British Journal of Pharmacology (2001) 134, 1328–1334

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Effects of carbamazepine on the first ovulation in gonadotropin-primed immature female rats

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1 The effects of carbamazepine (CBZ) and its possible mechanisms on the first ovulation were investigated in immature female rats. The first ovulation was induced by administration of equine chorionic gonadotropin (eCG) at 0800 h at 26 days of age.

2 A single s.c. injection of 360 mg kg^{-1} CBZ at 1300 h on the first pro-oestrus (day 28) completely inhibited the first ovulation on the morning of day 29. A marked elevation in 13, 14-dihydro-prostaglandin $F_{2\alpha}$ (13, 14H_2-$PGF_{2\alpha}$) forming capacity, a sensitive indicator of luteinizing hormone (LH) surge, was not detected in the CBZ-treated group at 0800 h on day 29 (72 h after eCG treatment). The elevation in serum LH levels at 57 h after eCG treatment was not observed in the CBZ-treated group, either. The blocking of the first ovulation and 13, 14H_2-$PGF_{2\alpha}$ forming capacity were recovered by an i.p. injection of human CG on day 28 in all animals.

3 However, the first ovulation was not blocked by repeated injections of 360 mg kg^{-1} CBZ at 1300 h once daily for 3 days (days 26–28). The repeated injections of CBZ caused a great fall (64% decrease) in the serum levels of CBZ at 4 h after the final CBZ injection as compared with the case of a single injection of CBZ and resulted in a delay for 5 h the occurrence of LH surge, which is normally observed around 57 h after eCG injection.

4 A significant increase in the activity of microsomal CBZ catabolism by the repeated injections of CBZ was quantitatively verified by the HPLC analysis. But, the activity of CBZ metabolism in the single injected-animals showed almost similar levels to that in the control.

5 The present results demonstrated that a single injection of CBZ blocks the ovulation by inhibiting LH surge but that the failure of the inhibition of ovulation by repeated injections of CBZ is due to a decrease in serum CBZ levels mediated through CBZ-induced hepatic enzyme induction. *British Journal of Pharmacology* (2001) **134,** 1328–1334

Keywords: Carbamazepine; ovulation; 13, 14-dihydro-prostaglandin $F_{2\alpha}$ (13, 14H_2-$PGF_{2\alpha}$); luteinizing hormone (LH)

Abbreviations: CBZ, Carbamazepiner; DHEAS, dehydroepiandrosterone sulphate; 15KD-$PGF_{2\alpha}$, 13, 14-dihydro-15-keto-$PGF_{2\alpha}$; 13, 14H_2-$PGF_{2\alpha}$, 13, 14-dihydro-prostaglandin $F_{2\alpha}$; DMSO, dimethyl sulphoxide; eCG, equine chorionic gonadotropin; LH, luteinizing hormone; PRL, prolactin; SHBG, serum sex hormone binding globulin; TRH, thyrotropin

Introduction

Carbamazepine (CBZ) is one of the antiepileptic drugs which is most widely used for patients with epilepsy. It has been reported that women with epilepsy often have anovulatory cycles (Mattson *et al*., 1985; Herzog *et al*., 1986; Isojarvi, 1990; Morrell, 1998), although it is not clearly demonstrated whether the disorder is due to epilepsy itself and/or to antiseizure medications. There are some clinical reports that antiepileptic drugs alter serum sex hormone levels (Levesque *et al*., 1986; Isojarvi, 1990; Isojarvi *et al*., 1995) and the function of the hypothalamic–pituitary axis (Dana-Haeri *et al*., 1984; Isojarvi, 1990). Serum sex hormone binding globulin (SHBG) levels increased, and dehydroepiandrosterone sulphate (DHEAS) levels and calculated free androgen index (FAI) values decreased, in female patients after 2 months of CBZ treatment (Isojarvi, 1990). Dana-Haeri *et al*. (1984) have reported increases in prolactin (PRL) responses to thyrotropin-releasing hormone (TRH) and in luteinizing hormone (LH) responses to LH-releasing hormone (LH-RH) in female epileptic patients receiving CBZ therapy. The PRL response to metoclopramide was enhanced after CBZ treatment in male patients with epilepy (Isojarvi *et al*., 1989). Further, a direct inhibitory effect of CBZ on the steroidogenesis in reproductive tracts has also been suggested (Kuhn-Velten *et al*., 1990). Our recent data showed that administration of phenytoin, which increases serum levels approximately double those reached with the clinical dose of this anticonvulsant drug for humans, inhibits uterine development as well as the first ovulation induced by gonadotropin (Tamura *et al*., 2000). But there are no available data so far concerning the acute effects of CBZ on ovulation and the fluctuation of reproductive hormone in experimental animals.

