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Effects of Psychotropic Drugs on Aldo-Keto Reductase Activity in Rat Ovary and Adrenal Gland

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ABSTRACT. We investigated the effects of minor and major tranquilizers on ovarian and adrenal aldo-keto reductase activity towards five substrates in relation to ovulation in mature cycling rats. Nitrazepam (NZP) did not alter ovarian and adrenal weights or body weight, although ovulation was inhibited at 5 and 10 mg/kg. NZP decreased ovarian 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ (15KD-$PGF_{2\alpha}$) and 4-benzoylpyridine (4BP) reducing activities. None of the doses of zopiclone (ZPC) influenced uterine and adrenal weights or body weight, but it increased ovarian weight at 10 mg/kg. No significant effects of ZPC on ovarian aldo-keto reductase activity were observed. NZP had inhibitory effects on adrenal aldo-keto reductase activity, whereas ZPC had a stimulatory effect. Chlorpromazine (CPZ) did not alter ovarian or adrenal weight, whereas the estrous cycles were abolished at 5 and 10 mg/kg. Reserpine (RSP) decreased ovarian weight and completely inhibited ovulation at 5 and 10 mg/kg, but it increased adrenal weight. Both CPZ and RSP decreased, dose dependently, ovarian aldo-keto reductase activity towards five substrates in agreement with the inhibition of ovulation. On the other hand, differences were found between the effects of CPZ and RSP on adrenal aldo-keto reductase activity. CPZ significantly increased 4BP reducing activity at 5 and 10 mg/kg, although no significant changes were observed in the other four reducing activities. RSP decreased 15KD-$PGF_{2\alpha}$ reducing activity in a dose-dependent manner, whereas the other four activities did not change. BIOCHEM PHARMACOL 52;10:1585–1591, 1996.

KEY WORDS. aldo-keto reductase; psychotropic drug; ovary; adrenal gland; estrous cycle

Carbonyl reduction of biologically and pharmacologically active xenobiotic carbonyl compounds to the corresponding alcohols is generally mediated by the cytosolic NADPH-dependent aldo-keto reductase superfamily [1, 2]. ALR‖ (EC 1.1.1.2) plays some roles in endogenous steroid metabolism, and AR (EC 1.1.1.21) is involved in glucose reduction during diabetic hyperglycemia [2]. CR (1.1.1.184), a member of the carbonyl reducing enzymes, is generally classified in the aldo-keto reductase superfamily for its catalytic function and in the short-chain dehydrogenase superfamily for its primary structure [1–3]. These enzymes have been isolated from a number of species and tissues including human [1, 2]. We have also purified and characterized some isoforms of CR from rat ovary [4], testis and vas deferens [5], adrenal gland [6] and human testis [7], AR from rat ovary [8] and adrenal gland [6], and ALR from rat adrenal gland [6]. Rat ovarian CR activity fluctuates largely during the estrous cycle; that is, the enzyme activity is increased significantly on the day of estrus [9] and is regulated by estrogen and LH [10, 11]. The rat adrenal gland contains six aldo-keto reductases [6], and the reductase activities fluctuate between the days of proestrus and estrus [12]. In addition to these changes, we have suggested that the adrenal enzymes may be regulated by the ACTH-corticosterone axis in rats [13].

It is well documented that many psychotropic drugs inhibit the estrous cycle in females, including ovulatory delay [14–17]. Benzodiazepines have been shown to suppress the estrous cycles in mice [15] and suppress PRL release in rats [18]. CPZ also inhibits ovulation and increases PRL release [17, 19], and RSP increases ACTH secretion as well as inhibition of gonadotropin secretion. In the present study, we evaluated the effects of minor and major tranquilizers on ovarian and adrenal aldo-keto reductase activity towards five substrates in rats.

