-cycle-controlled room (lights on 0600–1800 hr) throughout the course of the study, and were given rat chow (Wayne Feeds) and tap water *ad lib.*

*Experiment 1.* The effect of continuous LM administration on the PRL response to ether stress.

Thirty-five animals were divided into four groups for this experiment; controls ($n$ = 5) receiving 0.9% saline, and three experimental groups receiving LM at calculated rates of 2.5 ($n$ = 10), 5.0 ($n$ = 10), and 10.0 ($n$ = 10) μg/hr. Saline and LM were administered continuously via an osmotic minipump (Alzet No. 1701) implanted ip on Day 1 of the study. The pump was implanted immediately following a 3-min ether exposure and drawing of a 1.0-ml jugular blood sample. This pump is reported to release a constant 1.0 μl/hr for 7 days *in vivo*. On Days 2, 4, 6, and 7 following pump implantation, animals were subjected to a 3-min ether stress. A 1.0-ml blood sample was drawn from the jugular vein into a heparinized syringe immediately following ether exposure. Plasma was separated and stored at −20° until radioimmunoassay for rPRL.

*Experiment 2.* The effect of continuous LM administration on basal PRL levels.

Eight animals were divided into two groups: controls ($n$ = 4) receiving 0.9% saline, and experimental ($n$ = 4) receiving 10.0 μg LM/hr. Agents were administered via osmotic minipump implanted ip on Day 1 of the study. On Day 3 the right jugular vein of each rat was cannulated (Silastic, 0.025 in. i.d. × 0.047 in. o.d.) under ether anesthesia. Cannulae were passed sc anterior to the foreleg and externalized on the midline of the back between the scapulae. Cannulae were then flushed with heparinized saline and sealed until Day 7 to allow recovery from surgery.

On Day 7 the cannulae were opened, connected to a 25-cm section of Silastic tubing to allow blood withdrawal without disturbing the animal, and flushed with heparinized saline. Beginning at 0800, 0.45 ml blood was drawn every other hour for 24 hr into a heparinized syringe. Following removal of plasma from the sample, erythrocytes were resuspended in 38° Plasmanate (Cutter Biological) to a volume of 0.45 ml and reinjected in order to maintain hematocrit and blood volume. Plasma was stored at −20° until assayed for prolactin.

*PRL assay and data analysis.* Plasma rPRL was assayed according to the method of Birge *et al.* (12). Plasma PRL concentrations are expressed as nanograms per milliliter of NIAMDD RP-2 rat PRL. Data were analyzed using a one-way ANOVA. Where a significance of $P < 0.05$ or less was achieved, the Student $t$ test was utilized to determine which subgroups differed from the others.

**Results.** *Experiment 1.* (Fig. 1). Basal PRL levels in the male rats not exposed to

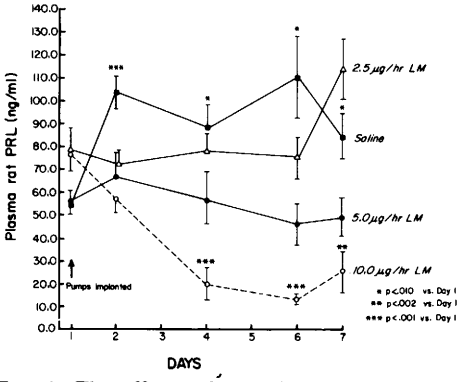


FIG. 1. The effects of a 3-min ether exposure on plasma PRL levels of adult male rats administered saline ($n$ = 5), or LM at calculated rates of 2.5 ($n$ = 10), 5.0 ($n$ = 10), and 10.0 ($n$ = 10) μg/hr via osmotic minipump implanted ip on Day 1. (Mean ± SE.)

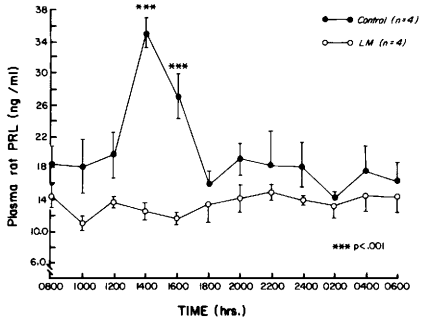


FIG. 2. Plasma PRL concentrations over a 24-hr period in chronically cannulated adult male rats, 7 days following ip implantation of osmotic minipumps releasing physiological saline ($n$ = 4) or 10.0 μg LM/hr ($n$ = 4).