Ovulation depends upon the preovulatory surge of LH which is triggered by a positive feedback mechanism *via* the

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elevation of oestrogen levels in connection with the growth of ovarian follicles. An enormously increased synthesis of prostaglandin is induced in granulosa cells of preovulatory follicles by LH surge (Hedin *et al*., 1987). In rat ovary, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is converted from 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ (15KD-$PGF_{2\alpha}$) *via* 15-keto-$PGF_{2\alpha}$ to 13, 14-dihydro-$PGF_{2\alpha}$ (13, $14H_2$-$PGF_{2\alpha}$) (Aizawa *et al*., 1980; Inazu *et al*., 1983). We have previously shown that the forming capacity for 13, $14H_2$-$PGF_{2\alpha}$ is stimulated by LH, and is useful as a sensitive indicator of appearance of LH surge in rats (Kogo *et al*., 1986; Tamura & Kogo, 1989). It may be associated with the process of ovulation (Kogo *et al*., 1992). The present study was designed to characterize the action of CBZ on the first ovulation and its possible mechanisms in the eCG-primed ovulation model using ovarian 13, $14H_2$-$PGF_{2\alpha}$ formation as an indicator of LH secretion.

Methods

Induction of the first ovulation

Immature female rats of the Wistar-Imamichi strain from the Imamichi Institute for Animal Reproduction (Ibaraki-ken, Japan) were used. The rats were maintained in temperature ($23\pm1^{\circ}C$), humidity ($55\pm5\%$) and light (12 h light/day) controlled quarters. To induce the first ovulation, the animals were given a single s.c. injection of 5 IU eCG in 0.2 ml saline at 0800 h at 26 days of age. These animals ovulated an average of nine oocytes on the morning of day 29. The number of oocytes was ascertained by examining in the ampulla of oviducts using a dissecting microscope.

Administration of CBZ

Immature rats pretreated with eCG on day 26 were given a single s.c. injection of CBZ ($72-720$ mg kg^{-1}) in 0.2 ml of dimethyl sulphoxide (DMSO) at 1300 h on day 28 or repeated s.c. injections of CBZ ($360-720$ mg kg^{-1}) at 1300 h once daily for 3 days (days 26–28). Animals for control group were injected the same volume of DMSO. Administration of 10 IU human CG (i.p.) to the CBZ-treated animals were performed at 1700 h on day 28.

Assay of the forming capacity of 13, $14H_2$-$PGF_{2\alpha}$ in the ovaries

Animals were killed at 1700 h on day 28 (57 h after eCG injection) and at 0800 h on day 29 (72 h after eCG treatment) under ether-anaesthesia. Ovaries were immediately removed and stored frozen in liquid nitrogen until assay for 13, $14H_2$-$PGF_{2\alpha}$ forming capacity. The forming capacity was measured by determining the conversion of [^{3}H]-15KD-$PGF_{2\alpha}$ to [^{3}H]-13, $14H_2$-$PGF_{2\alpha}$ using the cytosol fraction obtained by centrifugation of ovarian homogenates for 20 min at $12{,}000\times g$ as described before (Kogo *et al*., 1992).

Measurement of the CBZ levels in serum

The levels of CBZ in serum were measured by the simple and accurate high-performance liquid chromatographic method reported by Kouno *et al*. (1993). This method contains the system of a silica-gel column using a syringe-type mini-column, named Extrashot-Silica, packed with diatomaceous earth granules.

Radioimmunoassay (RIA) of gonadotropin (LH and FSH)

Blood was collected *via* the abdominal aorta under ether-anaesthesia at 1700 h on day 28 (57 h after eCG treatment) and allowed to clot at room temperature. Serum was separated by centrifugation and stored at $-80^{\circ}C$ until assay for the serum levels of gonadotrophin. LH and FSH levels were assayed with RIA kits supplied by NIDDK. Iodinated preparations were made from rat FSH-I-8 and LH-I-9. Anti-rat FSH-S-11 and anti-rat LH-S-10 were used as antisera. Results were expressed as NIDDK rat FSH-RP-2 and LH-RP-2. The intra- and inter-assay coefficients of variation were 5.7 and 20.4% for FSH and 8.6 and 9.8% for LH, respectively. The assays have been described previously (Tamura *et al*., 1998).

