EFFECT OF 13,14-DIHYDROPROSTAGLANDIN $F_{2\alpha}$ ON STEROID BIOSYNTHESIS IN RAT OVARY

Norihiisa INAZU, Hiroshi KOGO and Yoshio AIZAWA

Department of Pharmacology, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

Accepted December 18, 1980

We have recently reported that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was metabolized into 13,14-dihydro $PGF_{2\alpha}$ ($13,14H_2-PGF_{2\alpha}$) via 15-keto $PGF_{2\alpha}$ ($15K-PGF_{2\alpha}$) and 13,14-dihydro-15-keto $PGF_{2\alpha}$ ($15KD-PGF_{2\alpha}$) in the rat ovary (Fig. 1), and that catabolism in the rat ovary was different from that in rat uterus (1). Thus it was of interest to determine the significance of $13,14H_2-PGF_{2\alpha}$ formed in rat ovary, as related to the action on ovarian function. There are a few reports on the biological activity of PG metabolites in smooth muscle preparations. Although $15KD-PG$ has little biological activity on smooth muscle preparations, $13,14H_2-PG$ possesses an appreciable contractile activity on rat stomach and uterus (2, 3) and dog femoral vascular beds (4). Whether or not the metabolites formed by the catabolism of $PGF_{2\alpha}$ in rat ovary have any effect on steroidogenesis remains unknown. The present study was

designed to determine the possible role of the metabolites in the ovarian function by estimating the effect of $13,14H_2-PGF_{2\alpha}$ on the conversion of pregnenolone into progesterone and estrogen in rat ovary.

Female Wistar rats weighing approx. 200 g, were housed under controlled conditions of light and temperature. Vaginal smears were examined every morning and rats exhibiting the regular 4-day estrous cycle were used for the experiment. Animals were sacrificed on the day of diestrus, and ovaries obtained from each rat were homogenized in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4). The ovarian homogenate was incubated with 50 nCi of [$4-^{14}C$]-pregnenolone (55.7 mCi/mmol, New England Nuclear) in test tubes each containing 100 ng of $PGF_{2\alpha}$, $15K-PGF_{2\alpha}$, $15KD-PGF_{2\alpha}$ or $13,14H_2-PGF_{2\alpha}$ at $37^\circ C$ in 95% O_2 -5% CO_2 for 60 min. The incubation was terminated by the addition of 2 ml of chloroform-methanol (2:1) and the ovarian steroids formed in the incubation mixture were extracted. After evaporating the chloroform-methanol phase, the extracts were separated into the progesterone and estrogen fractions by Sephadex LH-20 column chromatography. Furthermore, each fraction of progesterone and estrogen was separated and purified by silica-gel 60 F_{254} thin-layer chromatography and the radioactivities of progesterone, estradiol and estrone area were counted in a scintillation spectrometer. The details of the above procedure have been described previously (5).

Fig. 1. Catabolic pathway of prostaglandin $F_{2\alpha}$ in rat ovary. 15K: 15-keto $PGF_{2\alpha}$, 15KD: 13,14-dihydro-15-keto $PGF_{2\alpha}$, $13,14H_2$: 13,14-dihydro $PGF_{2\alpha}$.

Both $\text{PGF}_{2\alpha}$ and $13,14\text{H}_2\text{-PGF}_{2\alpha}$ significantly increased the formation of progesterone, estradiol and estrone (Fig. 2). However, 15K- and $15\text{KD-PGF}_{2\alpha}$ had no effect on the conversion of pregnenolone into ovarian steroids. The important finding of this experiment was that the effect of $13,14\text{H}_2\text{-PGF}_{2\alpha}$ on steroid biosynthesis in rat ovary was similar to that of $\text{PGF}_{2\alpha}$. The catabolism of PG is generally initiated by the action of two enzymes, first 15-hydroxy-

prostaglandin dehydrogenase and then Δ^{13} -prostaglandin reductase, by which oxidation of the 15-hydroxy substituent (15-keto compounds) and reduction of the 13,14-double bond (13,14-dihydro compounds) occur. It is well known that the initial action has the greatest significance in the biological inactivation (6, 7). It has been reported that $13,14\text{H}_2\text{-PGF}_{2\alpha}$ formed by the catabolism of $\text{PGF}_{2\alpha}$ in rat ovary (1) possesses an appreciable contractile activity on smooth muscle preparations as well as $\text{PGF}_{2\alpha}$, although this activity is less than that of $\text{PGF}_{2\alpha}$ (2-4). However, the physiological role of $13,14\text{H}_2\text{-PGF}_{2\alpha}$ in the tissue was still unclear. It has been shown that $\text{PGF}_{2\alpha}$ *in vitro* has a steroidogenic action in corpus luteum in the bovine (8) and human species (9), however little is known of the effect of $13,14\text{H}_2\text{-PGF}_{2\alpha}$ on ovarian steroidogenesis. As we did not find a statistically significant difference between the effect of $\text{PGF}_{2\alpha}$ and that of $13,14\text{H}_2\text{-PGF}_{2\alpha}$ for steroid biosynthesis, it is strongly suggested that the reduction of the 15-keto group, namely the formation of $13,14\text{H}_2\text{-PGF}_{2\alpha}$ from $15\text{KD-PGF}_{2\alpha}$ which has no effect on steroidogenesis, may play some specific role for steroid biosynthesis, at least in the ovary.

Acknowledgement: We are grateful to Dr. John E. Pike of the Upjohn Company, Kalamazoo, Mich. for generous gifts of $\text{PGF}_{2\alpha}$ and its three metabolites.

Fig. 2. Effect of $13,14\text{-dihydroprostaglandin F}_{2\alpha}$, prostaglandin $\text{F}_{2\alpha}$ and the other prostaglandin $\text{F}_{2\alpha}$ metabolites on steroid biosynthesis in rat ovary. The ovarian homogenate with 50 nCi of pregnenolone was incubated in a test tube containing 100 ng of each compound at 37°C in $95\% \text{O}_2\text{-}5\% \text{CO}_2$ for 60 min. C: control, H_2 : $13,14\text{-dihydro PGF}_{2\alpha}$, K: $15\text{-keto PGF}_{2\alpha}$, KD: $13,14\text{-dihydro-15-keto PGF}_{2\alpha}$, F_2 : $\text{PGF}_{2\alpha}$. Each column represents the mean $\pm$ SE (vertical bar). N: number of determinations. * $p < 0.05$, ** $p < 0.01$: Significantly different from control.

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