MATERIALS AND METHODS

Animals

Mature female Wistar-KY rats (7 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and housed in

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‖ *Abbreviations:* ALR, aldehyde reductase; AR, aldose reductase; CR, carbonyl reductase; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; PRL, prolactin; CPZ, chlorpromazine; RSP, reserpine; PNAP, *p*-nitroacetophenone; PNBA, *p*-nitrobenzaldehyde; 4BP,4-benzoylpyridine; NZP, nitrazepam; ZPC, zopiclone; 15KD-$PGF_{2\alpha}$, 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$; 13,14H_2-$PGF_{2\alpha}$ 13,14-dihydroprostaglandin $F_{2\alpha}$; GABA, γ-aminobutyric acid; DTT, dithiothreitol; and CMC, sodium carboxymethyl cellulose.

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group cages (4 or 5 rats/cage) under controlled conditions of temperature (24°) and light (12 hr on, 12 hr off). Food and water were always available. Rats exhibiting regular 4-day estrous cycles were used for the experiments.

Chemicals

NADPH was obtained from the Oriental Yeast Co. (Tokyo, Japan). PNAP, PNBA, menadione, 4BP, DTT, EDTA and CMC were purchased from the Wako Pure Chemicals Co. (Osaka, Japan), and CPZ and RSP were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). NZP and ZPC were provided by the Rhone-Poulenc Rorer Co. (Tokyo, Japan). [5,6,8,9,11,12,14-^{3}H]-15KD-$PGF_{2\alpha}$ (sp. act. 80 Ci/mmol) was obtained from Amersham Int. plc (Buckinghamshire, UK) and authentic 15KD-$PGF_{2\alpha}$ from Upjohn Pharmaceutical Ltd. (Kalamazoo, MI, U.S.A.). Authentic 13,14H_2-$PGF_{2\alpha}$ was provided by the Ono Pharmaceutical Co. (Osaka, Japan). Other chemicals of reagent grade were obtained from the Wako Pure Chemicals Co. and the Bio-Rad Co. (Tokyo, Japan).

Drug Treatments

NZP, ZPC, and RSP were suspended in 0.5% CMC solution, respectively, and CPZ was dissolved in distilled water. Each drug (1, 5, and 10 mg/kg) was administered orally to rats once daily for 3 days from the first day of diestrus, and control rats were given orally the vehicle alone. The ovaries and adrenal glands of each rat were isolated at 9:00 a.m. on the day of expected estrus, and homogenized in 8 mL of ice-cold 10 mM phosphate buffer (pH 6.5) containing 0.154 M KCl, 1 mM DTT and 0.5 mM EDTA. The presence of ova in the oviduct was determined microscopically.

Enzyme Assay

The ovarian and adrenal homogenates were centrifuged at 4° for 60 min at 105,000 *g* by an Hitachi Automatic Centrifuge 70P-72, and the 105,000 *g* supernatant (cytosolic fraction) obtained was used as a crude enzyme preparation for the assay of enzyme activity. The reduction of PNAP (1 mM), PNBA (1 mM), menadione (0.2 mM) and 4BP (1 mM), respectively, was assayed in 1 mL of incubation mixture, consisting of 100 mM phosphate buffer (pH 6.5), cytosol, substrate solution, and NADPH (0.1 mM), for 3 min at 37° by an Hitachi 150-20 Spectrophotometer. One unit of enzyme activity was expressed as the amount of enzyme that oxidized 1 μmol of NADPH/min at 340 nm under the assay conditions. 15KD-$PGF_{2\alpha}$ reducing activity was determined by a radiochemical method as previously described [20] and expressed as picomoles per milligram of protein per 15 minutes of 13,14H_2-$PGF_{2\alpha}$ formed. Protein concentration in the ovarian and adrenal cytosol was determined by the method of Lowry *et al.* [21].

Statistical Analysis

All results are expressed as means ± SEM. Duncan's multiple range test was used for comparison between groups, with a P value of 0.05 or less considered to indicate a significant difference.

RESULTS

Effects of NZP and ZPC

Changes in body weight, and ovarian, adrenal, and uterine weights in relation to ovulation after treatment with NZP and ZPC are summarized in Table 1. No significant changes in body weight or adrenal weight were observed by treatment with both minor tranquilizers. NZP at 1 and 10 mg/kg increased uterine weight, and 5 mg/kg of NZP decreased significantly the number of ova, although ovarian weight was not influenced by this drug. One of five rats administered 5 mg/kg of NZP and two of five rats given 10 mg/kg of NZP were anovulatory, and the vaginal smears of these rats were diestrous. None of the doses of ZPC altered adrenal or uterine weights or the number of ova, although 10 mg/kg of ZPC significantly increased ovarian weight.

