NOTE

Effects of Chlorpromazine and Estradiol Benzoate on Prolactin Secretion in Gonadectomized Male and Female Rats

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Synopsis

The effects of chlorpromazine (CPZ) and estradiol benzoate (EB) on serum prolactin (PRL) levels were studied in gonadectomized male and female rats. In both sexes CPZ (25 mg/kg body weight) produced an elevation of PRL when measured 2 hr after the injection, but the elevated levels were higher in ovariectomized rats than in orchidectomized rats. These results reconfirm a sexual difference in the regulatory mechanism of PRL secretion in response to the dopamine receptor blocker. Pretreatment with 5 µg EB 48 hr before CPZ injection abolished this sexual difference in serum PRL concentration.

Several investigators have suggested that there is a sexual difference in the elevation of serum prolactin (PRL) concentration after the blockage of the hypothalamic inhibition (Bishop *et al.*, 1972; Krulich *et al.*, 1975; Ojeda *et al.*, 1977). It is claimed that dopamine (DA) is in itself a PRL-inhibiting factor and directly acts at the pituitary level (Brown *et al.*, 1976; Horowski and Gräf, 1976; Macleod, 1976). Ojeda *et al.* (1977) recently reported that serum PRL levels after the injection of pimozide, a DA receptor blocker, were higher in female rats than in male rats, and suggested that the sexual difference in PRL secretion was partly caused by the long-term effect of estrogen during the course of sexual development. Chlorpromazine (CPZ) is thought to be another DA receptor blocker (Horn and Snyder, 1971) and increased the basal serum PRL concentrations by the

blockage of dopaminergic inhibitory control (Lu *et al.*, 1970). In the present study, the effect of CPZ with or without estrogen treatment on serum PRL concentrations was compared between orchidectomized and ovariectomized rats. Gonadectomy was performed in all groups of rats to remove the difference in the circulating sex steroid hormones.

Materials and Methods

Male and female rats of the Wistar strain were used in the present experiments. At 8 weeks of age 16 male and 20 female rats were gonadectomized under ether anesthesia. Two weeks after gonadectomy 5 males and 7 females received an intraperitoneal injection of CPZ (Contomin, Yoshitomi Pharmaceutical Ind. Ltd., Osaka) in a dose of 25 mg/kg body weight (Groups B and E). Other 6 males and 6 females were given a subcutaneous injection of 5 µg estradiol benzoate (EB, Sigma) in 0.2 ml sesame oil 48 hr before CPZ treatment (Groups C and E). The remaining 5 males and 7 females re-

ceived an intraperitoneal injection of vehicle (consisting of 0.05% sodium pyrosulfite, 0.05% sodium sulfite and 0.075% vitamin C) of CPZ only (Groups A and D). Blood samples were collected under light ether anesthesia from the femoral vein before CPZ injection and from the carotid artery at autopsy 2 hr after CPZ injection. At autopsy the anterior lobe of pituitary was quickly taken out, and weighed on a torsion balance to the nearest 0.05 mg. It was homogenized in 2 ml of 0.05 M phosphate buffer at pH 7.0, frozen and thawed, then centrifuged at 3,000 rpm for 12 min. Serum samples and the supernatants of the homogenate were kept frozen at -20°C until assay for PRL. PRL levels were determined with NIAMDD radioimmunoassay kit from Rat Pituitary Hormone Distribution Program, NIAMDD, National Institutes of Health, Bethesda, Md, according to the procedure described in the instruction paper with a slight modification. The assay results were expressed as mIU of NIAMDD-rat prolactin-RP-1. The PRL levels between groups were analyzed by Student's *t* test.

Results

The serum PRL concentrations are presented in Fig. 1. In orchidectomized male rats there was some elevation in the level of serum PRL by CPZ injection, but the difference from the basal level before CPZ injection was statistically not significant ($0.05 < P < 0.1$, Fig. 1, Group B). In ovariectomized female rats the serum PRL level was significantly increased by CPZ injection ($P < 0.001$, Fig. 1, Group E). The serum PRL level of orchidectomized male rats 2 hr after CPZ injection was significantly lower than that of ovariectomized female rats ($P < 0.02$, Fig. 1, Group B *vs* E). There was no significant difference in the basal serum PRL levels between orchidectomized males and ovariectomized females before CPZ or vehicle injection.

In EB-pretreated gonadectomized rats the basal serum PRL levels were significantly higher than those in gonadectomized rats without EB pretreatment (Fig. 1, Group A *vs* C, Group D *vs* F). There was no difference in the basal serum PRL levels between EB-pretreated gonadectomized male

and female rats (Fig. 1, Group C *vs* F). In EB-pretreated orchidectomized males the serum PRL level significantly increased by CPZ injection ($P < 0.001$, Fig. 1, Group C). Likewise, in ovariectomized females the elevated level of serum PRL by EB pretreatment was further increased by CPZ injection ($p < 0.05$, Fig. 1, Group F).

Fig. 2 demonstrates the total PRL contents (mIU/gland) or concentrations (mIU/mg) of the anterior pituitary 2 hr after CPZ or vehicle injection. The pituitary PRL contents or concentrations did not show any significant changes by CPZ injection or EB plus CPZ injection, compared with those in the controls in both sexes.

Fig. 1. Effects of CPZ on serum PRL concentrations in gonadectomized male and female rats with or without prior EB treatment. A=orchidectomized male rats without EB and without CPZ; B=orchidectomized male rats without EB but with CPZ; C=orchidectomized male rats with both EB and CPZ; D=ovariectomized female rats without EB and without CPZ; E=ovariectomized female rats without EB but with CPZ; F=ovariectomized female rats with both EB and CPZ. The open columns indicate the serum PRL concentrations before CPZ injection and the shaded columns indicate the serum PRL concentrations 2 hr after the injection of CPZ (B, C, E and F) or vehicle of CPZ (A and D). Vertical lines depict standard error of the means, and the number in each column indicates the number of rats used.

Fig. 2. Comparison of the pituitary PRL contents (mIU/gland) and concentrations (mIU/mg) 2 hr after CPZ treatment or vehicle injection in gonadectomized male and female rats with or without EB treatment. For the abbreviation of Group A-F, see the legends in Fig. 1.

In female rat pituitaries PRL contents and concentrations were decidedly more than those in male rat pituitaries, regardless of prior treatments.

Discussion

The present study showed a sexual difference in the elevation of serum PRL concentrations by CPZ in gonadectomized rats. This finding is in agreement with those of Ojeda *et al.* (1977), who recently reported that in gonadectomized rats as well as in intact rats serum PRL concentrations were higher in female rats than in male rats after the injection of pimozide.

The difference in the basal serum PRL concentrations between gonadectomized

male and female rats was not observed. However, the pituitary PRL contents or concentrations were higher in ovariectomized females than in orchidectomized males. Therefore, as suggested by Ojeda *et al.* (1977), PRL release seems to be more strongly held in check in ovariectomized females than in orchidectomized males.

EB pretreatment tended to cause a more pronounced increase in the basal serum PRL concentrations in ovariectomized females than in orchidectomized males, but the difference was statistically not significant. In contrast, Mallampati and Johnson (1973) reported that the hypothalamo-pituitary axis in ovariectomized female rats was more responsive to a multiple injections of estrogen on PRL secretion than in orchidectomized male rats. In the present experiments, CPZ administration to EB-pretreated rats significantly increased the EB-pretreated levels of serum PRL in both sexes, but the significant sexual difference was not detected. The sexual difference in PRL secretion visualized by CPZ in gonadectomized rats was blunted by EB pretreatment.

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