Assay of enzymatic activity of CBZ metabolism

The activity of CBZ catabolism in hepatic microsome was determined by assessment of conversion of CBZ to CBZ 10′ 11′-epoxide. Preparation of microsomes was performed as previously described (Hosokawa *et al*., 1989). An assay mixture consisting of 50 μl of 500 mM potassium phosphate buffer (pH 7.4), 25 μl of 1 mM EDTA, 25 μl of NADPH generating system including 0.5 mM $NADP^+$, 2.0 mM glucose-6-phosphate, 1 IU/ml of glucose-6-phosphate dehydrogenase and 4 mM $MgCl_2$, 25 μl of rat liver microsomes at a suitable concentration, and CBZ solution (25 μl of 500 μM in methanol solution and then evaporated, final concentration of 50 μM solution) was incubated at $37^{\circ}C$ for 30 min. The reaction was performed in the linear range with respect to protein concentration and incubation time. After the reaction was stopped by addition of 200 μl of cold acetonitril, the mixture was centrifuged for 15 min at $10{,}000\times g$, and 100 μl of the supernatant was analysed by HPLC as described below (Kushida *et al*., 1983). CBZ 10′ 11′-epoxide was determined using the following HPLC method. The HPLC system consisted of a model L-6000 pump (Hitachi, Tokyo, Japan), a model L4000H UV detector (Hitachi), a model AS-2000 autosampler (Hitachi), a model D-2500 integrator (Hitachi), and a 4.6×150 mm Capcell pack C18 UG 120 column (Shiseido, Tokyo, Japan). For the determination of CBZ 10′ 11′-epoxide, the mobile phase consisted of H_2O: methanol (60 : 40, v v^{-1}) and was delivered at a flow rate of 1.0 ml/min. The eluate was monitored at a wavelength of 210 nm. A calibration curve was generated from 1.25 to 10 μM CBZ 10′ 11′-epoxide and phenacetine as an internal standard.

Statistics

Results are given as means $\pm$ s.e.mean. The significance of the differences between means was tested with unpaired Student's *t*-test or Cochran-Cox test (2-tailed). Differences with P value less than 0.05 were considered statistically significant.

Drugs

[5,6,8,11,12,14(n)-^{3}H]-15KD-$PGF_{2\alpha}$ (7.73 TBq/mmol) was purchased from Amersham Pharmacia Biotech U.K., Ltd. (Buckinghamshire, England). NADPH and DMSO were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Equine chorinonic gonadotrophin (eCG, PMSG; Serotropin) and human chorionic gonadotrophin (human CG, Gonatropin) were obtained from Teikoku Hormone MFG Company (Tokyo, Japan). Carbamazepine (CBZ) was donated by Fujinaga Pharmaceutical Co., Ltd. (Tokyo, Japan).

Results

Effects of CBZ on the first ovulation and the weights of reproductive organs

Effects of CBZ on the first ovulation induced by eCG injection were determined in immature female rats (Table 1). When a single injection of 360 or 720 mg kg^{-1} CBZ at 1300 h at 28 days of age was given to animals treated with eCG, the occurrence of the first ovulation was perfectly blocked in all rats. However, in the animals repeatedly treated with 360 mg kg^{-1} CBZ at 1300 h once daily for 3 days (days 26–28), the ovulation occurred normally in all animals. Table 2 shows the effect of CBZ on ovarian and uterine weight in eCG-primed rats. Ovarian weights showed a significant decrease in the rats whose first ovulation was blocked by CBZ treatment. But uterine weight showed a significant increase, as compared with control.

The mechanism of the block of ovulation induced by a single injection of CBZ

Figure 1 shows the effects of CBZ on ovarian 13, $14H_2$-$PGF_{2\alpha}$ forming capacity around the time of LH surge (57 h) and just after the time of ovulation (72 h) in eCG-primed rats. The 13, $14H_2$-$PGF_{2\alpha}$ forming capacity was markedly enhanced at 72 h after eCG treatment in control rats, indicating the occurrence of LH surge on the first pro-oestrus (day 28). The enhancement in the forming capacity

Table 1 Effects of carbamazepine on the first ovulation in eCG-primed immature rat

Treatment (mg kg^{-1} × days)	*Ovulating rats/ rats examined*	*No. of oocytes in ovulating rats*
Control	6/6	9.0±0.78
CBZ (72×1)	4/5	8.5±0.89
(144×1)	3/6	7.7±1.67
(360×1)	0/10	0
(720×1)	0/7	0
(360×3)	5/5	8.4±0.98
(720×3)	2/5	8.5

Immature female rats were given a single injection of eCG (5 IU) at 0800 h at 26 days of age. Carbamazepine (CBZ; 72–720 mg kg^{-1}) was injected 53 h (1300 h on day 28) after eCG treatment or 1300 h once daily for 3 days (days 26–28). Animals were sacrificed at 0800 h on day 29. The number of ovulation was determined by counting oocytes in the oviduct (means±s.e.mean of 5–10 rats).

