OVULATION BLOCK BY INDOMETHACIN, AN INHIBITOR OF PROSTAGLANDIN SYNTHESIS: A STUDY OF ITS SITE OF ACTION IN RATS

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Summary. Indomethacin, an inhibitor of prostaglandin (PG) synthesis, was used to assess the rôle of PG in ovulation and gonadotrophin release. The block to ovulation induced by indomethacin in normal pro-oestrous rats and immature rats primed with PMSG could be overcome by exogenous PG. The same block could not be overcome in cyclic rats and immature rats primed with PMSG by administration of LH-releasing hormone (decapeptide-LH-RH) or HCG at a dose level sufficient to induce ovulation in rats in which block had been induced by pento-barbital. Indomethacin did not interfere with the pro-oestrous surge of LH and FSH secretion although ovulation was prevented in 83% of animals. When indomethacin was given to cyclic rats at pro-oestrus, dioestrus persisted for 5 to 12 days until a return to the normal 4-day cycle occurred after reversal of the indomethacin-induced block to ovulation by treatment with PG. It is concluded that indomethacin, at least as a single injection, does not block LH release, but exerts its anti-ovulatory action directly on the ovary. It may also act at the hypothalamo-pituitary level.

INTRODUCTION

Indomethacin, an inhibitor of prostaglandin (PG) synthesis, has an inhibitory influence on the induction of ovulation in cyclic rats (Tsafriri, Lindner, Zor & Lamprecht, 1972), in immature rats pretreated with PMSG (Orczyk & Behrman, 1972; Armstrong & Grinwich, 1972; Behrman, Orczyk & Greep, 1972) and in rabbits (Grinwich, Kennedy & Armstrong, 1972; O'Grady, Caldwell, Auletta & Speroff, 1972). It is not clear, however, whether the inhibitory action of indomethacin is mediated by a blockade of gonadotrophin secretion (Orczyk & Behrman, 1972) or is exerted directly on the ovary (Tsafriri *et al.*, 1972; Armstrong & Grinwich, 1972; Behrman *et al.*, 1972; Grinwich *et al.*, 1972).

The purpose of this study was to ascertain the site of action of indomethacin in rats.

MATERIALS AND METHODS

All the animals were Holtzman-derived rats of the departmental colony and were housed in air-conditioned quarters ($25 \pm 2^\circ\text{C}$) with lighting between 05.00 and 19.00 hours. Pelleted food and water were offered without restriction. Adult rats were used only after having had at least two regular 4-day cycles. Blood samples were collected from the jugular vein under light ether anaesthesia. Serum was separated and stored at -20°C until assay for LH and FSH by NIAMD radioimmunoassay kits. The results were expressed in terms of the kits provided.

Indomethacin (Sumitomo Kagaku Pharmaceutical Co., Osaka) was prepared as a solution of 50 mg in 0.3 ml acetone, and diluted with 15% gelatin or isotonic saline. Solutions of $\text{PGF}_{2\alpha}$ and PGE_2 (Ono Pharmaceutical Co., Osaka) were prepared in 0.1 ml absolute ethanol and diluted with saline. The HCG (Primogonyl) and PMSG (Primantron) preparations were obtained from Schering AG, Berlin, and the LH-RH from Daiichi Pharmaceutical Co., Tokyo.

Experiment 1

Indomethacin in gelatin was administered subcutaneously at 13.00 hours on the day of pro-oestrus to adult rats at various dose levels (see Table 1).

Indomethacin (2 mg/rat) was also injected at various times (Table 2), and control animals received the vehicle alone.

Blood samples were taken at 18.00 and 23.00 hours on the day of the injection. The animals were killed the following day and their oviducts were examined under a dissecting microscope for the presence of ova in the distended ampulla.

Experiment 2

Indomethacin (2 mg/rat) in gelatin was administered subcutaneously at 13.00 hours on the day of pro-oestrus to adult rats and was followed by subcutaneous injections of $\text{PGF}_{2\alpha}$ or PGE_2 (0.8 mg/rat), HCG (20 i.u./rat) or LH-RH (400 ng/rat) at 15.30 hours on the same day. The PG-treated animals were bled at 18.00 and 23.00 hours and all the rats were killed the following day to check for the occurrence of ovulation (Table 3).

Experiment 3

Immature rats were injected subcutaneously at 25 days of age with 4 i.u. PMSG and then given indomethacin in gelatin 52 hr later. Prostaglandin $\text{F}_{2\alpha}$ or E_2 (0.8 mg/rat), HCG (20 i.u./rat) or LH-RH (400 ng/rat) were injected subcutaneously 55 hr after the PMSG injection and the animals were killed 72 hr after PMSG (Table 4).

