EFFECT OF INDOMETHACIN, AN INHIBITOR OF PROSTAGLANDIN BIOSYNTHESIS ON THE LENGTH OF PSEUDOPREGNANCY IN RATS AND HAMSTERS

By
I. F. Lau, S. K. Saksena and M. C. Chang

ABSTRACT

Pseudopregnancy (PSP) in rats was significantly lengthened after subcutaneous administration of 0.2–1.0 mg indomethacin/day, starting on day 5 of PSP. Injections of 1 mg indomethacin on days 5 and 6 or days 7 and 8, as well as a single injection of 2 mg indomethacin on day 6, also produced a significant increase of the duration of PSP. In hamsters, doses of 300–500 µg indomethacin per day starting on day 5 of PSP did not alter the length of PSP, but a significant prolongation varying from 11 to more than 30 days was observed after a treatment with 1 mg of indomethacin daily. It was further observed that the ovaries of hamsters autopsied on day 31 of PSP were very small and the uteri resembled those of ovariectomized animals. It is concluded that indomethacin lengthens pseudopregnancy in both rats and hamsters and that the latter species showed a more variable response. It seems that chronic administration of indomethacin in hamsters caused a long-lasting suppression of ovarian hormone production.

Hysterectomy prolongs the life span of corpora lutea in most laboratory and farm animals. It was suggested that uterus in an unknown manner controls the luteal functions (Asdell & Hammond 1933; Bradbury 1937; Wiltbank & Casida 1956; Perry & Rowlands 1961; Fischer 1965; Ginther et al. 1966; Duby et al. 1969; Orsini 1969). Recently, uterine luteolysin has been identified as prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}) in several animal species (Inskeep & Butcher 1966;
post-ovulatory

The starting of ovaries was done on pro-oestrous of animals (Gutknecht et al. 1969; McCracken et al. 1970; Bartke et al. 1972; Chang & Hunt 1972; Labhsetwar 1974; Lau et al. 1974; Lau et al., in press; Saksena et al., in press). Indomethacin, an inhibitor of PGs synthesis (Vane 1971) prolongs pseudopregnancy (PSP) in rabbits (O'Grady et al. 1972), extends oestrous cycle in guinea pigs (Marley 1973), delays parturition in rats and monkeys (see Labhsetwar 1974; Novy et al. 1974) and prevents implantation in mice (Lau et al. 1973). Here we report the effects of indomethacin administration on the duration of pseudopregnancy in rats and hamsters.

MATERIALS AND METHODS

Female CD rats (200–225 g) obtained from Charles River Breeding Laboratories and female hamsters (120–130 g) obtained from a local breeder were maintained in a light (12 h) and temperature (23 ± 1°C) controlled room. Vaginal smears were examined daily and only females showing at least 2 consecutive 4-day cycles were used in this study. In rats the pseudopregnancy (PSP) was induced by mechanical stimulation of the cervix in the evening of pro-oestrus and morning of oestrus and the day of vaginal cornification was assigned as day 1 of PSP (De Feo 1963). The duration of PSP was assessed by daily examination of vaginal smears and the appearance of pro-oestrous type smear was considered as the last day of PSP. In hamsters, PSP was induced by mating with vasectomized males on the day of pro-oestrus, and the following day was regarded as day 1 of PSP. All females were examined daily for post-ovulatory vaginal discharge (Orsini 1961) to determine the duration of PSP. Animals which did not return to oestrus within 30 days were killed on day 31, and ovaries and uteri were excised and examined.

Indomethacin (gift from Merck, Sharpe & Dohme) was injected in sesame oil, 0.2 ml in rats and 0.1 ml in hamsters. All treatments were given subcutaneously beginning on day 5 of PSP unless stated otherwise.

RESULTS

In the rat, the length of pseudopregnancy (PSP) was from 11 to 12 days with a mean of 11.7 ± 0.2 days. Daily subcutaneous injections of indomethacin starting from day 5 of PSP resulted in significant elongation of PSP (Table 1). The response appeared dose-dependent, since a dose of 200 µg indomethacin/day extended PSP to 14.6 days and the doses of 600 µg and 1 mg extended to 16.4 and 16.1 days, respectively. In all indomethacin treated groups the
Table 1.
Effect of indomethacin on the length of pseudopregnancy in rats.

<table>
<thead>
<tr>
<th>Dose/injection</th>
<th>Duration (days)</th>
<th>Number of animals</th>
<th>Mean length of pseudopregnancy in days ± SE (range)</th>
<th>Percentage prolongation of pseudopregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>9</td>
<td>11.7 ± 0.2 (11–12)</td>
<td>0</td>
</tr>
<tr>
<td>200 µg</td>
<td>From day 5</td>
<td>5</td>
<td>14.6 ± 0.2 (14–15)*</td>
<td>24.8</td>
</tr>
<tr>
<td>400 µg</td>
<td>of PSP until</td>
<td>6</td>
<td>15.5 ± 0.8 (13–18)*</td>
<td>32.5</td>
</tr>
<tr>
<td>600 µg</td>
<td>pro-oestrous</td>
<td>7</td>
<td>16.4 ± 0.4 (15–18)*</td>
<td>40.0</td>
</tr>
<tr>
<td>1 mg</td>
<td>smear</td>
<td>7</td>
<td>16.1 ± 0.4 (14–17)*</td>
<td>37.6</td>
</tr>
<tr>
<td>5, 6</td>
<td></td>
<td>5</td>
<td>13.2 ± 0.6 (12–15)*</td>
<td>12.8</td>
</tr>
<tr>
<td>1 mg</td>
<td>7, 8</td>
<td>6</td>
<td>13.5 ± 0.6 (12–15)*</td>
<td>15.4</td>
</tr>
<tr>
<td>9, 10</td>
<td></td>
<td>5</td>
<td>12.2 ± 0.4 (11–13)</td>
<td>4.3</td>
</tr>
<tr>
<td>2 mg</td>
<td>6</td>
<td>6</td>
<td>13.0 ± 0.4 (12–15)*</td>
<td>11.1</td>
</tr>
</tbody>
</table>

* Denotes a significant difference from controls at P < 0.01; Student’s t-test.