Figure 1 shows the effects of NZP and ZPC on ovarian aldo-keto reductase activity towards five substrates. 15KD-$PGF_{2\alpha}$ reducing activity was decreased to about 70% of control levels after NZP treatment. 4BP reducing activity was also decreased to about 60% after NZP. However, in anovulatory rats treated with 5 and 10 mg/kg of NZP, both enzyme activities were lower than those in ovulatory rats of the same group. ZPC had no significant effect on either 15KD-$PGF_{2\alpha}$ or 4BP reducing activities in the ovary. PNBA reducing activity was not altered by treatment with these psychotropic drugs.

Different effects between NZP and ZPC on adrenal aldo-keto reductase activity were observed (Fig. 2). NZP decreased adrenal aldo-keto reductase activity towards five substrates to 40–90% of the control levels. In contrast to the inhibitory effects of NZP, ZPC markedly increased each reducing activity to 1.2–1.5 times of the control levels.

Effects of CPZ and RSP

Table 2 summarizes the effects of CPZ and RSP on body weight, and ovarian, adrenal, and uterine weights in relation to ovulation. CPZ did not alter body weight or ovarian or adrenal weights at any of the doses tested, whereas the estrous cycle was lowered by treatment with 5 and 10 mg/kg. CPZ inhibited ovulation in one of four rats at 5 mg/kg and completely inhibited it at 10 mg/kg. A significant decrease in body weight was observed during the administration of 5 and 10 mg/kg of RSP. Furthermore, both ovarian and uterine weights also declined significantly with 5 and 10 mg/kg of RSP, whereas adrenal weight was increased. Ovulation in two of five rats was inhibited at 1 mg/kg of RSP, and ovulation was completely inhibited at 5 and 10 mg/kg.

Both CPZ and RSP decreased, dose dependently, ovarian

TABLE 1. Effects of NZP and ZPC on body weight, and ovarian, adrenal, and uterine weights in relation to ovulation

	Body wt (g)	Ovarian Wt (mg)	Adrenal wt (mg)	Uterine wt (mg)	No. of ova (ovulated rats)
Control (5)	194.6 ± 1.99	80.6 ± 1.69	83.6 ± 2.01	271.0 ± 6.14	13 ± 0.3 (5/5)
NZP					
1 mg/kg (5)	200.6 ± 3.23	87.2 ± 3.00	80.8 ± 3.72	302.0 ± 5.09*	12 ± 0.4 (5/5)
5 mg/kg (5)	193.4 ± 5.17	82.4 ± 2.50	87.2 ± 1.93	280.0 ± 15.17	8 ± 1.8† (4/5)
10 mg/kg (5)	201.0 ± 4.43	82.2 ± 5.03	85.4 ± 3.36	312.0 ± 12.63†	14 ± 1.7 (3/5)
Control (4)	195.3 ± 4.06	73.7 ± 2.33	81.3 ± 1.45	297.3 ± 15.08	11 ± 0.7 (4/4)
ZPC					
1 mg/kg (4)	200.0 ± 4.32	81.0 ± 2.48	84.5 ± 3.75	308.8 ± 6.77	10 ± 0.8 (4/4)
5 mg/kg (4)	206.3 ± 1.65	82.8 ± 3.79	87.3 ± 2.21	324.5 ± 2.50	12 ± 0.5 (4/4)
10 mg/kg (5)	204.4 ± 3.20	86.6 ± 1.44*	87.3 ± 2.06	315.0 ± 4.37	10 ± 1.4 (5/5)

NZP and ZPC, respectively, were suspended in 0.5% CMC solution, and administered orally to rats for 3 days from the first day of diestrus. Rats were killed on the day of expected estrus. Each value is the mean ± SEM.

* $P < 0.001$ vs control.