Table 2 Effects of carbamazepine on the weights of the reproductive tracts in eCG-primed immature rats

Groups	*Ovarian weight* (mg)	*Uterine weight* (mg)
Control	38.1±0.95	158.5±5.09
CBZ (360×1)	27.1±1.68***	200.1±7.35***
(720×1)	22.9±0.79***	210.0±6.21***
(360×3)	35.6±0.98	160.4±4.71

Treatments with eCG and carbamazepine (CBZ) were carried out as described in Table 1, and animals were killed at 0800 h on day 29 (72 h after eCG treatment). Each value shows means±s.e.mean of 5–10 rats. ***$P<0.001$; *vs* eCG.

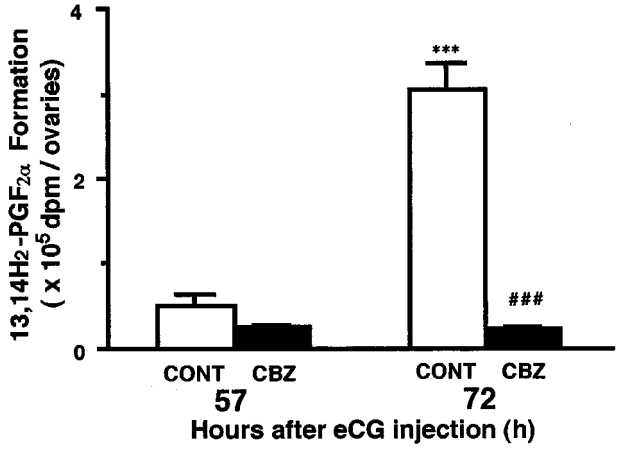

Figure 1 Effects of carbamazepine on ovarian 13, $14H_2$-$PGF_{2\alpha}$ forming capacity in eCG-primed immature rats. Immature female rats were given a single injection of eCG (5 IU) at 0800 h at 26 days of age. Carbamazepine (CBZ; 360 mg kg^{-1}) was injected s.c. at 1300 h on day 28. Ovaries were removed at 1700 h on day 28 (57 h after eCG) or at 0800 h on day 29 (72 h after eCG) to measure 13, $14H_2$-$PGF_{2\alpha}$ forming capacity. Each value shows means±s.e.mean of 4–7 rats. ***$P<0.001$; *vs* Control (CONT) at 57 h after eCG injection. ###$P<0.001$; *vs* Control (CONT) 72 h after eCG injection.

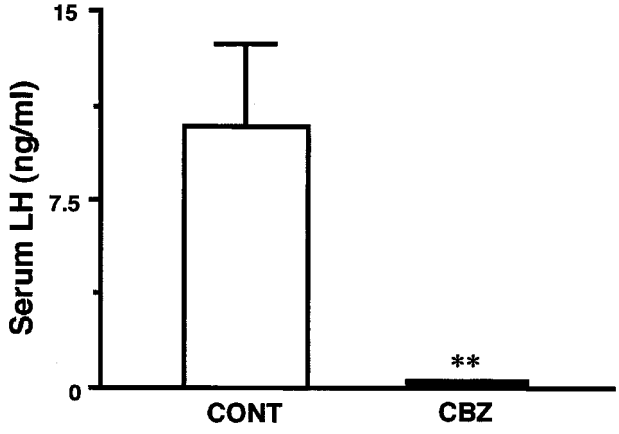

Figure 2 Effects of carbamazepine on serum LH levels in eCG-primed immature rats. Each treatment with eCG and/or carbamazepine (CBZ) was carried out as described in Table 1, and blood was collected at 1700 h on day 28 (57 h after eCG treatment). Each value shows means±s.e.mean of five rats. **$P<0.01$; *vs* Control (CONT).

disappeared with a single injection of 360 mg kg^{-1} CBZ at 1300 h on day 28. Actually, as shown in Figure 2, LH surge at 57 h after eCG treatment was not seen in CBZ-injected animals. The CBZ-induced inhibitions of the first ovulation and 13, 14H_2-$PGF_{2\alpha}$ forming capacity were completely restored by a single injection of hCG at 1700 h on 28 days of age (57 h after eCG) (Table 3 and Figure 3).

