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Effect of Propiomazine on Plasma Prolactin in the Rat: Counteraction by L-Dopa[1] (42049)

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Abstract. A single iv injection of 0.31, 0.62, 1.25, 2.5, 5, 10, or 20 mg/kg body wt of a phenothiazine derivative, propiomazine (PP), into male rats significantly ($P < 0.05$) increased plasma prolactin concentrations. The higher doses (5, 10, and 20 mg/kg body wt) produced increases that were greater in both magnitude and duration than those produced by the lower doses. The higher doses of PP, along with the elevations in plasma prolactin, also produced concomitant decreases in plasma luteinizing hormone (LH) levels. Pretreatment with L-dopa (100 mg/kg body wt) completely blocked the PP-induced stimulation of prolactin release, indicating that antidopaminergic action of PP either at the hypothalamic or anterior pituitary level was responsible for its effects on the release of prolactin.

Propiomazine hydrochloride (PP), or 2-propionyl - 10 - (3 - dimethylaminopropyl) - phenothiazine hydrochloride, is one of the more than 30 phenothiazine derivatives that have been shown to have a very wide spectrum of actions in the body. Besides their neurological, tranquilizing, and antipsychotic effects for which they are used extensively in man and animals, they produce a number of endocrine effects (1–3). Chlorpromazine, which is the most well-known phenothiazine derivative, has been shown to increase prolactin release, reduce gonadotropin release, and produce galactorrhea, amenorrhea, and gynecomastia in man (1–3); and block ovulation, disrupt the reproductive cycles, and cause infertility in rats, mice, and other animals (4–8).

Phenothiazine derivatives vary considerably in their potency, duration, and range of actions (7, 8). It was of interest to determine if PP, which is a commonly used tranquilizer in animals, has any effect on the release of prolactin and luteinizing hormone (LH).

Materials and Methods. Adult male Sprague–Dawley rats (Charles River, Wilmington, Mass.), weighing 250–300 g each, were housed in an air-conditioned (23 ± 2°C), light-controlled (lights on from 0700 to 1900 hr) room. They were fed Kansas State University rat chow and provided water *ad libitum*. Three days before experimentation, the animals were fitted with indwelling atrial cannulae and placed in individual cages. Each cannula was made of Silastic tubing (0.025 in i.d. × 0.047 in o.d.; Dow Corning, Midland, Mich.) and inserted into the right external jugular vein under light ether anesthesia (9). The free end of the cannula passed underneath the skin and exited near the base of the skull. The cannulae were rinsed daily with heparinized saline (10 U/ml). When not in use, the free ends of the cannulae were kept closed with a piece of paper clip. On the day of the experiment, extensions made of Silastic tubing were attached to the cannulae and the animals were left undisturbed for at least 60 min. In the first experiment, immediately after collection of a pretreatment blood sample (0.7 ml) in a heparinized syringe (5 U) at 1000 hr, the various groups of five animals each were injected iv with either 0.31, 0.62, 1.25, 2.5, 5, 10, or 20 mg of PP/kg body wt in a vol of 0.5 ml 0.85% NaCl at pH 6.6. The control rats were injected with pH 6.6 saline alone. Additional blood samples were collected at 15, 60, 120, and 180 min after PP or saline injections. Blood samples were centrifuged immediately after collection

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to separate cells and plasma. Plasma fractions were kept frozen for prolactin and LH radioimmunoassays later and the cells were returned to the animals in a total volume of 0.7 ml saline.

In the second experiment, a group of five cannulated rats was injected ip with 100 mg/kg body wt of L-dopa in 0.5 ml of 0.5 *N* HCl and 0.5 *N* NaOH at a pH of 3.0 (10), followed 15 min later with an iv injection of 5 mg/kg body wt of PP. Blood samples were collected immediately before L-dopa and PP injections (designated as −15- and 0-min samples, respectively) and then at 15, 60, 120, and 180 min after PP injection. Another group of five rats was treated similarly, except that the rats were not injected with PP.

