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Role of Prostaglandins in Ovulation in Rabbits

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Introduction

The mechanisms which cause follicular rupture and extrusion of the ovum have still not been completely elucidated. The enzymatic digestion of the follicular wall[2,22], local vasscular changes[1,19] and the contractility of ovarian smooth muscle[1,4] have been postulated as possible mechanism of ovulation. There are some good evidence indicated by studies in the laboratory animals[7,15,21] that prostaglandins are involved in ovulation. It has been demonstrated that the blockade of prostaglandin synthesis brought about by indomethacin prevents ovulation[23,24]. This effects can be reversed by the administration of prostaglandin $F_{2\alpha}$[4]. Furthermore, it has been shown both in vivo and in vitro that luteinizing hormone(LH) causes a marked preovulatory rise in both PGE and PGF in whole Graafian follicles obtained from rabbits[14]. While studies on the effects of prostaglandins on ovulation have been reported, mode of action and endogenous source of prostaglandin in ovulation process have not been reported. There are some reports that the uterine luteolytic hormone travels from the uterus to the ovary via the vascular system[3]. It has been reported that the tritiated $PGF_{2\alpha}$ appears to be transferred from the uteroovarian vein to the ovarian artery by a countercurrent mechanism[17]. Del Campo and Ginther[3] concluded from the anatomical arrangement of the blood vessels that blood vessels may not serve as the functional unilateral pathway for uterine luteolytic hormone on the ovary. The route by which the uterine luteolytic hormone reach the ovary has yet to be investigated in rabbits.

The ovulation process are, therefore, seems to be directly affected by the endogenous prostaglandins from ovary but not that of from uterus. The present studies were undertaken to determine the effect of bilateral hysterectomy and/or indomethacin treatment on hCG induced ovulation in rabbits.

Materials and Methods

Randomly bred albino rabbits weighing 2.0 to 3.0 kg were purchased from a local dealer. The animals were individually housed in wire cages in a room at 21° to 27°C, and received light between 05.00 to 19.00. Powdered food (CP : 19%) and tap water were offered without restriction, and in addition the rabbits received supplments of fresh green feed twice weekly. Ovulation was induced by the injection of 50 i.u. of human chorionic gonadotropin (HCG, Intervest) in 0.5 ml of sterile saline solution via the marginal ear vein. Ovulation process induced by HCG injection was studied under the effect of 3 different treatments; indomethacin, bilateral hysterectomy and in domethacin plus bilateral hysterectomy. In all experiments, the animals were killed by air injection via the marginal ear vein on the day after the HCG injection. Both ovaries were removed and examined under a stereomicroscope[23]. The number of ruptured follicles was recorded (Fig. 2).

The experimental animals were grouped as follours:

Group 1—0mg/kg bw (vehicle), Group 2—5 mg/kg bw, Group 3—10 mg/kg bw, Group 4—animals received only HCG, Group 5—bilateral hysterectomy, Group 6—indomethacin(10 mg/kg bw) plus bilateral hysterectomy.

Indomethacin (Sumitomo) was dissolved in distilled water containing Tween 80 (10%) and propyleneglycol (1%) and injected intramusculary immediatly before HCG treatment. The rabbits were hysterectomized under procaine hydrochloride (Vitarine) anaesthesia at least 3 weeks before HCG injection.

The efficiency of the operations was checked by post mortem. The data were statistically analyzed by the chisquare test for independent samples.

Results

Administration of indomethacin resulted in an inhibition of exogenously induced ovulation. As shown in Fig. 1, blockade of ovulation by indomethacin showed a dose dependent response relationship; the corelation coefficient was significant ($p<0.01$). As shown in Table 1, the dose of 10 mg/kg bw of indomethacin produced an

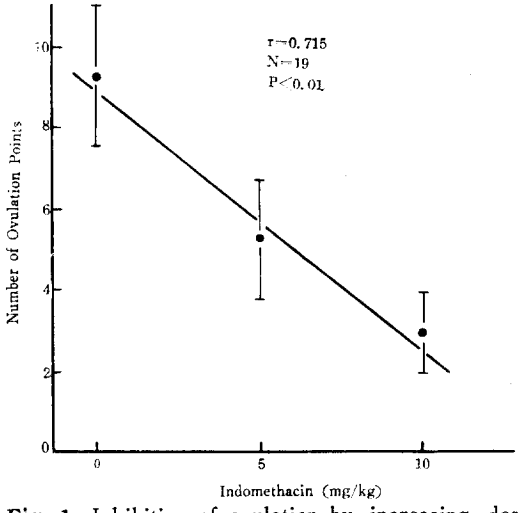

Fig. 1. Inhibition of ovulation by increasing doses of indomethacin. Vertical lines indicate ±SE.