The mechanism of the appearance of ovulation in animals repeatedly treated with CBZ

Effects of CBZ, which was given three times, on ovarian 13, 14H_2-$PGF_{2\alpha}$ forming capacity and serum LH levels were examined (Figure 4). The increase in 13, 14H_2-$PGF_{2\alpha}$ forming capacity was recognized in the CBZ (360 mg $kg^{-1} \times 3$) group (Figure 4a), although the preovulatory LH surge at 57 h after eCG injection was not observed in the CBZ (360 mg $kg^{-1} \times 3$)-treated group as well as the CBZ (360 mg $kg^{-1} \times 1$) group (Figure 4b). These results show that the preovulatory LH surge have been induced between 57 and 64 h to cause the ovulation. To know the reason why the elevation in ovarian 13, 14H_2-$PGF_{2\alpha}$ forming capacity was seen in the CBZ (360 mg $kg^{-1} \times 3$)-treated group in spite of the lack of LH surge which is normally observed at 57 h after eCG injection, changes in gonadotropin levels were examined after 57, 60, 62, and 64 h after eCG injection (Figure 5). The peaks in preovulatory LH and FSH surges were confirmed about 62 h (not 57 h) after eCG injection. These results mean a delay of the gonadotropin surge. Serum

Table 3 Effects on human CG on carbamazepine-induced blocking of the first ovulation in eCG-primed immature rats

Treatment	*Ovulating rats/ rats examined*	*No. of oocytes in ovulating rats*
Control	5/5	12.4±1.25
CBZ	0/5	0
CBZ+hCG	5/5	9.6±1.66

Immature female rats were given a single injection of eCG (CG; 5 IU) at 0800 h at 26 days of age. Carbamazepine (CBZ; 360 mg kg^{-1}) was injected at 53 h (1300 h on day 28) after eCG treatment. Human CG (hCG: 10 IU) was injected i.p at 1700 h on day 28. Animals were sacrificed at 0800 h on day 29. The number of ovulation, which is shown as means±s.e.mean of 5–10 rats, was determined by counting oocytes in the oviduct.

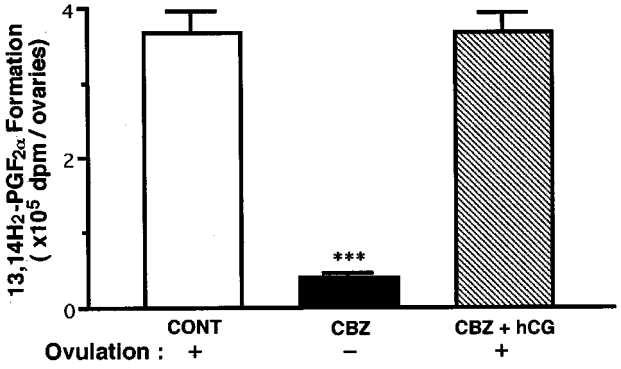

Figure 3 Effects of human CG on ovarian 13, 14H_2-$PGF_{2\alpha}$ forming capacity suppressed by carbamazepine in eCG-primed rats. Each treatment with eCG and carbamazepine (CBZ) was carried out as described in Table 1. Ovaries were removed at 0800 h on day 29 (72 h after eCG treatment) to measure the 13, 14H_2-$PGF_{2\alpha}$ forming capacity. Human CG (hCG) was injected at 57 h after eCG treatment (1700 h on day 28). Each value shows means±s.e.mean of 5–6 rats. Ovulation+ and − indicate that the number of oocytes was normal and that ovulation was not observed, respectively. ***$P<0.01$; *vs* Control (CONT).

