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Comparison of the Effects of Central Acting Drugs on Prolactin Release in Young and Old Male Rats (41178)

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Abstract. The effects of several central acting drugs on prolactin (PRL) secretion were compared in young (3–4 months) and old (18–19 months) Sprague–Dawley male rats. Administration of the catecholamine synthesis inhibitor, methyl-DOPA, or the dopamine receptor blocker, haloperidol, produced a significantly greater increase in plasma PRL in the old than in the young rats. Stimulation of the serotonergic system by quipazine or enhancement of opioid activity by morphine produced a significantly greater rise in plasma PRL in the young than in the old male rats. Administration of the specific opiate antagonist, naloxone, reduced plasma PRL levels more in old than in young male rats, but this difference was not significant. Measurement of pituitary content and concentration of PRL revealed that both were significantly greater in old than in young male rats. These results suggest that hypothalamic dopamine continues to be an important inhibitor of PRL release in old male rats, whereas serotonin and the opiates become relatively less effective as stimulators of PRL release.

Secretion of prolactin (PRL) in the rat is regulated mainly by hypothalamic hormones and neurotransmitters that can either increase or decrease PRL release (1, 2). As the rat ages, an elevation in basal levels of PRL has been observed in both sexes (3–5), together with a decrease in hypothalamic content and turnover of dopamine and norepinephrine, and an increase in content and turnover of serotonin (6, 7). These changes during aging may largely account for the increased PRL release in old rats, since it is well established that hypothalamic dopamine inhibits, whereas serotonin stimulates PRL release (1, 2). In the present study, drugs that influence brain dopamine, serotonin, or opioid activity, were compared for their effects on PRL release in old and young male rats. Pituitary content and concentration of PRL also were measured in young and old rats.

Materials and Methods. *Subjects.* Male Sprague–Dawley rats (Harlan Industries, Indianapolis, Ind.), 3–4 and 18–19 months old, respectively, were used in the present study. Animals were housed in a temperature-controlled room (22°) on a 14:10 hr light:dark cycle (lights on at 0500 hr). Food (Purina Laboratory Chow, Ralston Purina Co.) and water were provided *ad libitum*.

Surgery. Silastic indwelling atrial cannulas (0.1 mm × 0.2 mm) were implanted into the right external jugular vein of each rat, under ether anesthesia. The free end of the cannula was channeled subcutaneously (sc) to the back of the neck, where it emerged 1 cm posterior to the base of the skull. Immediately after surgery, animals were injected with 0.2 ml of penicillin (150,000 units; Longicil Fortified, Ft. Dodge Laboratories, Ft. Dodge, Iowa), to prevent infection, and placed in individual stainless-steel cages (18 × 18 × 24 cm). Body weights were monitored before surgery and immediately prior to drug administration. Animals showing a loss of body weight (BW) were not used.

Pharmacological agents. Morphine sulfate (Mallinckrodt, St. Louis, Mo), quipazine maleate (Miles Laboratories, Elk-

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hart, Ind.), naloxone (Endo Laboratories, Garden City, N.Y.), and methyldeoxyphenylalanine (Methyl-DOPA, Sigma Chemical Co., St. Louis, Mo.) were dissolved in 0.87% sterile NaCl. Haloperidol (McNeil Laboratories, Ft. Washington, Pa.) was dissolved in 0.3% tartaric acid.

Experimental procedures. Three days after the implantation of cannulas, animals were brought to the experimental room in their individual cages. A Silastic tubing extension (20 cm) was attached to each cannula and exited through wire mesh placed over the top of the cage. Animals were allowed free access to food and water throughout the experiment. After removal of the void volume (0.15 ml), blood samples (1 ml) were drawn into heparinized syringes. Samples were centrifuged at 2000 rpm in a Sorvall GLC-2B (Sorvall Inc., New Town, Conn.) table-top centrifuge (r = 9 cm) for 5 min. The plasma was removed and immediately frozen on dry ice. The remaining packed cells were resuspended in sterile physiological saline and reinjected. Plasma samples were stored at −20° until assayed for PRL by radioimmunoassay (RIA).

The ability of young and old rats to release PRL was compared after administering the following substances:

(*a*) *Methyl-DOPA.* At 45 and 15 min prior to injection of methyl-DOPA (200 mg/kg, ip), blood was collected from nine young and nine old rats. Four blood samples were withdrawn at 30-min intervals after drug administration.

