PITUITARY LH CONTENT DURING DELAYED IMPLANTATION INDUCED BY DIFFERENT TREATMENTS IN RATS

By

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ABSTRACT

The capacity of oestrogens to induce nidation during delayed implantation and the known dependence of oestrogen secretion on LH prompted a detailed study of the pituitary LH content of rats in which implantation of blastocysts was delayed by various procedures. Implantation was delayed by Provera (2 mg), reserpine (200 μg), oral dosing with the antioestrogen I.C.I. 46,474 (40 μg) or clomiphene (100 μg), ovariectomy followed by progesterone treatment and finally by permitting mothers mated postpartum to suckle a large litter (10 pups). The pituitary LH stores (OAAD method) of such animals were compared with those of rats killed in prooestrus, pseudopregnant or pregnant and on day 9 of pseudopregnancy, pregnancy or lactation. Hypophyseal LH levels were very low during delayed implantation induced by Provera or suckling and very high during that induced by ovariectomy followed by progesterone treatment compared with those in prooestrous, pseudopregnant or pregnant controls. By contrast, no significant change in LH stores was observed when nidation was delayed by single injections of I.C.I. 46,474 or clomiphene. Delayed implantation could not be induced in intact rats with chlormadinone or norgestrel even when given in large doses (2–8 mg/day). It is postulated that to delay implantation in the presence of the ovaries, a centrally acting agent must be capable of decreasing synthesis of LH in the pituitary.

While delayed implantation occurs regularly in several species (see Enders 1963, 1967), in rats it occurs naturally only when an animal mated postpartum is suckling a large litter (Weichert 1942). This condition can, however, be induced experimentally in this species by ovariectomy followed by daily treatment with progesterone (Cochrane & Meyer 1957; Mayer 1959). Even in the intact
rat implantation can be delayed by administration of Provera (Barnes & Meyer 1964) or some other progestins (Nutting & Sollman 1967), of neurodepressive agents such as trifluoperazine, chlorpromazine (Psychos 1963) or reserpine (Mayer 1963), of antioestrogens such as I. C. I. 46,474 (Harper & Walpole 1967) or of LH-antiserum (Madhawa Raj et al. 1968). In addition, autotransplantation of the anterior pituitary (Cochrane et al. 1962; Mayer 1963) or appropriate hypothalamic lesions (Gale & McCann 1961) lead to delayed implantation in pregnant rats.

Thus several seemingly unrelated treatments delay implantation. It is not known if they all act by a common pathway. Since exogenous oestrogen can induce nidation, it is probable that the treatments cause delay by creating a state of relative oestrogen deficiency. The secretion of oestrogen adequate to cause implantation of blastocysts can be induced by administration of LH in an absorption-delaying vehicle (Macdonald et al. 1967). This prompted an examination of the hypophyseal LH content of pregnant rats in which implantation had been delayed by several different procedures. A preliminary report of experiments with Holtzman rats has appeared (Labhsetwar 1969c).

**Materials and Methods**

The rats used were females of Alderley Park Strain 1, originally derived from Wistar rats and randomly bred for several generations under specific pathogen-free conditions. In addition some Holtzman rats were employed as indicated in Fig. 1. In either case, animals were housed under a 14 hours light/10 hour dark cycle and allowed free access to food and tap water. Vaginal smears were taken daily and rats in prooestrus were caged overnight with fertile males. The next day, when sperms were found in the smear, was taken as day 1 of pregnancy. Implantation of blastocysts was then delayed by one or other of the following treatments:

a) **Provera** (17α-acetoxy-6α-methyl-progesterone). The progestin was injected daily in oil (2 mg sc) from days 1 to 8 of pregnancy (Barnes & Meyer 1964).

b) **Reserpine.** The agent was injected sc in aqueous solution from days 1 to 8 of pregnancy (Mayer 1963). Each rat received 200 μg/day for the first few days; but as rats lost considerable weight the dose was reduced to 150 μg.

c) **I.C.I. 46,474 (40 μg)** or d) **Clomiphene (100 μg).** Both compounds are triphenylethylene derivatives (see Walpole 1968) for structural formulae) and are potent antioestrogens in rats. The drugs were given orally in single doses at 17:00 h on day 4 of pregnancy, and the rats killed on day 9 (Harper & Walpole 1967).

e) **Ovariectomy followed by progesterone treatment.** The rats were bilaterally spayed under ether anesthesia on the morning of day 3 of pregnancy and injected daily with progesterone (4 mg/day, sc in oil) until day 8 of gestation (Cochrane & Meyer 1957; Mayer 1959).

f) **Lactation delay.** Rats were mated postpartum and allowed to suckle a large litter (mean 10; range 9-11) (Weichert 1942).