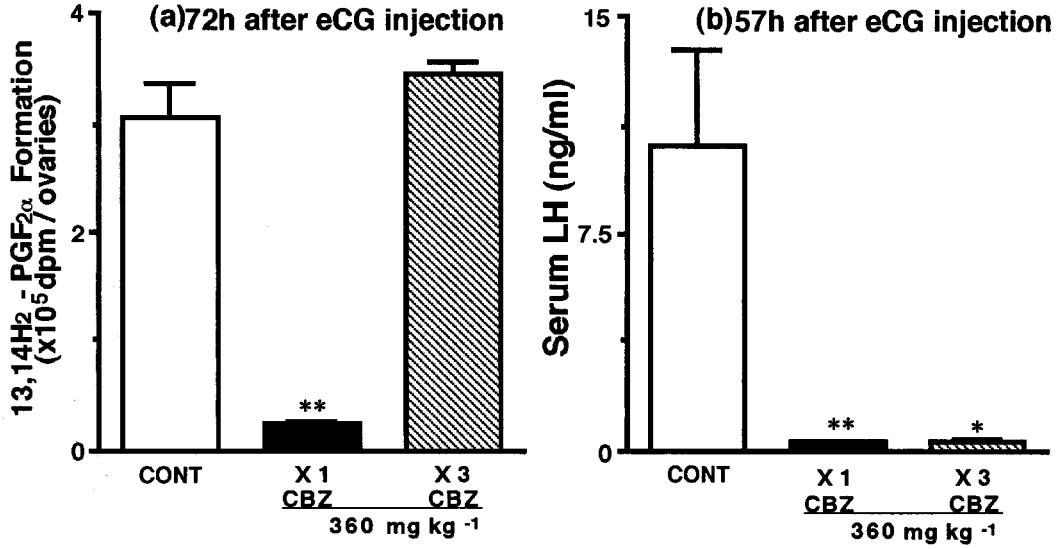

Figure 4 Effects of carbamazepine on ovarian 13, 14H_2-$PGF_{2\alpha}$ forming capacity and the serum LH levels in eCG-primed immature rats. Each treatment with eCG and/or carbamazepine (CBZ) was carried out as described in Figure 1 except the three-time injection of CBZ (CBZ×3) given with 360 mg kg^{-1} at 1300 h once daily for 3 days (days 26–28). (a) Animals were killed at 72 h after eCG treatment to measure 13, 14H_2-$PGF_{2\alpha}$ forming capacity. (b) Blood was collected at 57 h after eCG treatment and the serum LH levels were assayed by RIA. Each value shows means±s.e.mean of five rats. *$P<0.05$, **$P<0.01$, ***$P<0.01$; *vs* CONT.

concentration of CBZ at 4 h after the final injection of CBZ was determined between the two groups of 360 mg $kg^{-1} \times 1$ and 360 mg kg^{-1} g $\times 3$ (Table 4). The serum levels in the CBZ (360 mg $kg^{-1} \times 3$)-treated group were about one-third of those in a single injection group of CBZ (360 mg $kg^{-1} \times 1$). To determine the mechanism why repeated injections of CBZ caused a decrease in the serum levels of CBZ, the activity of hepatic catabolism against CBZ was measured at 4 h after the final injection of CBZ. When CBZ was given as three times at 360 mg kg^{-1}, the activity of 10′, 11′-epoxidation which is the main metabolic route clearly increased about 3 fold. However, the inducing effect of CBZ was not observed in a single injection of CBZ.

Discussion

The present data obviously demonstrated that a single administration of CBZ suppresses ovulation and ovarian 13, $14H_2$-$PGF_{2\alpha}$ formation in eCG-primed rats and the effects of CBZ were reversed by hCG treatment. In this study, the inhibition of LH release induced by CBZ treatment before 'the critical period' for LH surge on the first pro-oestrus was unequivocal. We have previously shown that the levels of ovarian 13, $14H_2$-$PGF_{2\alpha}$ forming capacity on the ovulation day (day 29) reflect the occurrence of LH surge during first pro-oestrus (Kogo *et al*., 1989). This forming capacity is significantly stimulated within 3 h after LH stimulation and then reaches a peak at 9–12 h. Thus, the measurement of 13, $14H_2$-$PGF_{2\alpha}$ formation is useful as a

Table 4 Serum concentrations of carbamazepine at the expected time (at 1700 h on day 28) of LH surge in eCG-primed immature rats

	Serum CBZ levels[(c)] (μg ml^{-1})	*Ovulation*[(d)]
CBZ (360 mg $kg^{-1} \times 1$)[(a)]	20.5±1.16	−
CBZ (360 mg $kg^{-1} \times 3$)[(b)]	7.3±0.41***	+

Immature female rats were given a single injection of eCG (5 IU) at 0800 h on day 26. (a): Carbamazepine (CBZ) was administered s.c at 1300 h on day 28. (b): CBZ was administered s.c at 1300 h once a day for 3 days (days 26–28). Blood was collected at 4 h after the final injection of CBZ. (c): Data show means±s.e.mean of five rats. (d): Ovualtion was confirmed on the morning of day 29. ***$P<0.001$; *vs* CBZ (360 mg $kg^{-1} \times 1$).

