DISPARITY BETWEEN THE STATES OF 
PSEUDOPREGNANCY INDUCED BY RESERPIE AND BY 
CERVICAL STIMULATION IN THE RAT

By
E. S. Kisch

ABSTRACT
The states of pseudopregnancy induced by electrical stimulation of the cervix uteri or by injection of reserpine were compared. Pseudopregnancy induced by either method can be interrupted by the administration of ergocornine, resulting in a return to oestrus. When the pseudopregnancy is induced by electrical stimulation, this oestrus is followed in about 70% of cases by a secondary pseudopregnancy: the stimulus for the induction of the initial pseudopregnancy seems to be »remembered« in the central nervous system. This phenomenon of secondary pseudopregnancy could not be elicited in animals treated with reserpine only. The findings point to a different central mode of action of the two methods of prolonging luteal function.

Many diverse stimuli are capable of eliciting pseudopregnancy in the rat by the initiation of a period of luteotrophic dominance of the hypothalamic-hypophyseal complex. The pseudopregnancy-inducing stimulus is »remembered« in the central nervous system (CNS) for about eight days (Zeilmaker 1965; Everett 1967; Kisch 1968).

A standard method for the induction of pseudopregnancy is electrical stimulation of the cervix uteri (Shelesnyak 1931). Another method is the injection of reserpine (Barraclough & Sawyer 1959). We have compared the effectiveness of these methods in the induction of pseudopregnancy as well as their capacity to activate the CNS »memory« as expressed by the incidence of secondary pseudopregnancy: when oestrus is induced within this period of CNS »memory«, the newly formed corpora lutea still benefit from the per-
sisting luteotrophic dominance and a new period of pseudopregnancy ensues, the so-called secondary pseudopregnancy.

The ergot alkaloid ergocornine (ECO) was used for the induction of such an oestrus in pseudopregant rats. This drug interrupts hypophysial luteo- 

trophin secretion and thereby causes luteal failure, followed by recurrence of oestrus within about three days (Shelesnyak 1955; Lindner & Shelesnyak 1967).

MATERIALS AND METHODS

Adult female rats from the Wistar-derived Biodynamics Colony, who had exhibited at least two consecutive regular 4-day cycles, were used. Pseudopregnancy was induced either by (1) electrical stimulation of the cervix uteri on the morning of the days of pro-oestrus and oestrus (this type of pseudopregnancy will be referred to as »EP«); or (2) by subcutaneous injection of reserpine (Serpasil®, Ciba) in different doses on the first day of leucocytic smear of the experimental cycle (»RP«). Vaginal smears were taken throughout the duration of the experiments. The length of the dioestrous period was expressed as the number of days of leucocytic vaginal smear following treatment until the next oestrus; these days of dioestrus are designated L_1, L_2, etc. An additional group of rats received both treatments (1) and (2).

Electrical induction of pseudopregnancy in this colony is virtually 100% effective, and the duration of the ensuing pseudopregnancy is 13.5 days ± 0.3 (mean and s; for 100 rats; range 11–21 days). Accordingly, true pseudopregnancy was considered as a dioestrous period of 11 days or longer.

In some groups, pseudopregnant rats were subjected to the subcutaneous injection of 1 mg of ergocornine methanesulphonate (ECO, Sandoz) in 0.25 ml of 70% ethanol on day L_1 of pseudopregnancy. After oestrus had recurred, about three days after injection of the alkaloid, further vaginal smears from those animals were taken, and the length of dioestrous interval between ECO-induced oestrus and the first sign of pro-oestrus was recorded.

In other groups, final confirmation of true pseudopregnancy was sought by attempts to elicit the decidual cell reaction. Deciduoma was induced on day L_4 of pseudopregnancy (1) by the intraperitoneal injection of 1 ml of an aqueous solution of pyrithiazine hydrochloride (2% w/v) (Shelesnyak & Kraicer 1961), or (2) by scratching the anti-mesometrial endometrium of both uterine horns with a needle after laparotomy of the ether-anaesthetized rat. Autopsy was performed on L_8 and the extent of the decidual cell reaction was scored as described by Shelesnyak & Kraicer (1961).

RESULTS

At the higher doses used (0.75–1.5 mg/kg body weight) reserpine was an effective inducer of pseudopregnancy: both the incidence of pseudopregnancy (34/36) and its mean duration (13.0 days) resembled the response to electrical stimulation of the cervix; a dose of 0.5 mg/kg was partially effective (Table 1). However, abnormal cycles with a prolonged dioestrous phase (3–7 days), short
of frank pseudopregnancy, were frequent after the administration of low doses of reserpine (0.1—0.5 mg/kg; see Table 1). The dose of 0.75 mg/kg of reserpine was chosen for further experimentation on the incidence of secondary pseudopregnancy.

When oestrus was caused by ECO injection during EP, secondary pseudopregnancy was seen in 8/11 rats (see Table 2); this is in agreement with previous data (cf. Kisch 1968). ECO-induced oestrus during RP was not followed by secondary pseudopregnancy in any of 24 rats, although reserpine, in the dose used in this experiment, effectively induced an initial pseudopregnancy. Even when reserpine was given in a dose of 3.0 mg/kg, none of the 24 rats thus made pseudopregnant responded to challenge with ECO; all the animals returned to oestrus and no secondary pseudopregnancy ensued.