In addition, attempts were made to induce delayed implantation in intact pregnant rats by injecting sc chlormadinone (2–8 mg/rat/day) or norgestrel (2–5 mg/rat/day) from days 1 to 8 (n = 2-10/group).
Pituitary LH content (µg equivalents of NIH-S-11) of rats in which implantation was delayed by several different treatments. The shaded columns refer to Holtzman rats and the open ones to Wistar derived rats. Asterisk indicates the control group killed without regard to the stage of oestrous cycle. Each value in the lower figure represents mean and 95% confidence limits.

For comparison control rats were killed during the oestrous cycle (prooestrus and oestrus), or on day 9 of pseudopregnancy, pregnancy or lactation. In the latter case mothers nursed an average of 10 pups (9–12 range).

Unless otherwise stated all the treated rats were killed by ether on the morning of day 9 of pregnancy. Uteri were weighed and then flushed to determine the presence of blastocysts. Any mated rat from which no blastocyst could be recovered was discarded. Anterior pituitary glands and ovaries (when present) were also weighed. The pituitaries were pooled within each experimental group (sometimes they were pooled in two lots/group) and stored at –20°C for bioassay of LH.

**LH assay**

On the day before assay the glands were thawed and homogenized in sterile physiological saline, diluted to the desired concentration and stored in the refrigerator. They were assayed by the OAAD method of Parlow (1961) employing oestrogenized assay rats (Bogdanove & Gay 1967). Immature rats were primed with PMS (75 IU) and HCG (75 IU) beginning on day 24–26 of life as described elsewhere (Labhsetwar 1967). Beginning 5 days after HCG injection, each rat received 10 µg of oestradiol undecylate (Schering A. G., Berlin) in oil by the subcutaneous route every 48 h for 7
days when either one or both ovaries of the rats were used for assay (Labhsetwar 1969d). When only one ovary was used, the other was employed 7 days later, injections of oestrogen being continued in the intervening period. A 2 + 2 assay design was used throughout this study and 5 rats were employed at each dose level. The interval between doses was 5 fold. The assay data were analysed by the method of Gaddum (1953) and Emmens (1962) for parallel line assays employing multiple assay design analysis of Borth (1960).

The pituitary glands from some of the experimental groups were assayed twice and the duplicate potency estimates were combined by the method of Sheps & Moore (1960). The indices of precision for results shown in Fig. 1 were all below 0.3.

RESULTS

All treatments used except for injection of chlormadinone or norgestrel were successful in delaying implantation as judged by the presence of unimplanted blastocysts at autopsy on day 9 of pregnancy. Changes leading to implantation in Holtzman rats can be detected by the electron microscope on the evening of day 5 of gestation (Enders & Schlafke 1967). It has been reported earlier that a single oral dose of the antioestrogen I.C.I. 46,474 delays implantation (Harper & Walpole 1967); in the present study clomiphene was also found to be similarly effective. In rats given daily doses of chlormadinone or norgestrel, implantation sites were invariably found at autopsy.

Organ weights. The ovarian weights of the pseudopregnant and pregnant controls were significantly lower than those of the prooestrous control rats (Table 1). Similarly uterine weights of pseudopregnant and lactating control rats were also significantly lower than those in the prooestrous control group (P < 0.01, Table 1). In the lactating control animals the ovaries and uteri were also lighter on a relative basis (mg/100 g b. w.) than those of the prooestrous controls.

In the animals with delayed implantation, except for the reserpine-treated group, ovarian and uterine weights were lower than in the prooestrous, but not the pseudopregnant controls (Table 1). In the reserpine treated rats uteri were lighter than in the prooestrous controls both on an absolute (Table 1) and a relative basis (mg/100 g b. w.). The ovarian weights, however, were lower only on an absolute (Table 1) and not on a relative basis.

Pituitary LH. The gonadotrophin level at oestrus was significantly lower than at prooestrus, confirming earlier observations of Schwartz & Bartosik (1962).

