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Temporal Relationship and Role of Dopamine in "Short-Loop" Feedback of Prolactin (39852)[1]

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Prolactin (PRL) can inhibit its own secretion by the pituitary ("short-loop" feedback) by acting at the hypothalamic level (1). Physiological elevations of PRL in rats, such as those occurring during the estrous cycle at proestrus (2), suckling (2), and pseudopregnancy (3), can be inhibited by a chronic hypothalamic implant of PRL. Yamada (4) recently used microiontophoresis and electrical recording techniques to demonstrate the presence of hypothalamic neurons that respond to infusion of PRL.

Neuropharmacological studies have shown that dopamine has an inhibitory effect on PRL release (1), and the work of Hökfelt and Füxe (5), using the Falck-Hillarp fluorescence technique, indicated that stimulation of the hypothalamic dopamine (DA) system may be involved in the "short-loop" feedback inhibition of PRL. It was the objective of this study to obtain information on the temporal effects of administered PRL on inhibition of PRL release and to further clarify the role of DA in this process.

Materials and methods. Animals and treatments. Male Sprague-Dawley rats (Spartan Research Animals, Haslett, Michigan) weighing 225-250 g each, were housed in a temperature (22°) and light-controlled room (lights on from 0600-1800 hr) and allowed access to food (Purina Rat Chow, Ralston Purina Co., St. Louis, Missouri) and water *ad libitum* for 3 to 4 days prior to each experiment. Ovine-PRL (oPRL, NIH, 28 IU/mg in 0.9% NaCl), pimozide (in 0.1 *N* tartaric acid) or the appropriate diluent were injected sc in 0.1 ml/100 g body wt. Restraint stress was carried out by taping the animals to wire test tube racks and placing them on their backs. Blood samples were removed by cardiac puncture under light ether anesthesia or from trunk blood after decapitation. Blood was allowed to clot overnight at 4° and was then stored at −20° until radioimmunoassays were performed.

Animals were subjected to one of the following experimental treatments: (a) injection with oPRL (4 mg/kg) and killed by decapitation 0, 1, and 4 hr later; (b) injection with oPRL and pimozide (2 mg/kg) and blood samples taken by cardiac puncture under light ether anesthesia 0, 2, and 4 hr later; (c) injection with oPRL 1, 4, or 12 hr prior to 3-min restraint stress, and killed by decapitation 5 and 15 min after the end of the stress period; (d) injection with oPRL 4 hr prior to 20-min restraint stress and killed by decapitation at the end of this period.

Radioimmunoassays and statistical analysis. Serum rat PRL was measured by the method of Niswender *et al.* (6) and serum oPRL by the method of Koprowski and Tucker (7). Values are expressed in terms of NIAMDD-RAT-PRL-RP-1 and NIH-β_1-PRL, respectively. Data were analyzed statistically using analysis of variance and Student's *t* test. The level of significance was chosen as $p < 0.05$.

Results. Figure 1 shows the concentration of rat and oPRL in male rats decapitated at different times after a sc injection of 4 mg of oPRL/kg body wt. Serum rat PRL remained at basal levels, whereas oPRL went from undectable values to almost 1200 ng/ml by 1 hr after oPRL administration and declined

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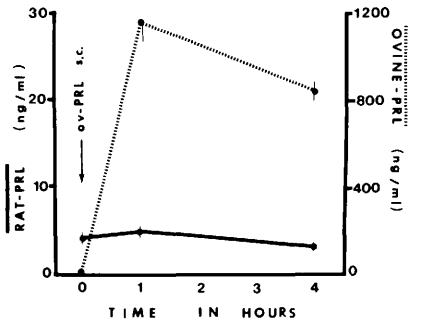

FIG. 1. Serum concentration of rat and ovine PRL in male rats decapitated at different times after a sc injection of 4 mg of oPRL/kg body wt. Serum oPRL levels before the injection (time zero) were undetectable in this and the following groups. Each point represents the mean of eight animals and the vertical lines the standard error of the mean (SEM).

slowly thereafter. It is clearly seen that ovine and rat PRL do not cross-react as shown previously (6).