Table 1. Number of Ovulation Points in Rabbits under Different Treatments

	No. of Animals Ovulating/Total	Total No. of Ovulation	Ovulation Points Mean±SE
HCG	6/6	59	9.83±1.70
Vehicle	6/6	56	9.33±1.85[a]
Indomethacin (10 mg/kg)	3/5	15	3.00±1.05[a]
Hysterectomy	5/5	44	8.80±1.54[a]
Indomethacin and Hysterectomy	0/3	0	0

a. Indomethacin vs vehicle of indomethacin and hysterectomy ($p<0.01$ and $p<0.02$, respectively).

The other groups did not show significant differences with their contrls.

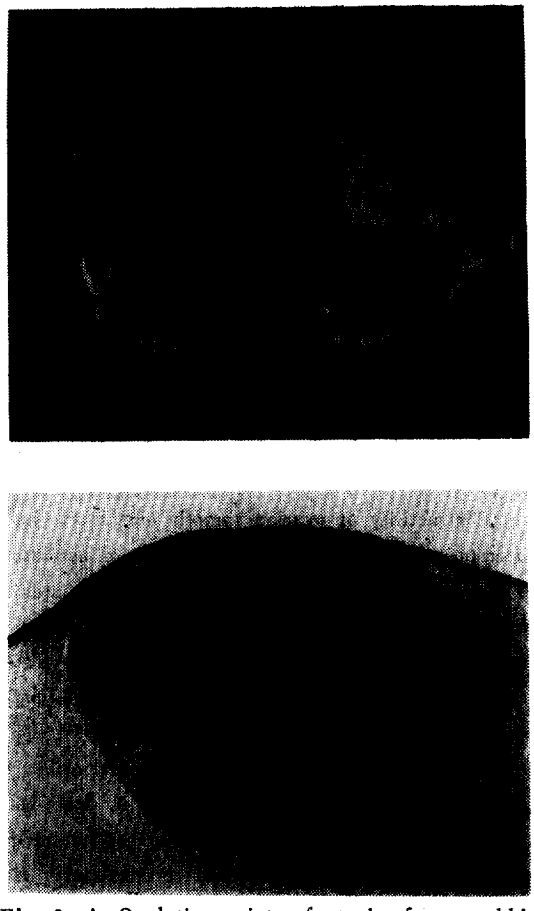

Fig. 2. A, Ovulation points of ovaries from a rabbit killed at 24 hours after hCG administration, showing an Graafian follicle and two ovulation fossa.
B, Section of an ovary, which received indomethacin entrapped ovum. H & E. ×12.5

inhibition of ovulation ($p<0.01$). The vehicle used for the injections of indomethacin had no detectable effect on ovulation ($p>0.05$). In animals treated with indomethacin the follicles were not ruptured and contained ovum until at the time of expected ovulation (Fig. 2).

Bilateral hysterectomized rabbits differ in the number of ovulation points from the rabbits treated with indomethacin ($p<0.02$).

Hysterectomized rabbits treated with indomethacin had no ovulation points.