Similarly in lactating control rats suckling a large litter pituitary LH stores were lower than in prooestrous controls (Fig. 1). On the other hand, the LH content on day 9 of pseudopregnancy or pregnancy was comparable with that at prooestrus — as has already been reported by Schwartz & Rothchild (1964) for pseudopregnant and Schwartz & Talley (1968) for pregnant rats. Most of
Table 1.
Organ weights of rats\textsuperscript{b)} in delayed implantation period induced by various procedures (Mean ± se).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>B. W. (g)</th>
<th>Ov. Wt. (mg)</th>
<th>Uterine Wt. (mg)</th>
<th>Pituitary Wt. (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prooestrous control</td>
<td>10</td>
<td>204 ± 4</td>
<td>79.8 ± 2.0</td>
<td>367 ± 19</td>
<td>9.5 ± 0.56</td>
</tr>
<tr>
<td>Oestrous control</td>
<td>10</td>
<td>208 ± 7</td>
<td>80.9 ± 5.2</td>
<td>362 ± 19</td>
<td>11.44\textsuperscript{a) }</td>
</tr>
<tr>
<td>Pseudopregnant control</td>
<td>8</td>
<td>225 ± 7</td>
<td>65.0 ± 2.8\textsuperscript{*}</td>
<td>273 ± 9\textsuperscript{*}</td>
<td>10.00 ± 0.37</td>
</tr>
<tr>
<td>Pregnant control</td>
<td>8</td>
<td>229 ± 4</td>
<td>61.3 ± 2.3\textsuperscript{*}</td>
<td>–</td>
<td>9.53 ± 0.46</td>
</tr>
<tr>
<td>Lactating control</td>
<td>7</td>
<td>325 ± 19\textsuperscript{***}</td>
<td>86.8 ± 3.3</td>
<td>313 ± 22\textsuperscript{*}</td>
<td>11.21 ± 1.20\textsuperscript{*}</td>
</tr>
<tr>
<td>Ovariectomy +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone delay (4 mg)</td>
<td>10</td>
<td>223 ± 3</td>
<td>–</td>
<td>238 ± 8\textsuperscript{***}</td>
<td>10.37 ± 0.20</td>
</tr>
<tr>
<td>Provera delay (2 mg)</td>
<td>7</td>
<td>227 ± 6</td>
<td>56.3 ± 2.8\textsuperscript{**}</td>
<td>289 ± 9\textsuperscript{*}</td>
<td>9.41 ± 0.34</td>
</tr>
<tr>
<td>I.C.I. 46,474 delay (40 µg)</td>
<td>8</td>
<td>219 ± 8</td>
<td>61.2 ± 1.8\textsuperscript{*}</td>
<td>278 ± 14\textsuperscript{**}</td>
<td>10.50 ± 0.30</td>
</tr>
<tr>
<td>Clomiphene delay (100 µg)</td>
<td>5</td>
<td>227 ± 5</td>
<td>61.2 ± 5.7\textsuperscript{*}</td>
<td>284 ± 16\textsuperscript{**}</td>
<td>10.80 ± 0.64</td>
</tr>
<tr>
<td>Reserpine delay (200 µg)</td>
<td>8</td>
<td>133 ± 3\textsuperscript{***}</td>
<td>48.6 ± 1.7\textsuperscript{***}</td>
<td>150 ± 6\textsuperscript{***}</td>
<td>8.42 ± 0.48</td>
</tr>
</tbody>
</table>

\textsuperscript{*} P < 0.05; \textsuperscript{**} P < 0.01; \textsuperscript{***} P < 0.005 when compared with prooestrous control.

\textsuperscript{a) } Pituitaries were not weighed individually.

\textsuperscript{b) } All rats of the Wistar-derived strain.
these observations were made on Sprague-Dawley derived rats. The present results indicate that similar fluctuations occur in the Wistar-derived rats used here, although the absolute level of LH found in these rats is higher than that at corresponding stages in Sprague-Dawley rats.

Pituitary LH during delayed implantation induced by Provera or by bilateral ovariectomy followed by progesterone treatment was studied both in Holtzman and Wistar-derived rats. The results were essentially similar (Fig. 1) and will therefore be considered together. Both lactation and Provera induced delay was associated with markedly lower levels of pituitary LH than those in prooestrous, pseudopregnant or pregnant controls (Fig. 1). On the other hand, when implantation was delayed by ovariectomy and progesterone treatment, pituitary LH stores were significantly higher than those present in the 3 control groups (Fig. 1). Both anti-oestrogens, although effective in inducing delay under the conditions used, failed to alter pituitary LH stores significantly (Fig. 1). The same was true with reserpine.