Experiment 4

The influence of indomethacin on the oestrous cycle was determined from examination of daily vaginal smears. Indomethacin (2 mg/rat) in gelatin or saline (to overcome the depot-effect of indomethacin in gelatin) was injected subcutaneously at 13.00 hours on the day of pro-oestrus. Prostaglandin $\text{F}_{2\alpha}$

(0.8 mg/rat) was given subcutaneously at 15.30 hours on the same day and vaginal smears were taken daily until the end of the experiment.

RESULTS

Indomethacin given in doses of 5 to 10 mg/rat at 13.00 hours on the day of pro-oestrus completely inhibited ovulation in adult cycling rats. Lower doses (0.5, 1 and 2 mg/rat) were less effective. All control rats which received the vehicle ovulated normally (Table 1). If indomethacin was given at any times other

Table 1. Effect of indomethacin in gelatin on ovulation in adult cycling rats

<i>Indomethacin*</i> (mg/rat)	<i>No. of rats ovulating/ no. treated</i>	<i>% Ovulation</i>	<i>Average no. of ova/ ovulating rat†</i>
0 (vehicle only injected)	6/6	100.0	8.3 ± 0.6
0.5	5/5	100.0	9.4 ± 0.2
1.0	2/5	40.0	7.0
2.0	1/6	16.7	3.0
5.0	0/5	0	
10.0	0/4	0	

* Indomethacin was administered subcutaneously at 13.00 hours on the day of pro-oestrus.

† Mean ± S.E.

Table 2. Effect of time of administration of indomethacin in gelatin on ovulation in adult cycling rats

<i>Time of dosage with 2 mg indomethacin/rat (hours)</i>	<i>No. of rats ovulating/ no. treated</i>	<i>% Ovulation</i>	<i>Average no. of ova/ ovulating rat*</i>
09.00	3/5	60.0	6.0 ± 0.6
13.00	1/6	16.7	3.0
14.00	1/5	20.0	3.0
15.00	3/5	60.0	4.3 ± 0.9
17.00	3/6	50.0	5.0 ± 1.7
19.00	3/5	60.0	3.7 ± 0.7
21.00	2/5	40.0	6.5 ± 0.5
23.00	4/4	100.0	6.3 ± 1.1

* Mean ± S.E.

than 13.00 and 14.00 hours, more than 40% of the rats ovulated, but the number of ova shed was reduced (Table 2). The block to ovulation was not overcome by administration of 20 i.u. HCG or 400 ng LH-RH, doses which were sufficient to induce ovulation in rats treated with pentobarbital. The ovulation-blocking action of indomethacin was reversed in 60 to 100% of animals by subsequent administration of PGE₂ or PGF_{2α} (Table 3).

All animals treated with PMSG ovulated 72 hr later. Ovulation was completely inhibited in the rats which received 2 mg indomethacin 52 hr after injection of PMSG. A block of ovulation was observed in 50% and 40% of the animals by subsequent administration (55 hr after PMSG injection) of PGF_{2α} or PGE₂, respectively. The indomethacin-induced block to ovulation

was not overcome, however, by administration of HCG or LH-RH at a dose level (20 i.u. or 400 ng) sufficient to induce ovulation in rats treated with pentobarbital (Table 4).

Serum LH levels in rats which received the vehicle only showed an apparent increase at 18.00 hours and a sharp decrease at 23.00 hours on the day of pro-oestrus in cyclic rats. In adult rats which received indomethacin at 13.00 hours on the day of pro-oestrus, the serum LH levels did not differ significantly from

Table 3. Prostaglandin, HCG and LH-RH reversal of indomethacin-induced block to ovulation in adult cycling rats

<i>Treatment</i>	<i>No. of rats</i>	<i>No. of rats ovulating</i>	<i>% Ovulation</i>	<i>Average no. of ova/ovulating rat*</i>
Control	6	6	100.0	8.3 ± 0.6
Indomethacin (2 mg/rat)	6	1	16.7	3.0
Indomethacin + HCG (20 i.u./rat)	6	0	0	
Indomethacin + LH-RH (400 ng/rat)	5	0	0	
Indomethacin + PGF _{2α} (0.8 mg/rat)	5	5	100.0	8.6 ± 0.5
Indomethacin + PGE ₂ (0.8 mg/rat)	5	3	60.0	7.0 ± 0.6

* Mean ± S.E.