Plasma samples were assayed in duplicate for PRL and LH using radioimmunoassay reagents supplied by the NIADDK. The reference preparations for PRL and LH were NIADDK-rPRL-RP-3(AFP-4459B) and NIADDK-rLH-RP-2, respectively. The biological potency of prolactin standard was 30 IU/mg. The sensitivity of the LH RIA was 0.1 ng of the standard and 50 μl of plasma was used for LH determinations. Significance of differences between hormone levels within a group and between groups was determined by analysis of variance and Student's *t* test.

Results. Fig. 1 shows that single iv injections of 0.31, 0.62, 1.25, or 2.5 mg of PP/kg body wt produced four- to sixfold increases ($P < 0.05$) in plasma prolactin levels in male rats, while saline injection had no effect. The highest increase in plasma PRL was produced by the highest dose of PP. Within 15 min, all doses of PP produced increases in plasma prolactin which were followed by sharp declines at 60 min.

Both the magnitudes and durations of prolactin increases were drastically increased when the doses of PP injected were increased to 5, 10, or 20 mg/kg body wt. These increases were 25- to 30-fold and prolactin remained 7- to 18-fold above basal levels at 3 hr after injection (Fig. 2, top). In response to PP treatment, plasma LH decreased significantly ($P < 0.05$) within 60 min and remained depressed during the next 2-hr period of observation (Fig. 2, bottom).

The 25-fold increase in plasma prolactin produced by 5 mg/kg body wt of PP was completely blocked by pretreatment with L-dopa (Fig. 3). L-Dopa treatment alone resulted in a significant decrease in plasma prolactin.

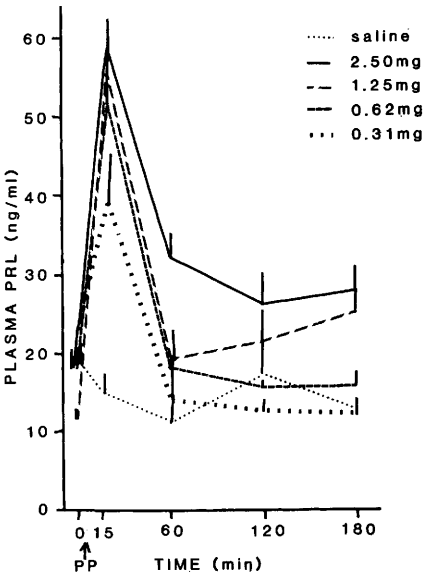

FIG. 1. Effects of a single iv injection of saline or 2.5, 1.25, 0.62, or 0.31 mg/kg body wt of propiomazine (PP) on plasma prolactin (PRL) concentrations in male rats. The injections were given immediately after collection of a pretreatment sample at time 0. Each point represents mean ± SEM (vertical bar) of five animals.

Discussion. These results demonstrate that PP is a potent stimulator of prolactin release. All doses of PP produced severalfold increases in plasma prolactin levels. The higher doses not only increased the magnitudes but also the durations of prolactin elevations. These results add PP to the list of phenothiazine derivatives, like chlorpromazine, acepromazine and perphenazine, that increase plasma prolactin levels in a number of species, including the rat, sheep, and man (8, 11–14).

In our experiments, along with the elevations in plasma prolactin levels, PP produced marked and concomitant decreases in circulating levels of LH. This effect is consistent with previous reports, which indicate that phenothiazine derivatives, especially chlorpromazine, can reduce the release of LH and FSH and disrupt the menstrual cycle in women (1–3, 7, 14), inhibit ovulation, suppress the estrous cycles, retard gonadal development in the rat (4, 5), and delay sexual maturity in the mouse (6).