† $P < 0.05$ vs control.

aldo-keto reductase activity towards five substrates (Fig. 3). At a dose of 10 mg/kg, CPZ decreased 15KD-PGF$_{2\alpha}$ reducing activity to 74% of control levels, PNBA to 70%, menadione to 63%, and 4BP to 51%. Similarly, RSP decreased 15KD-PGF$_{2\alpha}$ reducing activity to 54%, PNAP to 25%, PNBA to 37%, menadione to 60%, and 4BP to 27%.

The effects of CPZ and RSP on adrenal aldo-keto reductase activity were different from those on ovarian enzyme activity (Fig. 4). CPZ did not exert any influence on 15KD-PGF$_{2\alpha}$, PNAP, PNBA, or menadione reducing activity in the adrenal gland, whereas 4BP reducing activity was increased significantly. On the other hand, no significant changes in PNAP, PNBA, menadione, or 4BP reducing activity were observed at any of the RSP doses tested; how-

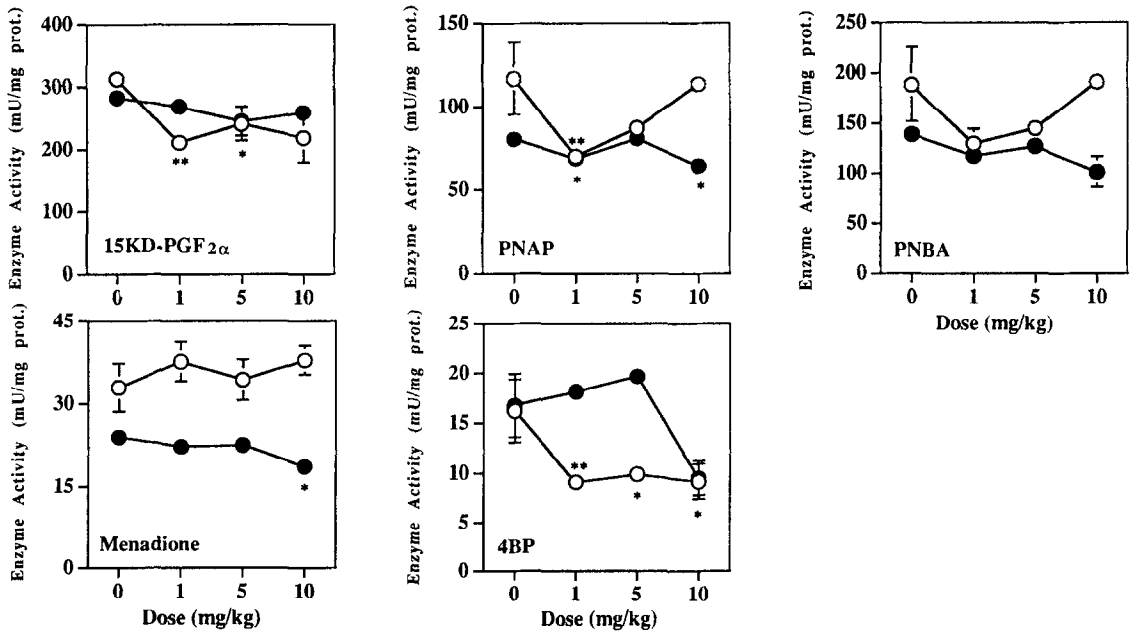

FIG. 1. Effects of NZP and ZPC on ovarian aldo-keto reductase activity towards five substrates in rats. The experimental conditions are the same as described in the legend of Table 1. Each point is the mean ± SEM of 4–5 rats. Key: (○) NZP-treated, and (●) ZPC-treated; (*)$P < 0.05$, and (**)$P < 0.01$ vs control (0 mg/kg).