Figure 2 shows the temporal profile of serum rat PRL levels after injection of a single supramaximal dose of pimozide, or pimozide + oPRL, as compared with control values. Blood samples were obtained by cardiac puncture under light ether anesthesia. Basal levels of serum rat PRL did not change after injection of diluent alone, but they decreased slowly after injection of diluent + oPRL. The decrease was statistically significant 4 hr after injection of oPRL. Serum PRL levels showed a six-fold increase by 2 hr after injection of pimozide alone. The rise in serum PRL observed in animals that received the combination of pimozide + PRL was reduced only by about 25% ($p \simeq 0.05$).

Figure 3 shows the PRL levels after 3 min of restraint stress in animals that received a single dose of 4 mg of oPRL/kg body wt at different times before stress and killed by decapitation at various times after stress. It can be seen that about 50% of the rise in serum PRL induced by restraint stress was significantly blocked only when oPRL was injected 4 hr before stress but not when injected 1 or 12 hr before stress. Basal levels of PRL at time 0 were not significantly different among the groups of rats.

Figure 4 shows serum PRL levels after 20 min of restraint stress in animals pretreated with 4 mg of oPRL/kg body wt 4 hr before stress and killed by decapitation at the end of the stress period. About a 50% inhibition in the stress-induced release of PRL was observed in animals pretreated with oPRL, and there was only a small but insignificant reduction in control values.

Discussion. These results indicate that administration of oPRL is able to inhibit endogenous release of PRL in male rats in a nonacute manner by acting mainly through a dopaminergic system. Since basal levels of PRL in male rats are very low, it is difficult to demonstrate inhibition by oPRL unless an increase in PRL release is induced. Hence the rats were subjected to restraint stress to increase PRL release. Under this condition, prior injection of ovine PRL inhibited PRL release from the rat pituitary. Inhibition of PRL release by the "short-loop" feedback does not appear to be a rapid mechanism for controlling PRL release, since it required more than 1, but less than 4 hr to become fully active.

It is well known that DA is an important inhibitor of PRL release (8). It can act directly on the anterior pituitary to inhibit PRL release (8), and it recently was reported to be present in the portal vessels (9). DA also was observed to stimulate

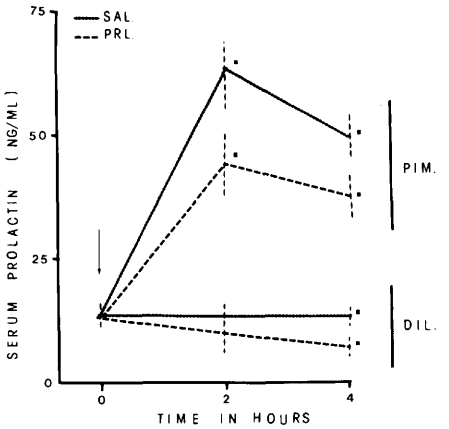

FIG. 2. Serum concentration of rat PRL at different times after injection of a single supramaximal dose of pimozide or pimozide + oPRL, as compared with control values. Blood samples were obtained by cardiac puncture under light ether anesthesia. SAL = saline control. Black squares show results significantly different from saline control $p < 0.05$.

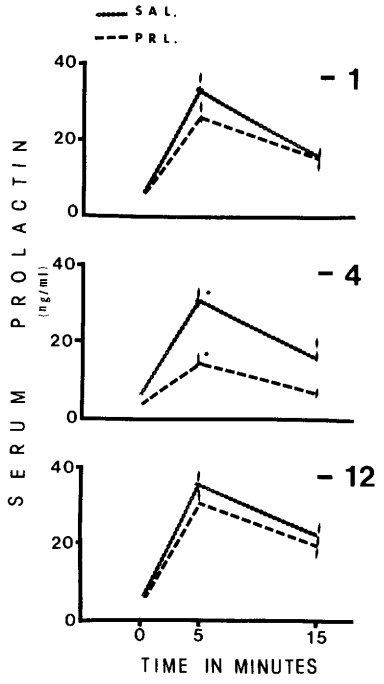

FIG. 3. Serum concentration, of rat PRL at different times after a 3-min restraint stress period ended in animals that received a single sc injection of oPRL (4 mg/kg) 1, 4, or 12 hr before stress. Animals were killed by decapitation. SAL = saline control. Asterisks show results significantly different from saline control $p < 0.05$.