ether were 18.1 ± 1.9 ng/ml ($\bar{x}$ + SE). The plasma PRL response to initial ether exposure in control animals on Day 1 (55 ± 4 ng/ml) was followed by a persistently greater increase in the PRL response upon subsequent ether exposures on Day 2 (104 ± 7 ng/ml, $P < 0.001$), 4 (89 ± 11 ng/ml, $P < 0.01$), 6 (111 ± 18 ng/ml, $P < 0.01$), and 7 (85 ± 9 ng/ml, $P < 0.01$). LM suppressed the PRL response to ether in a dose-dependent manner. The administration of LM at a rate of 2.5 μg/hr prevented the subsequent enhancement of PRL secretion to ether exposure on Days 2, 4, and 6 following minipump implantation while LM at a rate of 5 μg/hr prevented the enhancement on Days 2, 4, 6, and 7 following pump implantation. Administration of LM at the rate of 10.0 μg/hr significantly suppressed ether-induced PRL secretion to basal levels on Days 4 (21 ± 10 ng/ml, $P < 0.001$), 6 (13 ± 2 ng/ml, $P < 0.001$), and 7 (25 ± 10 ng/ml, $P < 0.002$) in comparison to Day 1 (77 ± 8 ng/ml). These data suggest that constant LM administration at the rate of 10.0 μg/hr is sufficient to suppress the PRL response to ether stress.

*Experiment 2.* (Fig. 2). Animals receiving physiological saline via osmotic minipump demonstrated a diurnal fluctuation in plasma PRL levels, with a surge occurring late in the light phase from 1400 to 1600 hr. Plasma PRL values rose from a presurge level of 19.7 ± 2.7 ng/ml at 1200 to a peak of 35.1 ± 1.6 ng/ml at 1400, falling to 15.8 ± 1.5 ng by 1800 and remaining low throughout the dark phase. Treatment with LM, administered via minipump at the rate of 10.0 μg/hr, completely abolished the diurnal surge observed in control animals at 1400 and 1600 ($P < 0.001$). The mean 24-hr plasma PRL concentration of animals receiving saline was 19.7 ± 1.7 ng/ml, while that of the LM-treated group was 13.2 ± 0.4 ng/ml ($P < 0.005$).

*Osmotic pumps.* Upon completion of Experiments 1 and 2, the osmotic pumps were visually examined to determine whether they had released their contents. All pumps appeared to have released more than 90% of their contents by Day 7 following implantation.

**Discussion.** Ergot alkaloids are commonly used to suppress endogenous PRL secretion for the purpose of studying the effects of chronic hypoprolactinemia in animals. However, the usefulness of these agents is limited by several factors. First, these compounds are toxic to the animal, and they lack specificity to the PRL secretory pathway. In addition to suppressing PRL secretion, ergot alkaloids may independently alter renal electrolyte excretion (13), increase peripheral smooth muscle tone (7, 8, 14), alter blood pressure (2, 7, 8), and alter CNS control of respiration, body temperature, cardiac output, and the responses of baroreceptors and chemoreceptors (7, 8, 15). These effects vary depending upon the specific drug used, dosage, route of administration, and the species involved.

Second, the handling and pain associated with repeated administration of these drugs increases the amount of stress on animals during an experiment, possibly affecting results.

LM is less toxic than many of the commonly used ergot derivatives, having an acute iv $LD_{50}$ = 320 mg/kg in rats, in comparison to 95 mg/kg for ergocornine (8), and 120 mg/kg for bromocryptine (8). The use of LM is less harmful to the rat than equivalent doses of the latter drugs, while it achieves a similar degree of PRL suppression (9). Furthermore, LM lacks the potent vasoconstrictor activity found in ergot derivatives which are not hydrogenated at the double bonds of C9 and C10 of the molecule (9), eliminating a hypertensive effect with its use. However, LM is a potent dopamine agonist which may have inherent peripheral actions that have not been studied. Theoretically, these may mimic or antagonize the actions of PRL and should be considered when LM is used to suppress PRL secretion.

The results of the present study suggest that administration of LM by osmotic minipump at a rate of 10.0 μg/hr (0.69 mg/kg/day) produces chronic hypoprolactinemia in male rats by abolishing the diurnal rise in PRL levels seen in control animals 7 days following pump implantation, reducing mean 24-hr plasma PRL levels by 33%. This method of administration also consistently suppresses the PRL response to ether exposure in a dose dependent manner on Days 4–7 following pump implantation, thus suppressing PRL secretion under both basal and stimulated conditions. The hourly rate of LM administration necessary to suppress enhanced PRL secretion in the present study agrees with the findings of Clemens *et al.* (11) in reserpine-treated male rats. Further suppression of spontaneous PRL secretion may be possible with an increased rate of LM administration. Clemens *et al.* found that 3.0 mg LM/kg daily for 28 days lowered serum PRL levels in six ovariectomized female rats to less than 2.0 ng/ml (10).

The route of LM administration for the induction of chronic hypoprolactinemia used in the present study is preferable to single daily injection as it decreases the required amount of LM administered by 77% (0.69 vs 3.0 mg/kg/day). This is desirable as it will reduce the toxic effects of the drug on the animal, and may reduce the degree of side effects from the drug. Furthermore, prolonged suppression of endogenous PRL secretion by this route of administration requires handling of animals only once over a 7-day period, in contrast to the daily manipulation required with sc or ip injection, minimizing stress on the experimental animals during the course of an experiment.

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