Discussion

It is widely accepted that prostaglandins play an

important role in ovulation[15]. The present study has reconfirmed that prostaglandins are necessary for the process of ovulation as indicated by the facts that inhibitor of prostaglandin synthesis such as indomethacin has inhibited ovulation in the rabbit (Table 1). It is not clear, however, whether the inhibitory action of indomethacin is mediated by a blokade of gonadotropin secretion or is exerted directly on the ovary[7,21,24]. This study demonstrated that major site of action of indomethacin on the ovulation block appears to be exerted on the ovary, either directly or by some mechanism not involving the availability of gonadotropin. Although it is not clear that indomethacin inhibited the synthesis of porstaglandins in the rabbits, the doses used in this study were equivalent to those reported to prolong the life span of the corpus luteum in pseudopregnant rabbits and are considerably higher than the therapeutic dose which resulted in a 77 to 98% inhibition of prostaglandin metabolites in human urine[9]. It is well known that a nongravid uterus causes regression of the corpus luteum and uterine luteolysin is $PGF_{2\alpha}$ or a closely related substance[8,10]. A local uteroovarian pathway named countercurrent mechanism is involved in uterine-induced luteolysis in cattle, sheep and swine[6]. However, in the rabbit the luteolytic effect of the uterus involves a systemic pathway; if a local pathway is present it apparently plays only a minor role and is not readily demonstrated[6]. But why the rabbit should differ is yet to be elucidated. This study demonstrated that bilateral hysterectomy did not alter the number of ovulation point in the rabbit (Table 1). Thus if endogenous prostaglandins are important in ovulation, they must come from an extrauterine source. Our finding that bilateral hysterectomy did not alter ovulation point in the rabbit does not support the hypothesis that endogenous prostaglandins come from the uterus in the process of ovulation in the rabbit[12]. Furthrmore, previous studies provided inderect evidence that endogenous prostaglandins come from the ovary in the process of ovulation in the rabbit[13,26]. The prostaglandins are 95 to 99% metabolized after one passage of the blood through the lungs and has a very short half life[5]. If prostaglandins are indeed local hormones with local actions it is resonable to suppose that they are formed by particular tissues within an organ; some of these tissues may produce or destroy an prostaglandin derivative in particular. Recently, it has been suggessted that the prostaglandins are synthetized by the cellular elements of the follicle and released into the fluid[25]. The present study suggest that endogenous prostaglandins are important in ovulation and come from the ovary in the rabbit. This suggestion is controversial to the countercurrent mechanism in cattle and sheep[17].

Conclusion

Role of prostaglandins in ovulation was examined in indomethacin and/or bilateral hysterecto mized rabbits. Indomethacin at a dose of 10 mg/kg intramusculary interfered with hCG induced ovulation but bilateral hysterectomy alone does not alter the number of ruptured follicles in the rabbits. Hysterectomy plus indomethacin adminisrtation inhibited ovulation. In addition, some of ovaries treated with indomethacin was examined histologically.

Indomethacin interfered follicular rupture and the inhibition of follicular rupture resulted in entrapment of ova. This interruption is dose dependent response.

It is concluded that prostaglandins may play an important role in ovulation and may come from ovary but not from uterus. The possible role of prostaglandins in ovulation process are discussed.

Although the exact mechanism by which luteinizing hormone induces ovulation is not clear, recent studies with laboratory animals have provided evidence to suggest that cyclic adenosine 3,5-monophosphate (cyclic AMP) and prostaglandins play important roles in the sequence of events leading to follicle rupture and ovulation in reponse to LH[16].

Indomethacin administration has been shown to interfere with follicular rupture (Fig.2). This result suggest that prostaglandin may have a role in follicular rupture in the ovulatory process. In the present experiments it is not clear whether the inappropriately high level of PG or the change in the ratio

of PGEs to PGFs or other prostanoid substances was responsible for the inhibition of follicular rupture[11]. Nevertheless, it seems likely that a PG-mediated mechanism is involved in the failure of follicular rupture[11]. We conclude that endogenous prostaglandins may play an important role in ovulation process such as follicular wall rupture and they may come from ovary but not from uterus in the ovulation process in the rabbit.

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Prostaglandin이 가토의 배란에 미치는 영향

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초 록

Prostaglandin이 배란에 미치는 영향을 관찰하기 위하여 정상군과 양쪽 자궁을 제거한 가토에 indomethacin (prostaglandin 합성억제제)을 투여한 후 HCG에 의해 유발된 배란부위를 조직학적으로 관찰하였던 바 아래와 같은 결과를 얻었다.

1. indomethacin (10 mg/kg bw) 투여로 배란은 억제되었으며 투여량에 비례하여 배란은 억제되었다($p<0.01$).
2. 자궁적출 단독으로는 배란에 영향을 주지 못하였다($p>0.05$).
3. indomethacin은 난포파열을 억제함으로써 배란을 억제한다.
4. 토끼에서는 자궁이 아닌 장기에서 유래된 prostaglandin이 난포파열과정에 작용함으로써 배란을 시키는 작용이 있는 것으로 추정된다.