release of hypothalamic PIF (10). Since blockade of DA receptors by a supramaximal dose of pimozide overcame almost 80% of the inhibiting action of ovine PRL on rat PRL release, it appears that DA is the main neurotransmitter involved in the "short-loop" feedback mechanism of PRL. Furthermore an increase in DA turnover has been reported to occur 4 hr after ovine PRL administration into hypophysectomized rats (5). Since the increase in DA turnover in the median eminence after administration of haloperidol, a DA receptor blocker, can be inhibited by hypophysectomy (11), it is possible that the time required for the "short-loop" feedback to become fully operative reflects activation of a DA system. Only 25% of the increase in PRL induced by pimozide was prevented by oPRL administration, indicating that a DA mechanism mainly mediates this inhibitory response. However, the possibility exists that non-DA systems also may be involved in the "short-loop" feedback mechanism.

Serotonin has been shown to increase PRL release (8) and was reported to increase during restraint stress (12). Therefore the possibility exists that a serotoninergic mechanism also may be operative in the "short-loop" feedback of PRL. However, we were unable to detect by a fluorimetric method any changes in content or turnover of serotonin in the brain, hypothalamus, or midbrain raphe nucleus [the main source of serotonin in the brain (13, 14)] after injection of ovine-PRL (unpublished). Furthermore, no change was noted in the multiple unit activity of the midbrain raphe neurons after ovine PRL injection.

Our results therefore confirm previous reports that DA is involved in the "short-loop" feedback of PRL (5) and suggest that at least 75% of this inhibitory mechanism is mediated through DA receptors. The possibility also exists that non-DA and nonserotonergic systems are involved. The time course for the full effects of injected PRL to be manifested suggests that the "short-loop" feedback mechanism probably is not involved during the time of acute release of PRL, when it is high in the circulation.

Summary. In order to study the time relationship and role of dopamine (DA) in feedback inhibition of prolactin (PRL) by PRL, (a), ovine PRL (oPRL) (4 mg/kg) was given to male rats 1, 4, and 12 hr before restraint stress; and (b) oPRL was given together with pimozide (2 mg/kg), a specific dopaminergic receptor blocker. Four hours after

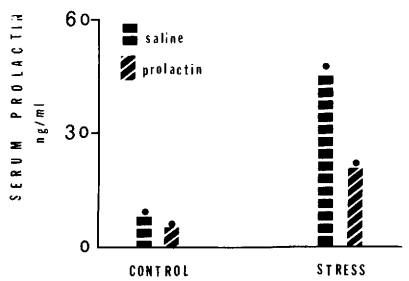

FIG. 4. Serum concentration of rat PRL after 20 min of continuous restraint stress in animals pretreated with 4 mg of oPRL/kg body wt 4 hr before stress. Control animals also received oPRL but were not restrained. Animals were killed by decapitation.

injection of oPRL, basal levels of serum rat PRL were significantly reduced. Restraint stress elevated serum PRL levels about tenfold; pretreatment with ovine PRL prevented about 50% of this rise when given 4 hr but not 1 or 12 hr prior to stress. Blockade of dopamine receptors by administration of pimozide produced a six-fold rise in serum prolactin levels by 2 and 4 hr after injection. o-PRL given in combination with pimozide blocked only 25% ($p \simeq 0.05$) of the increase of endogenous PRL by pimozide at 2 and 4 h. These observations suggest that (a) the "short-loop" inhibitory feedback on PRL release requires more than 1 but less than 4 hr to become active, and (b) a dopaminergic mechanism is mainly responsible for inhibition of PRL release by PRL.

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