**DISCUSSION**

When implantation was delayed by bilateral ovariectomy and progesterone treatment, pituitary stores of LH were significantly higher than those in any of the control groups (Fig. 1). Bilateral ovariectomy of cyclic rats is known to result within two weeks in increased synthesis and release of LH (Parlow 1964; Labhsetwar 1969b; Gay & Midgley 1969). Injection of progesterone in the doses used here into rats ovariectomized during the oestrous cycle does not affect the accumulation of LH in the pituitary, but does reduce plasma levels of the hormone (Labhsetwar 1969a). Thus the response of the pituitary of the pregnant animal to ovariectomy plus progesterone treatment does not seem to differ materially from that of the cyclic animal. However, this needs to be confirmed by concurrent assay of pituitary glands from both groups.

Both the suckling stimulus (Rothchild 1967) and reserpine (Meites & Nicoll 1966) obviously act through a neural pathway. Implantation delayed by suckling was associated with lower levels of LH than those found in control prooestrous, pseudopregnant or pregnant rats. It has already been reported that suckling (Parlow & Rothchild 1960; Kobayashi et al. 1965; Minaguchi & Meites 1967) and reserpine treatment (Labhsetwar 1967) reduce synthesis of LH in the pituitary. With reserpine, however, decreased synthesis is not reflected in a decreased pituitary LH concentration (µg/mg) (Fi. 1; Labhsetwar 1967). Data obtained in this study suggest that the effect of these treatments are similar during pregnancy. It is probable that this results in a lower secretion of oestrogen by the ovaries with consequent delay of implantation.
Of the 3 progestins used, only Provera proved to be effective in delaying implantation. Barnes & Meyer (1964) attributed this effect of Provera to inhibition of synthesis of LH which is seen also in non-pregnant rats (Labhsetwar 1966). The fact that pituitary LH stores in Provera-induced delay were significantly lower than in the corresponding stages of pseudopregnancy or pregnancy adds further support to this view.

Chlormadinone and norgestrel, although used in relatively large doses, proved ineffective in delaying nidation. Both of these compounds are either equally or more potent than Provera as progestational agents in rabbits (Clauberg assay) and also as anti-oestrogens in mice (Brennan & Kraay 1963; Edgren et al. 1966). This suggests that progestational activity as shown in rabbits or anti-oestrogenicity as judged in mice is not adequate to delay implantation in rats. Both of these progestins, however, are considerably weaker as inhibitors of pituitary gonadotrophin secretions than Provera in parabiotic or unilaterally spayed rats (Brennan & Kraay 1963; Edgren et al. 1966). In fact, chlormadinone causes a marked storage of LH (and FSH) in the pituitary of intact rats (Labhsetwar 1968) similar to that caused by progesterone (see Rothchild 1965; Labhsetwar 1969a). Thus it appears that to be fully effective in inducing delay, a progestin must be capable of decreasing synthesis of LH.

The mode of action of non-steroidal anti-oestrogens as exemplified by I.C.I. 46,474 and clomiphene seems to be entirely different from that of the progestins. Harper & Walpole (1967) have postulated that I.C.I. 46,474 when given on the afternoon of day 4 of pregnancy as in this study delays implantation not by blocking the oestrogen surge but by preventing the action of secreted oestrogen on the uterus. This implies that the agent acts peripherally rather than centrally. This hypothesis is consistent with our failure to detect any significant changes in the pituitary LH stores during the delay induced by these agents. It is further supported by the fact that the oestrogen surge has already occurred by the time of injection of the anti-oestrogens on day 4 (Yoshinaga et al. 1969) and that the role of the pituitary in initiating implantation is believed to have been completed by the morning of day 4 (Alloiteau 1961; Madhawa Raj et al. 1968) although Zeilmaker (1963) reported different results.

In summary, the data presented in this study show that a variety of experimental manipulations results in rats in delay of implantation and this is associated with variable changes in pituitary LH stores. It seems that centrally acting agents such as certain progestins, neurodepressive agents and suckling delay implantation in the presence of the ovaries by decreasing synthesis of LH in the pituitary with a consequent lowering of oestrogen secretion by the gonads. By contrast, anti-oestrogens cause delay without altering the gonadotrophin content of the pituitary or, presumably, the oestrogen output of the ovaries: these prevent the action of secreted oestrogen on the uterus.
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