(*b*) *Haloperidol.* Haloperidol (1 mg/kg, sc) was given to eight young and eight old rats. Blood was sampled 45 and 15 min prior to injection of the drug. Subsequent blood samples were removed at hour intervals for 3 hr after haloperidol administration.

(*c*) *Quipazine maleate.* Ten young and ten old rats were given quipazine maleate (10 μg/kg, ip). Blood was withdrawn at 60, 40, and 20 min prior to administration of the drug and 10, 30, 50, and 70 min after its administration.

(*d*) *Morphine sulfate.* Morphine sulfate (5 mg/kg, iv) was administered to 16 young and to 16 old male rats. Three blood samples were obtained at 20-min intervals prior to the administration of morphine, and blood was withdrawn 10, 30, 50, 70, and 90 min after injection of the drug.

(*e*) *Naloxone.* Preinjection blood samples were obtained from nine young and nine old male rats, 40 and 20 min before naloxone was given (2 mg/kg, ip). Four blood samples were taken at 20-min intervals beginning 10 min after naloxone was administered.

(*f*) *Pituitary prolactin measurement.* Eight young and eight old male rats were decapitated and their pituitaries were removed. The posterior pituitary was discarded and the anterior pituitary was placed in 2 ml of cold 0.01 *M* phosphate-buffered saline (pH 7.6). Each anterior pituitary was homogenized and immediately frozen at −20° until assayed for PRL content by RIA. Pituitary protein concentration was determined by the method of Lowry *et al.* (8), using RIA grade bovine serum albumin as the standard.

(*g*) *Prolactin radioimmunoassay.* PRL was assayed by RIA with the NIAMDD kit provided by the National Pituitary Agency, NIAMDD. The rat PRL antibody used was a gift from Dr. C. L. Chen (University of Florida, College of Veterinary Medicine, Gainesville, Fla.). Bound and free hormone were separated using IGg-Sorb (The Enzyme Center Boston, Mass.). The minimal detectable dose of the assay was calculated to be 0.5 ng PRL per tube, and 50% inhibition of tracer binding was 1.8 ng per tube. Samples were parallel to the standard curve from 20 to 400 ng/ml using plasma volumes of 25, 50, and 100 μl. The intra- and interassay coefficients of variations were 4 and 6%, respectively.

Statistics. Statistical analysis was accomplished using repeated measures analysis of variance with a subject/age × repeated blood sampling design (9). Significant differences between age, trial, or interaction were further analyzed by the Newman–Keul's procedure. One-way analysis of variance and Student's t test were used to analyze the data on pituitary content and concentration of PRL. In all experiments the level of significance chosen was $P < 0.05$.

Results. (*a*) Methyl-DOPA. Injection of the catecholamine synthesis inhibitor,

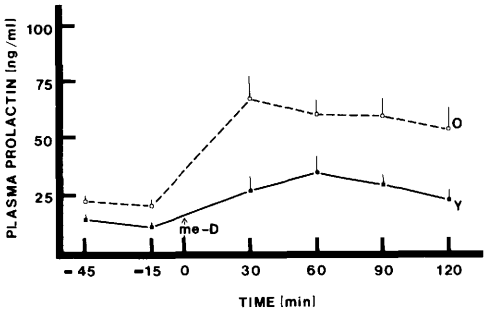

FIG. 1. Plasma prolactin in nine young (solid line) and nine old (broken line) male rats injected ip with 200 mg/kg methyl-DOPA. Each point represents the mean ± 1 SE.

methyl-DOPA, produced a significant rise in plasma PRL in the young ($P < 0.05$) and old ($P < 0.01$) male rats 30 min after administration (Fig. 1), and this increase in plasma PRL was maintained throughout the 120-min sampling period. The old male rats consistently demonstrated significantly higher ($P < 0.01$) elevations of plasma PRL in response to the drug compared to young male rats. Plasma PRL values in the old male rats ranged from a maximum of 68 ± 10 ng/ml at 30 min to a minimum of 54 ± 10 ng/ml at 120 min, whereas in the young male rats, maximum and minimum values for plasma PRL were 35 ± 7 ng/ml at 60 min and 23 ± 4 ng/ml at 120 min.

(*b*) *Haloperidol.* Sixty minutes after administration of the dopamine receptor blocker, haloperidol, plasma PRL rose significantly ($P < 0.01$) in the young and old male rats (Fig. 2). The increase in plasma PRL in the old male rats did not differ significantly from that observed in the young male rats at 60 min, but was significantly greater than in the young male rats at 120 and 180 min ($P < 0.01$ and $P < 0.05$, respectively). The maximum rise in plasma PRL in the old male rats (130 ± 15 ng/ml) was observed 120 min after haloperidol administration, whereas the highest value for plasma PRL in the young male rats (93 ± 4 ng/ml) was seen 60 min after injection of the drug. Plasma PRL values remained significantly elevated ($P < 0.05$) above preinjection values throughout the 180-min period of observation in both young and old male rats.