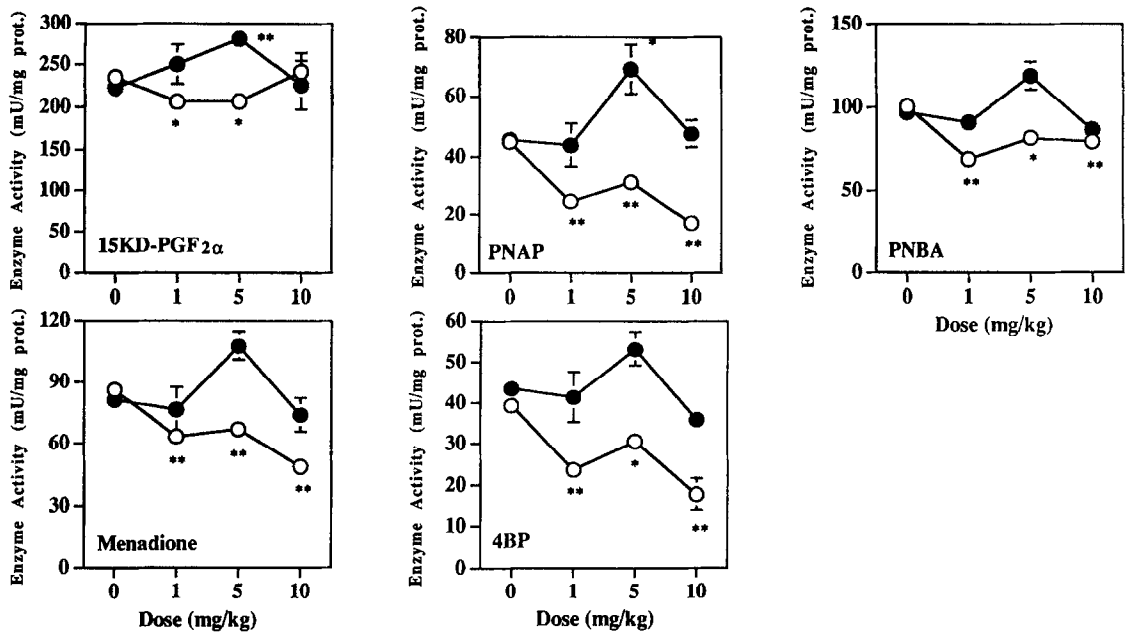

FIG. 2. Effects of NZP and ZPC on adrenal aldo-keto reductase activity towards five substrates in rats. The experimental conditions are the same as described in the legend of Table 1. Each point is the mean ± SEM of 4–5 rats. Key: (○) NZP-treated, and (●) ZPC-treated; (*)$P < 0.05$, and (**)$P < 0.01$ vs control (0 mg/kg).

ever, 15KD-$PGF_{2\alpha}$ reducing activity was decreased significantly to 56% of the control at 10 mg/kg.

DISCUSSION

Minor and major tranquilizers are widely prescribed as sedatives, and recently benzodiazepines are the most prescribed. Both NZP and ZPC are used clinically at a dose between 5 and 10 mg, CPZ between 30 and 100 mg, and RSP between 0.3 and 2.5 mg. In the present study, female rats were administered 1–10 mg/kg of these psychotropic drugs orally.

Whereas central benzodiazepine receptors are coupled with the GABA receptor and chloride channel, peripheral receptors are not coupled to GABA receptors and exhibit

TABLE 2. Effects of CPZ and RSP on body weight, and ovarian, adrenal, and uterine weights in relation to ovulation

	Body wt (g)	Ovarian wt (mg)	Adrenal wt (mg)	Uterine wt (mg)	No. of ova (ovulated rats)
Control (4)	196.3 ± 1.03	76.8 ± 2.59	84.8 ± 1.31	289.0 ± 3.39	11 ± 0.3 (4/4)
CPZ					
1 mg/kg (4)	195.8 ± 6.64	78.3 ± 5.30	80.0 ± 2.04	304.8 ± 10.85	10 ± 0.3 (4/4)
5 mg/kg (4)	198.3 ± 2.59	71.5 ± 1.19	82.3 ± 2.06	307.0 ± 5.74*	11 (1/4)
10 mg/kg (4)	198.0 ± 2.94	70.8 ± 4.29	85.0 ± 1.47	293.5 ± 13.57	0 (0/4)
Control (5)	199.8 ± 2.06	78.8 ± 2.08	81.2 ± 1.66	298.8 ± 5.17	11 ± 0.6 (5/5)
RSP					
1 mg/kg (5)	197.8 ± 5.76	71.0 ± 3.49	85.6 ± 3.34	264.2 ± 29.46	10 ± 0.6 (3/5)
5 mg/kg (5)	187.2 ± 3.60*	61.4 ± 2.11†	93.8 ± 4.04*	209.6 ± 8.45†	0 (0/5)
10 mg/kg (5)	157.6 ± 2.18†	55.4 ± 1.83†	95.0 ± 5.93	166.2 ± 6.94†	0 (0/5)

CPZ was dissolved in distilled water and RSP was suspended in 0.5% CMC solution, and each drug was administered orally to rats for 3 days from the first day of diestrus. Rats were killed on the day of expected estrus. Each value is the mean ± SEM.

