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Effect of Indomethacin on the Progesterone Secretion of Hysterectomized Pseudopregnant Rats

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The length of pseudopregnancy and the progesterone levels in rats pseudopregnant and hysterectomized on their 2nd day and injected daily with indomethacin (500 g/0.2 ml) from the 2nd to 10th day do not differ from those exhibited by oil treated rats. On the contrary, indomethacin treatment from 11th day on, results in both a lengthening of the diestrous phase and a delayed luteolysis. These findings point out to an extrauterine prostaglandin synthesis mechanism participating in the functional luteolysis in histerectomized pseudopregnant rats.

Key words: Progesterone, Pseudopregnancy, Prostaglandins inhibitors, Luteolysis.

The corpus luteum (CL) life span of pseudopregnant (PSP) rats depends on both the luteotrophic action of the two daily prolactin (PRL) surges (20) and the luteolytic effects of the uterus. The latter can be exercised directly, through prostaglandins (PGs), and indirectly, through the release of a PRL inhibiting factor (8). Hysterectomy, as well as PGs synthesis inhibitors, delays the functional luteolysis in PSP rats (9, 12).

Both, the PRL supported CL progesterone secretion and the regression of CL progesterone secretion phases, are longer in hysterectomized (HX) PSP rats than in ordinary PSP rats (5). When PRL secretion ceases, progesterone secretion declines in absence of any known luteolytic factor (functional luteolysis). The ability of either the post-ovulatory follicle or the CL to make PGs (16), and the participation of intraluteal PGs in functional (23) as well as in structural luteolysis (18) in autopituitary transplanted rats, have been reported.

The present work was undertaken to determine the effect of indomethacin (a PGs synthesis inhibitor) on the luteal regression of PSP rats deprived of the luteolytic action of the uterus, in order to

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see whether the inhibition of PGs synthesis at extrauterine sites may account for luteal regression.

Materials and Methods

Adult female Wistar rats (200-250 g) bred in this Department, kept in a light (12 L/12 D, light on at 07.00) and temperature controlled room, with free access to Sanders rat chow and tap water, were used. Vaginal smears were monitored daily and only females showing at least two consecutive four-day cycles were used.

Pseudopregnancy (PSP) was induced by mechanical stimulation of the cervix in the evening of proestrus and morning of estrus. The day of vaginal cornification was assigned day 1 of PSP. The duration of PSP was assessed by daily examination of the vaginal smears up to the appearance of epithelial nucleated cells.

Hysterectomy (HX) was done through a mid line abdominal incision on day 2 of the cycle under ether anaesthesia. Sham HX consisted in laparotomy and manipulation of the uterus without removing it.

Indomethacin (IM) (Sigma) was prepared in oil solution at 2.5 g/l. From this solution 0.2 ml were subcutaneously injected according to the experimental plan.

Less than 0.5 ml of blood were obtained under light ether anaesthesia by direct jugular venopuncture on days 2, 5, 8 and 11 of the cycle; afterwards, blood samples were allowed to clot and centrifuged at 4°C, and the serum stored at –20°C until assayed by RIA for progesterone using the GDN-337 antiprogesterone serum as previously described (6). Samples were assayed in duplicate in the same assay. The coefficient for intra-assay variability was 13.6 %.

Experimental plan. — Day 2 HX-PSP rats injected daily with 0.2 ml of oil (n = 11) up to the end of PSP served as control of both, the effect of HX on CL function after its comparison with Sham-HX-PSP rats (n = 12) and the effect of daily IM injections either from day 2 to day 10 (n = 5) or from day 11 to the end of PSP (n = 6) in day 2 HX-PSP rats. The effect of HX and IM treatment on both the duration of vaginal diestrous and the progesterone levels, were analyzed by two way analysis of variance (ANOVA) to look for any significant differences between groups. When found, they were compared by Newman-Keuls multiple range tests. $P > 0.01$ was considered to be non significant.

Results

Hysterectomized PSP rats treated with oil, as well as those treated with IM, had longer PSP periods than sham HX-PSP rats. HX-PSP rats treated with IM during the second half of PSP displayed slight but significant prolongation of duration (table I).

No differences in serum progesterone levels were observed between groups up to day 8 of PSP. On the contrary, HX induced the maintenance of high serum progesterone levels up to day 15. On day 17 of PSP, serum progesterone levels of both HX-PSP rats treated with oil and HX-PSP rats treated with IM during the first half of PSP, were lower than those of HX-PSP rats treated with IM during the second half of the PSP (table I).

Discussion

In ordinary pseudopregnant rats, CL progesterone secretion ceases mainly because of the effect of the uterine PGs, that reach the CL through the utero-ovarian circulation. The two uterine PGs surges on days 7 and 10 (17) seem to increase the ovarian activity of

Table I. *Effect of day 2 hysterectomy (HX) and indomethacin treatment (IM) from day 2 to day 11 or from day 11 to the end of pseudopregnancy (PSP) on the duration of PSP and on the progesterone levels.*

The values are shown as mean ± SE. Hysterectomy was done on day 2 of PSP. Indomethacin (500 µg/0.2 ml) was injected once/day, either on days 2-10 (IM-OIL) or on days 11 onward (OIL-IM). a: $P < 0.01$ vs ordinary PSP, b: $P < 0.01$ vs HX-PSP (OIL-OIL) and vs HX-PSP (IM-OIL). Analysis of variance and Newman-Keuls multiple range test. n: number of determinations.