PSP length was significantly different from that in vehicle treated control animals (P < 0.01). In another experiment where a dose of 1 mg of indomethacin was given either on days 5 and 6 or on days 7 and 8, all animals showed a prolonged PSP. The same dose failed to alter the length of PSP when administered on days 9 and 10. However, a single injection of 2 mg of indomethacin on day 6 significantly increased the duration of PSP.

In hamsters, the normal length of PSP was 9.1 ± 0.1 days. Daily subcutaneous administration of 300 or 500 µg of indomethacin, starting from day 5 of PSP, did not alter the length of PSP, but a significant elongation varying from 11 to more than 30 days was observed after a treatment with 1 mg of indomethacin (Table 2). The animals which were pseudopregnant for over 30 days had very small ovaries with no developing follicles and uteri resembling those from ovariectomized females.

**DISCUSSION**

The mean length of pseudopregnancy in the vehicle treated (control) hamsters and rats agrees with values reported earlier (Lau et al. 1974; Saksena et al. 1974b). A prolonged subcutaneous treatment with indomethacin at a dose of 200 µg/day/rat (approximately 1 mg/kg b.w.) resulted in a prolonged PSP.
Table 2.
Effect of indomethacin on the length of pseudopregnancy in hamsters.
(Treatment started on day 5 of PSP).

<table>
<thead>
<tr>
<th>Treatment dose/injection</th>
<th>Number of animals</th>
<th>Length of pseudopregnancy in individual animals (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame oil (controls)</td>
<td>8</td>
<td>9, 9, 9, 9, 9, 10, 9, 9</td>
</tr>
<tr>
<td>300 μg*</td>
<td>8</td>
<td>9, 9, 9, 9, 9, 10, 9</td>
</tr>
<tr>
<td>Indomethacin 500 μg*</td>
<td>10</td>
<td>9, 9, 9, 10, 9, 9, 9, 10</td>
</tr>
<tr>
<td>1 mg**</td>
<td>7</td>
<td>11, 23, 23, &gt; 30, &gt; 30, &gt; 30, &gt; 30</td>
</tr>
</tbody>
</table>

* Indomethacin was administered until the return of post-ovulatory discharge.

** Indomethacin treatment was continued from day 5 until post-ovulatory discharge or until day 22 of PSP. Vaginal smears were checked up to day 31 and the animals were killed on the same day.

It was further observed that increase in the length of PSP was related to the dose of indomethacin. A single injection of 2 mg of indomethacin (8 mg/kg b. w.) increased the length of PSP, but prolonged administration of this dose was lethal and all treated females died within 5 days of treatment.

In an earlier study we have observed two peaks of prostaglandins F in the plasma of pseudopregnant rats; one on day 7 and another of shorter duration on day 10 (Saksena et al. 1974b). In the present study, we recorded a lengthened PSP when indomethacin was administered on days 5 and 6 or 7 and 8 but not when it was given on days 9 and 10 of PSP. It seems that administration of indomethacin on days 5 and 6 or 7 and 8 prevented or delayed the peaks of PGF and consequently, corpora lutea were not exposed to PGF; and functional luteolysis, a phenomenon where progesterone output is depressed, was prevented. Presumably by day 9 of PSP, functional luteolysis already could have taken place, and therefore, administration of indomethacin did not have any effect on PSP.

Since in the rat PG synthetases were reported to be resistant to inhibitors of PG's synthesis (see Labhsetwar 1974), it is reasonable to assume that endogenous production of PG might have not been completely suppressed after prolonged administration of indomethacin. The termination of PSP in spite of continued administration of indomethacin could indicate that presumably depressed PGs production was sufficient to cause luteal regression.

In contrast to the situation observed in the rat, the duration of PSP in hamsters was not affected after daily injections of 300–500 μg of indomethacin. However, 1 mg indomethacin/day/hamster caused a significant increase in
PSP length. The elongation of PSP in hamsters varied from 11 to more than 30 days (Table 2). Hamsters are the most sensitive species to the luteolytic action of PGF$_2\alpha$. It, therefore, appears possible that 300–500 µg dose of indomethacin did not completely inactivate the PG-synthetase enzymes and sufficient amounts of PGs were produced to cause luteolysis at the normal time. Another possibility is that indomethacin was metabolised rapidly and 300–500 µg doses were, therefore, not adequate for suppression of PGs production. One mg dose of indomethacin suppressed PGs production and PSP was maintained. Moreover, 1 mg indomethacin in hamsters seems to have altered the ovarian functions, since on day 31 of PSP the ovaries and uteri were considerably smaller than those of normal cyclic hamsters at any stage of the cycle. There were no corpora lutea or developing follicles. It seems that the damage to the ovaries was extensive and impaired their endocrine secretory functions. The assumption that the ovarian functions might have been impaired is further supported by the observation that the indomethacin-treated PSP rats did not have a normal receptivity. These animals did not mate when paired with fertile males even though they showed pro-oestrous type vaginal smear. In contrast, the normal control rats mated normally on the day of PSP termination. The present study clearly shows that indomethacin prolongs PSP in rats and hamsters. Hamsters showed a more diverse response to the indomethacin treatment. From this study it is not clear whether indomethacin acted at the ovarian level, on the pituitary level, or had a general toxic effect causing suppression of ovarian functions in the hamster.

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