* $P < 0.05$ vs control.

† $P < 0.01$ vs control.

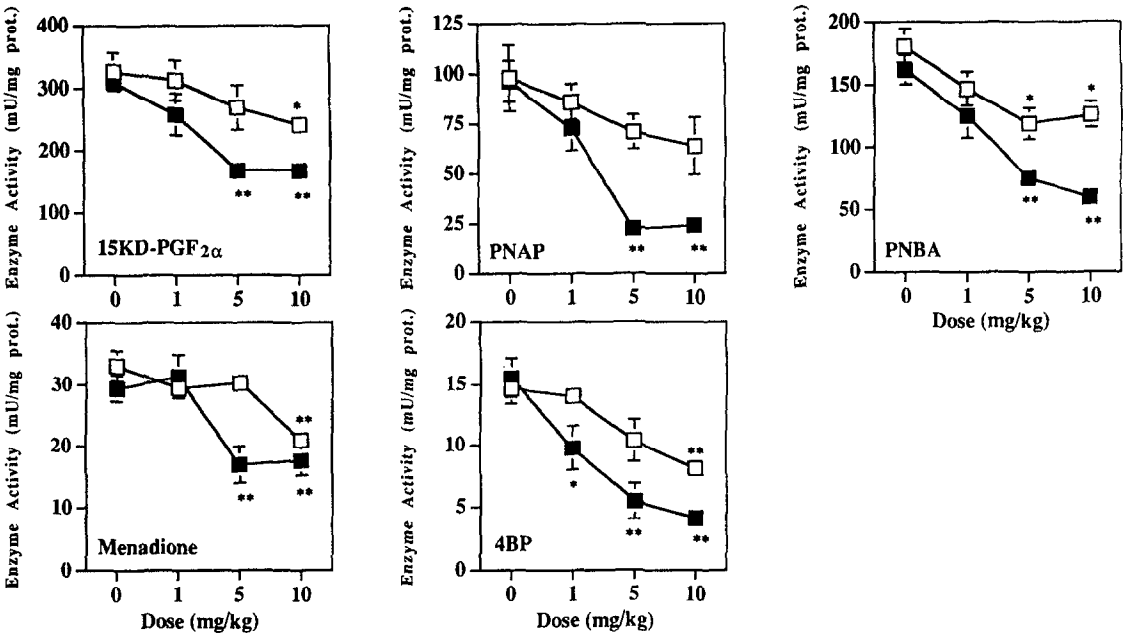

FIG. 3. Effects of CPZ and RSP on ovarian aldo-keto reductase activity towards five substrates in rats. The experimental conditions are the same as described in the legend of Table 2. Each point is the mean ± SEM of 4–5 rats. Key: (□) CPZ-treated, and (■) RSP-treated; (*)$P < 0.05$, and (**)$P < 0.01$ vs control (0 mg/kg).

distinctly different ligand specificity. These receptors are detected in various tissues including the ovary, oviduct, uterus, pituitary, testis, adrenal gland, and kidney [22–24]. Fares *et al.* [22, 25] have reported that the concentrations of peripheral benzodiazepine binding sites of the ovary and uterus, but not kidney, of female rats are elevated by sexual maturation, exposure to gonadotropin, and cyclic increases of estradiol and progesterone. Although the relationship between the dose of NZP and uterine weight is not clear at present, we have considered that the effect of this drug on uterine weight may be influenced by the dose administered. Namely, 1 mg/kg of NZP may directly increase uterine weight, as ovulation was not inhibited and the estrous cycle was not lowered. At 5 and 10 mg/kg, ovulation was inhibited in a few animals, and 10 mg/kg of NZP lowered the estrous cycle. The increase in uterine weight at 10 mg/kg

