The Mode of Action of Indomethacin, Aspirin and Melatonin on the Blockage of the First Ovulation in Immature Rat Pretreated with PMSG

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Abstract—The purpose of this paper was to evaluate the anti-ovulatory effects of indomethacin, aspirin and melatonin by examining the LH sensitive 13,14-dihydro-prostaglandin F2α forming capacity in rat ovary. When ovulation was blocked by aspirin or melatonin, the forming capacity was strongly suppressed, and these effects were reversed by hCG injection. However, the ovulation blockage by indomethacin did not accompany the inhibition of the forming capacity. These results show that aspirin and melatonin block the ovulation via the hypothalamus-pituitary level, and indomethacin acts directly on the ovary.

It is well-known that indomethacin (INDO) (1-4) or aspirin (ASP) (1, 2), both being an inhibitor of prostaglandin synthesis, has an inhibitory action on the induction of ovulation in cycling rats or in immature rats pretreated with pregnant mare serum gonadotropin (PMSG). Some studies on the action of pineal gland amines have indicated that melatonin (MEL), a physiological substance which has an inhibitory influence on reproductive function, also inhibited ovulation (5-7). Some investigators have shown that the inhibitory response of INDO may result from the direct actions at the ovarian level (2, 4), but ASP and MEL may block ovulation via their inhibitory action on the release of gonadotropin. We previously reported that in adult cycling rats, the ovarian 13,14-dihydro-prostaglandin F2α (13,14H2-PGF2α) forming capacity was markedly stimulated by gonadotropin (8), and it was inhibited, together with ovulation blockage, by chlorpromazine (CPZ) injection just before the LH surge in proestrus (9, 10). The inhibition of the forming capacity and ovulation was clearly reversed by hCG injection. Recently, we also observed that the ovarian 13,14H2-PGF2α forming capacity and the first ovulation induced by PMSG in immature rats were blocked by CPZ in agreement with our previous report with the cycling rats. The inhibition of the forming capacity, together with the ovulation blockage induced by CPZ, was completely recovered by injection of hCG (data not shown). Further, we have some evidence that the 13,14H2-PGF2α forming capacity in rat ovary may be closely associated with the ovulation process (11, 12). The purpose of the present study was to estimate the mode of action for the anti-ovulatory effect of INDO and ASP, representing anti-inflammatory drugs, and MEL, as a physiological substance, by using the ovarian 13,14H2-PGF2α forming capacity as a sensitive indicator of gonadotropin secretion.

To induce the first ovulation, immature female rats of the Wistar strain were given a single s.c. injection of 5 IU PMSG (Serotropin, Teikoku Hormone MFG Co., Ltd.) at 8:00 at 26 days of age. These animals ovulated an average of 11 oocytes on the morning of day 29. INDO, ASP and MEL were obtained from the Sigma Chemical Company. INDO (1.5 mg/rat) was dissolved in sesame oil. ASP (700 mg/kg) was suspended in 15% gelatin. These drugs were injected s.c. at 13:00 at 28 days of age. MEL which was dissolved in ethanol and diluted to 10% with saline was repeatedly given s.c. in the dose of 1.5 mg/rat every hour from 13:30 to 17:30 on day 28.
after 24 hour fasting. Each dose of these anti-ovulatory drugs was a sufficient amount for obtaining an ovulation blockage ratio of more than 90% (2, 5). An i.p. administration of hCG (Gonatropin, Teikoku H. MFG Co., Ltd.) was carried out at 17:00 on day 28 (57 hr after PMSG treatment). These animals were sacrificed at 8:00 on day 29, and the oviducts and ovaries were removed immediately after decapitation. The occurrence of ovulation was determined by microscopically examining the oviducts for the presence of oocytes. After weighing, the ovaries were homogenized with 0.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) at 4°C using a glass homogenizer, and used for estimation of the forming capacity of 13,14H2-PGF$_{2\alpha}$. The 13,14H2-PGF$_{2\alpha}$ forming capacity was measured by determining the conversion of $^3$H-13,14H$^2$-15keto-PGF$_{2\alpha}$ to $^3$H-13,14H$_2$-PGF$_{2\alpha}$ in the ovarian tissue homogenate containing NADPH as reported previously (8, 11). Protein was determined by the method of Lowry et al. (13) employing bovine serum albumin as a standard. All data were expressed as the mean±S.E.M., and Student's $t$-test was used to assess the statistical significance of the group comparison. Comparisons with $P<0.05$ were considered significant.