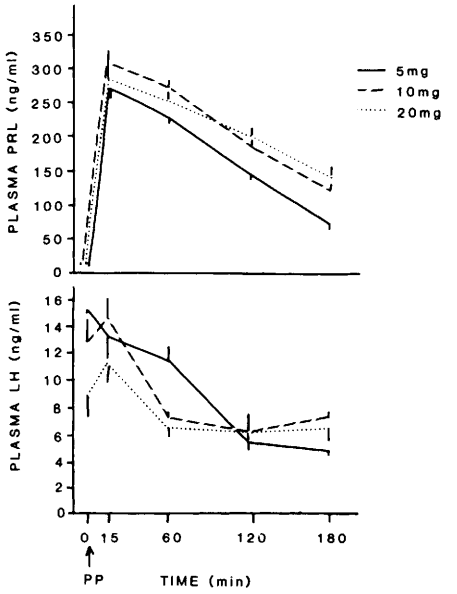

FIG. 2. Effects of a single iv injection of 5, 10, or 20 mg/kg body wt of propiomazine (PP) on plasma prolactin (PRL; top) and luteinizing hormone (LH; bottom) concentrations in male rats. The injections were given immediately after collection of a pretreatment sample at time 0. Each point represents mean ± SEM (vertical bar) of five animals.

Phenothiazines have a wide range of effects. Besides affecting the release of prolactin, LH, and other hormones, they produce a variety of neurological and antipsychotic effects and influence functions of the kidney, liver, and cardiovascular system (7). The precise mechanisms responsible for this broad spectrum of activities are not clear, although it is known that phenothiazines affect all levels of the nervous system, including the cortex, basal ganglia, limbic system, brain stem, spinal cord, and peripheral nerves (7, 8). Their endocrine effects, especially the stimulation of prolactin release, may be brought about through blockade of the tubero-infundibular dopaminergic system, by a direct antagonism of the dopamine receptors in the anterior pituitary, or by a combination of the two.

Several studies have indicated that perphenazine enhanced plasma prolactin levels in the rat by suppressing the release of prolactin inhibiting factor (PIF) from the hypothalamus (16–19). In these studies, (a) perphenazine increased the content of PIF in the hypothalamus, (b) hypothalamic fragments from perphenazine-treated rats were unable to suppress prolactin release from pituitary glands *in vitro,* and (c) addition of perphenazine to pituitary cell cultures had no effect on prolactin release. Other studies, however, have pointed out the possibility that perphenazine can influence prolactin release by a direct action on the pituitary. In these studies, perphenazine increased prolactin release from pituitaries transplanted under the kidney capsule (19). These results, however, also can be explained on the basis of release of a prolactin releasing factor of hypothalamic or peripheral origin. The possibility of a direct action of phenothiazine derivatives on the pituitary is supported by the studies in which chlorpromazine was able to increase prolactin release in rats with medial basal hypothalamic lesions (20, 21). The same phenothiazine derivative also has been shown to enhance adenylate cyclase levels in prolactin secreting GH_3 cells (22).

Whatever the exact locus of action of phenothiazine derivatives, there is ample evidence to indicate that they enhance prolactin release by their antidopaminergic action. Their effects on prolactin release can be blocked by pretreatment with the dopamine precursor, L-dopa, as has been shown in this study, or by treatment with dopamine agonist brom-ergocryptine, as has been shown in

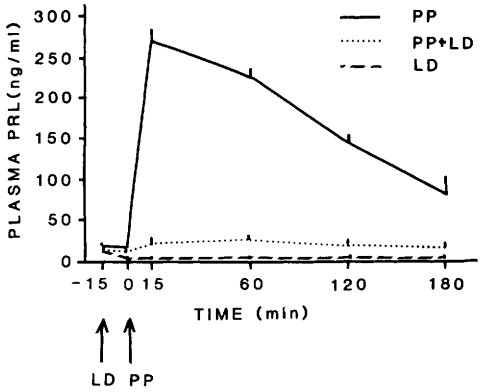

FIG. 3. Counteraction of propiomazine (PP)-induced increase in plasma prolactin (PRL) by L-dopa (LD). The iv injections of LD (100 mg/kg body wt) at −15 min and of PP (5 mg/kg body wt) at 0 min were given alone or in combination. Each point represents mean ± SEM (vertical bar) of five animals.

women who were undergoing treatment with phenothiazine derivatives (23).

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