(*c*) *Quipazine.* Administration of the serotonin receptor stimulator, quipazine, resulted in a significant elevation ($P < 0.01$) of plasma PRL in the young and old male rats (Fig. 3). At 10, 30, and 70 min after quipazine administration, plasma PRL was significantly higher ($P < 0.01$) in the young than in the old male rats. Peak values of plasma PRL were attained by 20 min after administration of the drug to the young (128 ± 14 ng/ml) and 10 min after administration of the drug to the old (73 ± 12) animals. In the young rats, plasma PRL remained elevated at 30 min and then declined. The old male rats demonstrated elevated levels of plasma PRL 30 and 50 min after quipazine was administered. Although lower than peak values, plasma PRL was still significantly higher ($P < 0.05$) than baseline values in the young and the old male rats at the end of the experimental period (70 min).

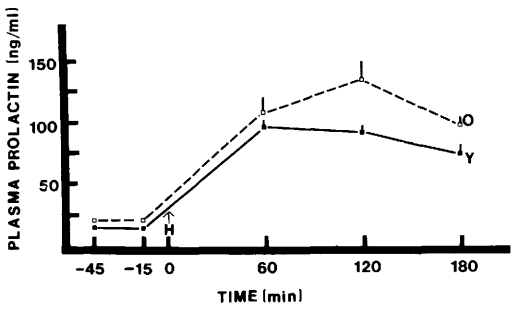

FIG. 2. Plasma prolactin in eight young (solid line) and eight old (broken line) male rats injected sc with 1 mg/kg haloperidol. Each point represents the mean ± 1 SE.

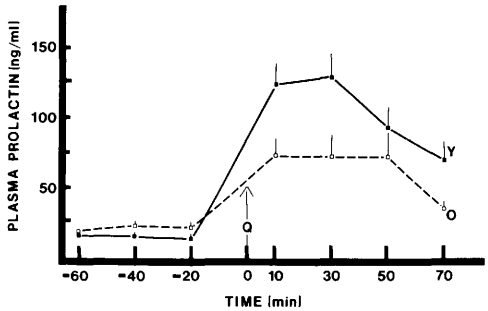

FIG. 3. Plasma prolactin in 10 young (solid line) and 10 old (broken line) male rats injected ip with 10 μg/kg quipazine. Each point represents the mean ± 1 SE.

(*d*) *Morphine.* Injection of morphine significantly increased ($P < 0.01$) plasma levels of PRL, as compared to preinjection values, in both young and old male rats (Fig. 4). The increase in plasma PRL in the young rats was significantly greater ($P < 0.01$) than that observed in the old rats at 10, 30, and 50 min after administration of the drug. Plasma PRL levels reached a maximum in the young rats (164 ± 14 ng/ml) by 10 min after morphine administration. The maximum increase in plasma PRL in the old male rats (90 ± 10 ng/ml) was observed 30 min after morphine was administered.

(*e*) *Naloxone.* The opiate antagonist, naloxone, significantly decreased ($P < 0.05$) plasma PRL levels, as compared to mean preinjection values, in both young and old rats (Fig. 5). Minimum values for plasma PRL were seen 30 min after naloxone injection in the young (7 ± 1 ng/ml) and old (8 ± 1 ng/ml) animals. When plasma PRL levels observed 30 min after naloxone administration were compared to mean preinjection values (young = 12 ± 1 ng/ml; old = 15 ± 2 ng/ml), it was found that naloxone produced a 47% decrease in plasma PRL in the old rats, and a 42% reduction in the young rats. This difference was not significant.

(*f*) *Pituitary prolactin.* Pituitary PRL content was significantly greater ($P < 0.05$) in the eight old than in the eight young male rats when expressed as micrograms per gland (young = 12.56 ± 0.78; old = 20.0 ± 3.29), or as micrograms per milligrams protein (young = 7.06 ± 0.41; old = 10.87 ± 0.92).