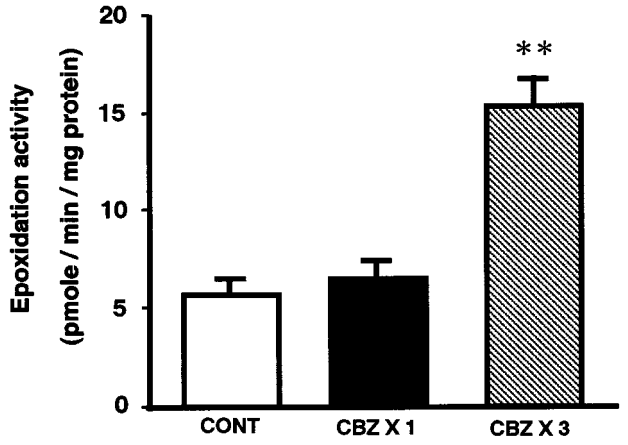

Figure 6 Effects of carbamazepine on hepatic activity of 10′, 11′-epoxidation against carbamazepine in eCG-primed immature rats. Each treatment with eCG and/or carbamazepine (CBZ) was carried out as described in Table 1. The liver was removed at 1700 h on day 28 (57 h after eCG treatment) and homogenized to prepare microsome fractions in each animal. Each value shows means±s.e.mean of five rats. **$P<0.01$; *vs* Control (CONT).

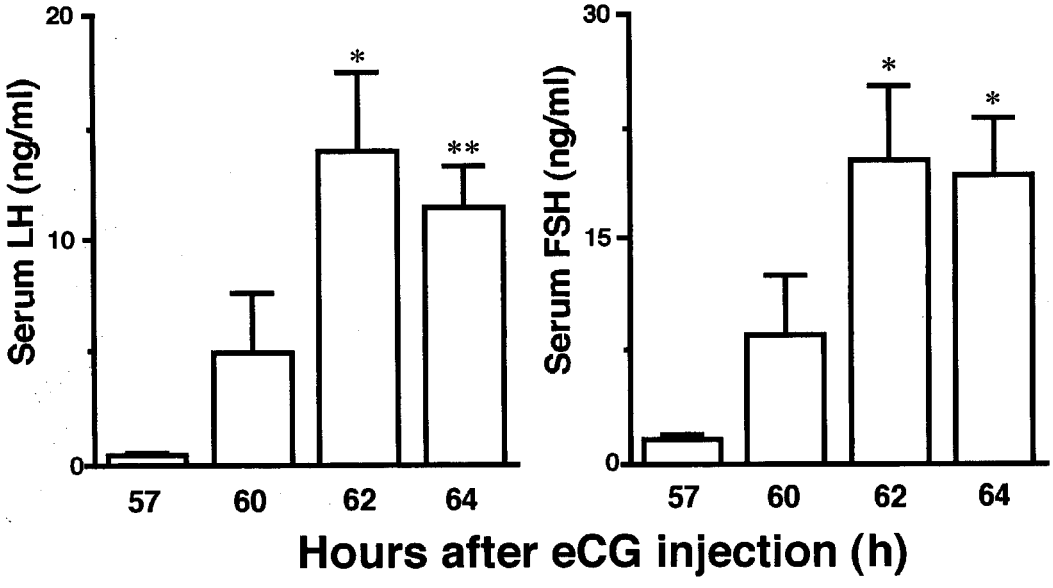

Figure 5 Delayed effects on the gonadotropin surge induced by three injections of carbamazepine in eCG-primed immature rats. Immature rats were given a single injection of eCG (CG; 5 IU) at 0800 h at 26 days of age. Animals were given a s.c. injection of carbamazepine (CBZ; 360 mg kg^{-1}) at 1300 h once daily for 3 days (days 26–28). Blood was collected at 1700, 2000, 2200 and 2400 h on day 28 (57, 60, 62, 64 h after eCG). Each value shows means±s.e.mean of 5–6 rats. *$P<0.05$, **$P<0.01$; *vs* Group at 57 h after eCG treatment.

sensitive indicator of LH secretion. This enzyme which converts 15KD-$PGF_{2\alpha}$ to 13, $14H_2$-$PGF_{2\alpha}$ has been identified as a carbonyl reductase in our laboratory (Iwata *et al*., 1990), and further it has been confirmed that the activity (Iwata *et al*., 1989) and mRNA expression (Espey *et al*., 2000) of the enzyme are correlated to LH secretion and the process of ovulation. We used this 13, $14H_2$-$PGF_{2\alpha}$ marker to monitor LH secretion on the first pro-oestrus in addition to the direct measurement of serum LH levels at the expected time of LH surge. Therefore, from the results in Figure 4, it was confirmed that the LH surge arose in the subsequent time to 57 h after eCG injection.