		Progesterone levels (nmol/l) on days:								
Group	n	2	5	8	11	13	15	17	19	PSP (days)
Ordinary (PSP)	12	29.9 ± 4.8	171.1 ± 13.3	137.1 ±10.8	23.2 ± 5.1	—	—	—	—	11.6 ± 0.8
HX-PSP (OIL-OIL)	11	23.2 ± 2.2	167.7 ± 8.6	161.9 ± 8.9	159.7 ± 10.2[a]	130.8 ± 6.7	110.3 ± 10.2	14.6 ± 2.5	—	17.0 ± 0.6[a]
HX-PSP (IM-OIL)	5	22.9 ± 7.6	158.1 ± 10.5	154.9 ± 7.0	158.1 ± 6.7[a]	142.2 ± 6.0	114.9 ± 8.3	9.5 ± 2.9	—	17.2 ± 0.4
HX-PSP (OIL-IM)	6	17.5 ± 2.2	168.3 ± 18.7	159.1 ± 18.4	166.0 ± 18.1[a]	167.6 ± 31.4	145.4 ± 25.4	104.1 ± 10.5[b]	19.7 ± 7.0	19.0 ± 0.7[ab]

20 α-hydroxysteroid dehydrogenase (20 α-OHSD) (4) responsible for converting progesterone to its less active metabolite 20 α-hydroxyprogesterone (20 α-OHP). Hysterectomy delays the onset of augmented 20 α-OHSD activity (2), prolongs the duration of pseudopregnancy (13) and abolishes the increase in ovarian PGs (9). Indomethacin treatment lengthens the diestrous period of pseudopregnant rats to that found in hysterectomized PSP ones (12).

It has also been demonstrated in pregnant rats near delivery that indomethacin treatment inhibits both the increased production of PGs which occurs near term (3) and the normal increase in luteal 20 α-OHSD activity (21), and protracts the antepartum luteolysis (7).

In contrast to both, the regression of the luteal phase in ordinary PSP, which is initiated by a PG-mediated uterine mechanism, and the antepartum luteolysis in pregnancy near term, which is probably initiated by the release of PGs from the uterus (3, 15, 19), the exact nature of luteal regression in hysterectomized PSP rats is poorly understood. Progesterone levels in hysterectomized PSP rats consist in a rising phase (day 2-5), a plateau phase (day 5-11) and a regression phase (day 11 onwards) (table I). Indomethacin treatment during the first part of PSP (rising and plateau phases) did not affect the duration of the PSP or the serum progesterone levels. On the contrary, indomethacin treatment during the regression phase did prolong the length of PSP and postponed the functional luteolysis (table I) which is an expression of the CL autonomy to make progesterone.

Prostaglandins are not stored in the tissues, and their half-life in the circulation is very short (11), so they should act locally, with the only exception of prostaglandins secreted by the uterus. Therefore, the effect of the treatment with indomethacin on the secretion of progesterone by the CL could be interpreted as a consequence of the prostaglandins synthesis inhibition at a local level.

It has been postulated that once the CL has reached its maximum progesterone secretion capacity, it turns to produce prostaglandins, which are responsible for luteolysis (16). The results presented here show that prostaglandins synthesis inhibition in hysterectomized PSP rats during the regression phase of progesterone secretion by the CL brings about a prolongation of pseudopregnancy duration. It might be argued that this effect could be due to an indomethacin action on the release of gonadotrophins. Since prostaglandins synthesis inhibitors treatment has no effect *in vivo* on the preovulatory LH release in the rat (22), and taking into account that hysterectomized PSP rats given with indomethacin during the regression phase of progesterone secretion do prolong the period of secretion of this hormone, the latter effect is almost certainly due to the inhibition of ovarian prostaglandins. During the first part of pseudopregnancy, the CL has a high potential ability to produce progesterone, which keeps the intraluteal prostaglandins sythesis mechanism and/or prostaglandins effect depressed (16, 23). This fact seems to explain by itself the lack of effect of indomethacin treatment during the early phase of CL life (table I).

The present experiment evidences that prostaglandins synthesis inhibition at extrauterine sites postpones the functional luteolysis in hysterectomized pseudopregnant rats, and supports that, as it has been demonstrated both in other models (14) and in other species (1), the intraluteal prostaglandins are involved in the luteolytic process.

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Resumen

La duración de la pseudopreñez y los niveles de progesterona en ratas pseudopreñadas e histerectomizadas en el día 2 y tratadas diariamente con 500 µg/0,2 ml de indometacina hasta el día 10, son similares a los de las ratas tratadas con aceite. Por el contrario, el tratamiento con indometacina desde el día 11 hasta el final de la pseudopreñez produce una prolongación de la fase de diestro y un retraso de la luteolisis funcional. Se interpretan estos resultados como indicativos de la existencia de un mecanismo extrauterino de síntesis de prostaglandinas que participan en la luteolisis funcional en las ratas pseudopreñadas e histerectomizadas.

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