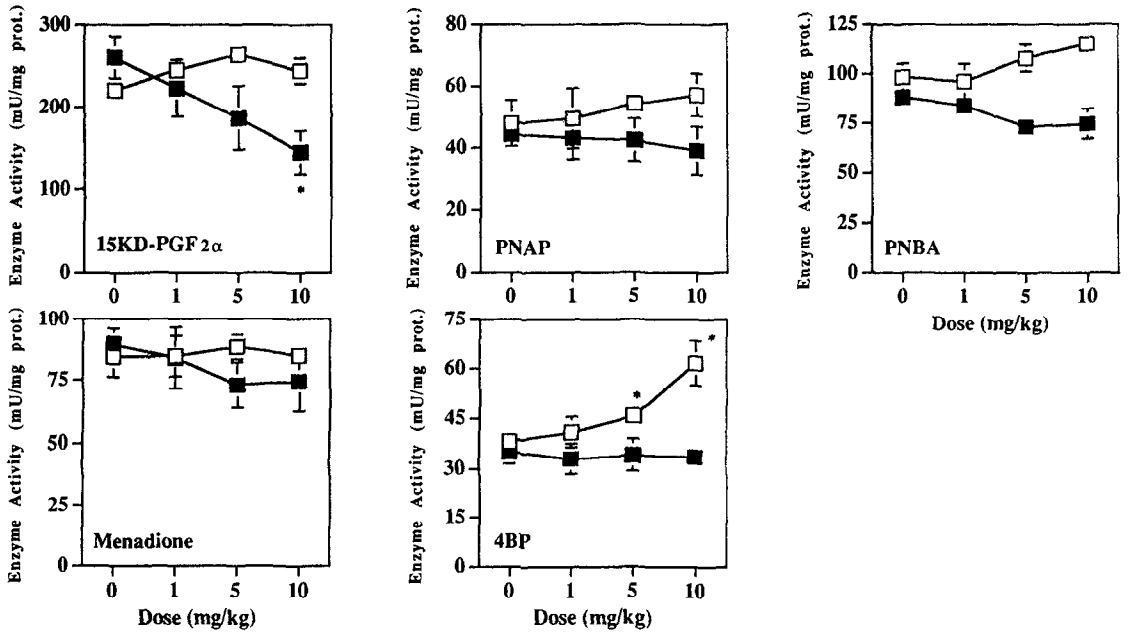

FIG. 4. Effects of CPZ and RSP on adrenal aldo-keto reductase activity towards five substrates in rats. The experimental conditions are the same as described in the legend of Table 2. Each point is the mean ± SEM of 4–5 rats. Key: (□) CPZ-treated, and (■) RSP-treated; (*)$P < 0.05$ vs control (0 mg/kg).

was due to an increase in the uterine luminal fluid, but 5 mg/kg of this drug did not cause the increase in luminal fluid. These results suggest that 1 mg/kg of NZP may reveal peripheral action, and the higher dose may influence uterine weight by mediating the central action. On the other hand, it seems that the decrease in the number of ova at 5 mg/kg may be caused by a decrease in Graafian follicles, although the action at this dose of NZP remains unclear. The ovarian 15KD-$PGF_{2\alpha}$ and 4BP reducing activities, which reflect rat ovarian CR activity and are LH dependent [8–10, 13], were decreased without relation to ovulation. The results of the present study indicate that the effects of NZP on both of the above ovarian enzyme activities may occur via peripheral benzodiazepine receptors, and Calvo *et al.* [26] have reported that plasma testosterone levels in male rats are altered by NZP independently of changes in LH levels. Furthermore, the inhibition of ovulation by NZP may be due to the suppression of preovulatory LH surge. Accordingly, it is possible that benzodiazepine has both a direct and indirect action on ovarian function by mediating peripheral benzodiazepine receptors and central receptors. On the other hand, the results indicating that the ovarian PNAP, PNBA, and menadione reducing activities were not influenced by NZP as compared with changes in the other two reducing activities suggest the presence of a regulatory mechanism other than both the central and peripheral benzodiazepine receptors in rat ovary. The suppression of all reducing activities in the adrenal gland by all doses of NZP without inhibition of adrenal weight suggests that peripheral benzodiazepine receptors in the adrenal gland may be closely involved in the inhibitory regulation of aldo-keto reductase.