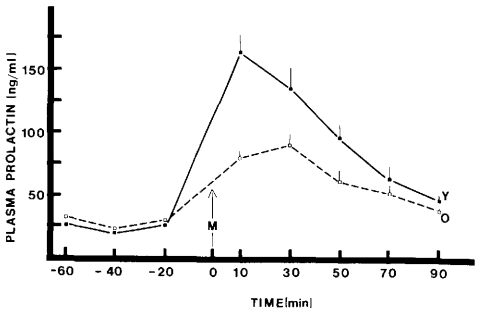

FIG. 4. Plasma prolactin in 16 young (solid line) and sixteen old (broken line) male rats injected iv with 5 mg/kg morphine. Each point represents the mean ± 1 SE.

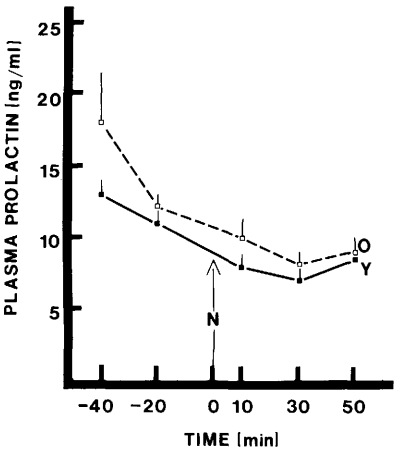

FIG. 5. Plasma prolactin in nine young (solid line) and nine old (broken line) male rats injected ip with 2 mg/kg naloxone. Each point represents the mean ± 1 SE.

Discussion. The anti-dopaminergic drugs, methyl-DOPA and haloperidol, each produced a greater increase in plasma PRL in the old than in the young male rats. To our knowledge, this is the first indication that any drug can produce a greater release of a pituitary hormone in old than in young rats. Drugs or other treatments that normally increase gonadotropin or GH release, previously were found to be less effective in old than in young rats ((10, 11); Sonntag, Forman, Miki, Ramos, Arimura, and Meites, unpublished observation). The present results suggest that despite the increase in pituitary and plasma levels of PRL normally seen in old rats ((3–5); the present study), dopamine continues to be an active inhibitor of PRL release and prevents PRL from rising to even higher levels in the blood of old rats.

There is ample evidence that dopamine acts directly on the pituitary to inhibit PRL release (12), and dopamine has been shown to be present in the hypothalamic–hypophysial portal vessels (13). In old male rats, an increase in the affinity of pituitary binding sites for dopamine has been reported (14). The increased release of PRL in the old rats in response to administration of the anti-dopaminergic drugs, methyl-DOPA and haloperidol, may be due in part

to the antagonism of the high-affinity binding sites for dopamine by these drugs, and to the greater initial pituitary content of PRL in these animals. In addition, reduced hypothalamic dopamine activity in the old rats (6, 7) may also contribute to the increased PRL release.

Serotonin, its precursors, and several serotonin agonists, each have been shown to induce release of PRL by the rat (1). In the present study, the serotonin receptor stimulator, quipazine, produced a greater elevation of plasma PRL in young than in old male rats. It is possible that the lower PRL response of old animals to quipazine is due in part to a reduction in number of serotonin postsynaptic receptors, their affinity for serotonin, or both. A decrease in number of high-affinity binding sites for serotonin has been found in the brain of aged human subjects (15).

Morphine is a potent stimulator of PRL release in the rat, and is believed to act by depressing dopamine and increasing serotonin turnover in the hypothalamus (16). In our old male rats, morphine increased plasma PRL to a greater extent in young than in old male rats. Similarly, we observed that morphine stimulated a greater release of growth hormone in young than in old male rats (Sonntag, Forman, Miki, Ramos, Arimura and Meites, unpublished observations). Morphine may be less effective in increasing PRL in the old male rats because hypothalamic dopamine activity already is depressed, serotonin activity is increased (6, 7), and the number or affinity of serotonin receptors may be reduced. There also may be a deficiency in the number and/or affinity of hypothalamic opioid postsynaptic receptors in old male rats, similar to that observed in the number and affinity of opioid receptors in the hypothalamus of aging female rats (17).

Naloxone, a specific opioid antagonist significantly decreased plasma PRL in both young and old male rats. Although the magnitude of the reduction in the old male rats was somewhat greater in the old than in the young rats, this difference was not significant. This, together with the data on the effect of morphine on PRL release, suggests that the brain opiates have a decreased capacity to stimulate PRL release in old male rats.

In general, this study provides further evidence that there are significant changes in the neuroendocrine regulation of PRL secretion in aging rats. These results show the ability of anti-dopaminergic drugs to increase PRL release in old male rats is enhanced, whereas the ability of serotonin and the opiates to promote PRL release is reduced.

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