The CBZ-induced blocking of ovulation appears to be mainly due to the suppression of preovulatory gonadotropin surge, because ovulation was recovered by an additional treatment with hCG. However, two-time injections of CBZ before CBZ treatment on pro-oestrus counteracted the blocking. The repeated treatments of CBZ resulted in the occurrence of LH surge which was delayed about 5 h as compared with normal animals, and caused a decrease in serum CBZ levels. Administration of 360 mg kg^{-1} CBZ which blocked ovulation resulted in about 80 μM (20 μg/ml) CBZ in the serum levels at 4 h after injection. For the therapeutic serum concentration, 20–40 μM of CBZ is thought to be needed for performing epileptic therapy. Our data indicate that the serum levels of CBZ for blocking LH surge and ovulation in rats is comparable to 2–4 times the clinical concentration for the therapy in humans. The clinical significance of the suppressed LH surge caused by CBZ is not clear. But it has been shown that after CBZ treatment serum LH levels decreased and the LH-RH-stimulated LH secretion diminished as a direct effect of CBZ on the hypothalamic-pituitary axis in epileptic patients (Isojarvi *et al*., 1989). The inhibitory effect of CBZ on LH release by affecting the pituitary responsiveness for LH secretion in the central nervous system may explain anovulatory cycles during the early stage of CBZ therapy for epilepsy. Kuhn-Velton *et al*. (1990) have reported that CBZ inhibited dibutyryl-cyclic AMP- or hCG-stimulated testosterone formations in rat Leydig cells *in vitro*. Interestingly, testosterone levels were suppressed by 50% at 40 μM CBZ, suggesting a direct inhibition of steroidogenesis in rat testis at the clinical dose of CBZ. Although we have not examined the levels of ovarian steroids in the present study, the CBZ-induced blocking of ovulation was completely reversed by a hCG injection when a physiological dose of hCG was injected to CBZ-treated animals. The number of oocytes was normal. These data suggest that the action of CBZ on the ovary is reversible by hCG even if steroidogenesis is directly suppressed by CBZ. This reversible effect was different from that in indomethacin-treated animals (Tamura *et al*., 1989; 1991). In humans, no changes were found in the serum levels of 17β-oestradiol, testosterone, and cortisol during short-term or long-term CBZ treatment, although there was an elevation in progesterone levels in the early follicular phase of the menstrual cycle (Isojarvi, 1990). It is suggested that a decrease in serum DHEAS levels in CBZ-treated patients is due to an acceleration of DHEAS catabolism in the microsomal drug metabolizing enzyme system in the liver as well as to the inhibitory effect on the androgen synthesis in the adrenal cortex (Richens, 1984). We suggest here that repeated administrations of CBZ increased the metabolism of CBZ mediated by inducing the microsomal enzyme in liver and that metabolism of CBZ caused by pretreatment with CBZ itself is responsible for the counteraction of the ovulation blocking. The results imply that CBZ may alter the balance of reproductive hormones in female patients with epilepsy by mediating the induction of liver enzymes for the catabolism of hormones, and that the difference in CBZ metabolism might explain part of the difference in the effect of CBZ on endocrine functions between the short-term and long-term administrations. Thus, CBZ-induced enzyme might lead the serum levels of hormones to fluctuate, as seen in the clinical study.

In conclusion, this paper demonstrates that CBZ, which induced double the serum levels of the clinical dose for epilepsy therapy, inhibits the first ovulation mainly by suppressing gonadotropin surge. Furthermore, repeated administrations of such a dose of CBZ before preovulatory gonadotropin surge decrease the serum levels of CBZ and cause eCG-induced ovulation, implying that the frequency of CBZ administration is associated with the influence of CBZ on the reproductive physiology.

We are grateful to Dr A.F. Parlow (Pituitary Hormone and Antisera Center) for radioimmunoassay materials. We thank Prof S. Saida for his critical reading of the manuscript and Dr K. Takeuchi of Fujinaga Pharmaceutical Co., Ltd. for providing carbamazepine.

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(Received July 26, 2001
Accepted September 5, 2001)