ZPC is one of the sedatives different from benzodiazepine, but this drug has the same properties as benzodiazepines in the CNS [27, 28]. Little, however, is known about the relationship between ZPC and peripheral benzodiazepine receptors. Ovarian weight was increased by ZPC, and, in particular, significantly increased by 10 mg/kg, although body weight and adrenal and uterine weights were not altered and ovulation completely occurred with all doses of ZPC. The ovarian reductase activity towards PNAP, menadione, and 4BP was decreased differently from the effect of NZP, but the respective changes in these activities were small. Furthermore, the adrenal reductase activity was increased at a dose of 5 mg/kg of ZPC, and the increase in both 15KD-$PGF_{2\alpha}$ and PNAP reducing activities was significant. It is suggested that ZPC has a mainly direct action on the ovary and adrenal gland in rats as far as aldo-keto reductase activity is concerned, but this is not mediated by peripheral benzodiazepine receptors, as the effects of ZPC on ovarian weight, ovulation, and aldo-keto reductase activities were different from those of NZP.

CPZ inhibited ovulation at 5 and 10 mg/kg without decreasing either ovarian or adrenal weights, and the luminal fluid remained in the uterus. This indicates that the preovulatory LH surge is inhibited completely by CPZ. In addition, CPZ causes an increase in PRL release by blockade of the dopaminergic receptor [19, 29]. As Kogo *et al.* [17] reported that the ovulation inhibited by CPZ is restored in 60% of rats treated with bromocryptine, an increase in PRL secretion also may be involved in anovulation in rats treated with CPZ. The decrease in both 15KD-$PGF_{2\alpha}$ and 4BP reducing activities in the ovary is due to inhibition of the preovulatory LH surge by CPZ. Although both PNBA and menadione reducing activities were also decreased, this may be different from changes in 15KD-$PGF_{2\alpha}$ and 4BP reducing activities. We have already observed that the ovarian reductase activity towards PNBA and menadione is not correlated with the action of gonadotropins and ovarian steroids [30–32]. Accordingly, it is suggested that PRL may be directly, at least in part, involved in the decrease in both PNBA and menadione reductions. On the other hand, the finding that only 4BP reducing activity was increased significantly in the adrenal gland may mean that it participates in ACTH secretion decreased by CPZ, although the adrenal weight did not change with any dose of CPZ tested.

The dose-dependent decrease in body weight after RSP treatment suggests a general toxicity of this drug. Although RSP decreases gonadotropin secretion, this drug increases PRL and ACTH secretion differently from the action of CPZ on the anterior pituitary hormone. Our data showing that ovarian weight was decreased, that adrenal weight was increased, and that ovulation did not occur were also associated with the effect of RSP. RSP revealed inhibition of ovulation at 1 mg/kg, and this inhibition by RSP was the most powerful action of the psychotropic drugs tested. As was expected, both 15KD-$PGF_{2\alpha}$ and 4BP reducing activities in the ovary were decreased in accordance with inhibition of the preovulatory LH surge of RSP. The results indicating that the other three activities were also inhibited by RSP may be due to a different action, such as an increase in PRL secretion, as described for CPZ action. We have reported that glucocorticoid treatment, which inhibits ACTH secretion, increases adrenal 15KD-$PGF_{2\alpha}$ reducing activity in female rats, and suggested that ACTH demonstrates an inhibitory effect on this activity [13]. Accordingly, it is suggested that 15KD-$PGF_{2\alpha}$ reducing activity in the adrenal gland may be decreased by the increase in ACTH secretion after RSP treatment. It is obvious that reductase systems for the other four activities are not influenced by RSP.

In conclusion, NZP and ZPC, both minor tranquilizers, influenced ovarian and adrenal aldo-keto reductase activities with peripheral and central actions, and CPZ and RSP, both major tranquilizers, influenced these reductase activities with action on the CNS.

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