Table 4. Prostaglandin, HCG and LH-RH reversal of indomethacin-induced block to ovulation in immature rats

<i>Treatment</i>	<i>No. of rats</i>	<i>No. of rats ovulating</i>	<i>% Ovulation</i>	<i>Average no. of ova/ovulating rat*</i>
PMSG (4 i.u./rat)	5	5	100	8.8 ± 0.6
PMSG + indomethacin (2 mg/rat)	5	0	0	0
PMSG + indomethacin + HCG (20 i.u./rat)	5	0	0	0
PMSG + indomethacin + LH-RH (400 ng/rat)	7	0	0	0
PMSG + indomethacin + PGF _{2α} (0.8 mg/rat)	6	3	50	3.3 ± 0.9
PMSG + indomethacin + PGE ₂ (0.8 mg/rat)	5	2	40	4.5 ± 0.5

* Mean ± S.E.

those in the control rats which received the vehicle alone at 18.00 and 23.00 hours on the same day, but ovulation was prevented in 83% of the animals. Serum LH levels in adult rats treated with indomethacin and PG (Exps 1 and 2) did not differ significantly from those in rats which received the vehicle or indomethacin alone at 18.00 and 23.00 hours on the same day. Serum FSH levels at 18.00 and 23.00 hours in rats treated with indomethacin, or indomethacin and PGF_{2α}, did not differ significantly from those in control rats (Table 5).

Table 5. Effect of indomethacin and $\text{PGF}_{2\alpha}$ on serum LH and FSH levels in pro-oestrous rats

Time of blood collection (hours)	Hormone assayed	Vehicle alone	Indomethacin (2 mg/rat)	Indomethacin and $\text{PGF}_{2\alpha}$ (0.8 mg/rat)	P†
18.00	LH	1552.8 ± 248.6* (5)	1032.9 ± 423.4 (9)	930.0 ± 209.1 (7)	N.S.
	FSH	528.6 ± 88.8 (5)	396.2 ± 73.3 (10)	392.0 ± 76.9(7)	N.S.
23.00	LH	92.0 ± 21.8 (5)	107.3 ± 34.9(5)	188.8 ± 71.1 (5)	N.S.
	FSH	411.5 ± 96.7 (5)	645.9 ± 31.3 (5)	510.6 ± 66.1 (5)	N.S.

* Mean values expressed in ng/ml ± S.E.; the number of rats is indicated in parentheses.

† $P > 0.01$.

TEXT-FIG. 1. The influence of indomethacin (arrow) on the oestrous cycle of rats. Daily vaginal smears were recorded from rats treated with indomethacin in gelatin or saline or with indomethacin and $\text{PGF}_{2\alpha}$. D-I=dioestrus-I; D-II=dioestrus-II; P=pro-oestrus; E=oestrus.

As shown in Text-fig. 1, dioestrus persisted for 5 to 12 days in rats treated with indomethacin in gelatin with or without $\text{PGF}_{2\alpha}$, and for 5 to 9 days in rats treated with indomethacin in saline. One of the nine rats treated with indomethacin in gelatin did not return to oestrus and there was no disturbance of the normal cycle in one rat treated with indomethacin in saline.

DISCUSSION

The experiments reported in this paper demonstrate that the administration of indomethacin to normal pro-oestrous rats and immature rats primed with

PMSG prevented ovulation, and that this block was overcome by exogenous PG. The block to ovulation could not be overcome by giving LH-RH or HCG at a dose level sufficient to induce ovulation in rats treated with pentobarbital. These observations confirm those of a number of investigators including Tsafiriri *et al.* (1972), Armstrong & Grinwich (1972) and Behrman *et al.* (1972). By contrast, Orczyk & Behrman (1972) reported that inhibition of ovulation by indomethacin was reversed by LH when a minimal effective dose of indomethacin was given 3 hr before LH injection. Our results (Tables 3 and 4) strongly suggest that the ovulation-inducing action of PG is exerted directly on the ovary, rather than by stimulating LH release. Additional support for an ovarian site of action was provided by the observations that the oocytes held in the follicle completed their maturation division (Tsafiriri *et al.*, 1972) and that animals treated with indomethacin did not have fluid-filled uteri (Behrman *et al.*, 1972)—an effect that must be attributed to the availability of endogenous LH. Recently, Tsafiriri, Koch & Lindner (1973), using a radioimmunoassay technique, reported that indomethacin did not interfere with the pro-oestrous surge of LH.

On the basis of the results of the present experiments, it seems clear that indomethacin does not interfere with the pro-oestrous surge of LH secretion although ovulation was prevented in 83% of animals. Hence, a 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 gonadotrophins.

It has been found, however, that PGE₁, PGE₂ or PGF_{2α} significantly increased the serum LH level 10 to 60 min after a single injection in spayed rats primed with oestrogen and progesterone (Sato, Taya, Jyujo, Hirono & Igarashi, 1974). When indomethacin was given to cyclic rats at pro-oestrus, dioestrus persisted for 5 to 12 days before return to the normal 4-day cycle.

We conclude that indomethacin, at least when given as a single injection, does not block LH release, and exerts its antioviulatory action directly on the Graafian follicle under the conditions described. It also seems possible that indomethacin may act at the hypothalamo-pituitary level.

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