First, we determined the dose-response effect of hCG on the ovarian 13,14H$_2$-PGF$_{2\alpha}$ forming capacity in immature rat (Fig. 1). These animals received 1, 10 and 100 IU of hCG at 8:00 on day 27 (24 hr after PMSG) to avoid the influence of an endogenous LH surge on the ovarian 13,14H$_2$-PGF$_{2\alpha}$ forming capacity. The 13,14H$_2$-PGF$_{2\alpha}$ forming capacity was dose-dependently increased with 0.1 and 10 IU of hCG. Ovaries were sensitive to 1 IU of hCG, which was the lowest dose tested, and the forming capacity showed the maximal level by injection of 10 IU of hCG. Figure 2 shows both the change of the ovarian 13,14H$_2$-PGF$_{2\alpha}$ forming capacity and the effect of hCG on the forming capacity and ovulation when ovulation was inhibited by each anti-ovulatory drug. A single injection of INDO or ASP completely suppressed the occurrence of ovulation. Although a single s.c. injection of MEL did not block ovulation, MEL administration (s.c.) at a dose of 1.5 mg/rat 5 times on day 28 most effectively blocked the ovulation. The ovulation blockage ratio of MEL was approximately 90% of the control (number of rats ovulated/total rats: 1/7). As shown in Fig. 2, the ovarian 13,14H$_2$-PGF$_{2\alpha}$ forming capacity was markedly inhibited when ovulation was blocked by injection of ASP or MEL ($P<0.001$, compared with that in the control group). However, INDO exercised no influence on the ovarian 13,14H$_2$-PGF$_{2\alpha}$ forming capacity, although the ovulation was blocked and the ovarian PGF$_{2\alpha}$ level was considerably lowered by INDO treatment (i.e., 0.55±0.09 ng vs. 2.4±0.41 ng in the control, $P<0.01$). The ovulation blockage observed in the ASP and MEL groups was restored in all rats by hCG administration, and the suppressed 13,14H$_2$-PGF$_{2\alpha}$ forming capacity in the ASP or MEL
group was also markedly increased up to the control level by hCG treatment. The ovarian 13,14H2-PGF2α forming capacity in rats that received 10 IU of hCG at 17:00 on day 28 following PMSG treatment on day 26 was almost the same level as that of PMSG treatment alone (control level) (11). In contrast, the blockage of ovulation induced by INDO was not counteracted by hCG administration.

Berman et al. reported that an injection of INDO (14 mg/kg) or ASP (400 mg/kg) on the day corresponding to proestrus inhibited ovulation in PMSG-treated immature rats (100% and 80%, respectively) (2). They observed that the INDO-induced blockage of ovulation was not reversed by LH or gonadotropin-releasing hormone, but it was reversed in the case of the ASP-induced blockage of ovulation, indicating that the mode of action of INDO was different from that of ASP. Even though INDO was injected at a sufficient dosage to inhibit ovulation in adult rats, the gonadotropin level in the serum was almost the same as that of the control (4). Ying and Greep (6) have examined the effect of MEL on the proestrus surge of LH in female rats and supported the concept that the pineal gland exerts control over reproductive functions via an inhibiting action of MEL on the release of gonadotropin from the pituitary. In the present study using the 13,14H2-PGF2α forming capacity as a sensitive indicator of LH secretion, the results clearly confirmed that INDO does not block LH release. Although LH secretion occurred in the INDO-treated group, these animals did not ovulate (even if the treatment of exogenous hCG was performed). The inhibition of the 13,14H2-PGF2α forming capacity induced by ASP or MEL was due to the suppression of endogenous LH secretion.

These results are consistent with the interpretation that ASP and MEL exert their ovulation blocking action via an inhibitory effect on the LH surge at the hypothalamus-pituitary level, whereas INDO blocks ovulation by directly affecting the ovarian function rather than by inhibition of LH release at the pituitary level.

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