When an initial pseudopregnancy was induced by both electrical stimulation and reserpine, a subsequent ECO injection on L4 caused secondary pseudopregnancy in 8 out of 22 rats, and prolonged the dioestrous periods (6–10 days) in four other rats. This number of secondary pseudopregnancies is lower than after EP only, but not significantly so (0.25 > P > 0.1; by χ² test). The absence of secondary pseudopregnancy after RP can therefore not be ascribed to a blocking action by this drug on the phenomenon of secondary pseudopregnancy.

Definitive evidence of actual pseudopregnancy – rather than the designation of a certain period of dioestrus – is the ability to elicit the decidual cell reaction (DCR). The data obtained by either method of deciduoma induction (trauma or pyrathiazine; see Table 3) completely confirm the results reported

Table 1.
Effectiveness of induction of pseudopregnancy in the rat by reserpine; duration of dioestrus interval as function of dose.

<table>
<thead>
<tr>
<th>Dose of reserpine (mg/kg)</th>
<th>No. of rats</th>
<th>true pseudopregnancy</th>
<th>normal cycle</th>
<th>other</th>
<th>average duration of true pseudopregnancy (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>5'</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>6</td>
<td>-</td>
<td>4''</td>
<td>12.3</td>
</tr>
<tr>
<td>0.75</td>
<td>24</td>
<td>23</td>
<td>-</td>
<td>1'''</td>
<td>12.6</td>
</tr>
<tr>
<td>1.5</td>
<td>12</td>
<td>11</td>
<td>-</td>
<td>1'''</td>
<td>13.7</td>
</tr>
</tbody>
</table>

' 3 days of dioestrus
'' 4–7 days of dioestrus
''' 8–9 days of dioestrus
### Table 2.

Incidence of secondary pseudopregnancy following ergocornine methanesulphonate (ECO) administration to rats made pseudopregnant by electrical stimulation and/or reserpine.

<table>
<thead>
<tr>
<th>Method of induction of initial pseudopr.</th>
<th>No. of rats</th>
<th>Results</th>
<th>no secondary pseudopregnancy</th>
<th>average duration of second pseudopregn.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>second pseudopregn.</td>
<td>cycle</td>
</tr>
<tr>
<td>Reserpine only</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>--</td>
</tr>
<tr>
<td>Reserpine plus electr. stimul.</td>
<td>22</td>
<td>8</td>
<td>10</td>
<td>4'</td>
</tr>
<tr>
<td>Electr. stimul. only</td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>1'</td>
</tr>
</tbody>
</table>

Electrical stimulation to cervix on days of pro-oestrus and oestrus; reserpine (0.75 mg/kg body weight injected sc) on the first day of dioestrus; ECO (1 mg/rat) on the fourth day of leukocytes in the vaginal smear after treatment cycle.

‘6–10 days of dioestrus following ECO-induced oestrus.

### Table 3.

Decidual cell response (DCR) of rats made pseudopregnant by various methods.

<table>
<thead>
<tr>
<th>Method of induction of initial pseudopregnancy</th>
<th>Method of challenge for DCR</th>
<th>Initial pseudopregnancy</th>
<th>Secondary pseudopregn.</th>
<th>Decidual reaction</th>
<th>Decidual reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Incidence</td>
<td>Decidual reaction</td>
<td>Incidence</td>
<td>Score</td>
</tr>
<tr>
<td>(a) Electrical stimul.</td>
<td>Pyrithiazine Trauma</td>
<td>12/12</td>
<td>11/11'</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>(b) Reserpine</td>
<td>Pyrithiazine</td>
<td>12/12</td>
<td>10/11'</td>
<td>2.4''</td>
<td></td>
</tr>
<tr>
<td>(c) Electrical stimul. + reserpine</td>
<td>Pyrithiazine Trauma</td>
<td>12/12</td>
<td>8/12</td>
<td>2.0''</td>
<td></td>
</tr>
</tbody>
</table>

' One rat died before completion of the experiment.

'' Significantly different from group «a» (P < 0.05).

Experimental procedures were the same as in Table 2.
above, and provide proof that the secondary period of dioestrus following ECO administration constitutes a true pseudopregnancy.

**DISCUSSION**

A striking difference between the pseudopregnancy-inducing action of electrical stimulation of the cervix uteri and that of reserpine injection is the graded response to the latter treatment: depending on dose-rate, reserpine causes states of prolonged dioestrus ranging from that of a normal 4-day cycle (= 2 days of dioestrus) to true pseudopregnancy. Similar results have been reported for reserpine by Coppola et al. (1965). Such transitional cycles are never seen when electrical stimulation is used to induce pseudopregnancy: this method causes an all-or-none response (Kisch, unpubl. observations).

Another aspect of the different mode of action of electrical stimulation and of reserpine in the induction of pseudopregnancy is the different effect of the two treatments on the CNS «memory». The phenomena of secondary pseudopregnancy has been observed after any type of interruption by oestrous of the initial pseudopregnancy, irrespective of the means by which such oestrous was induced (Bruce 1962; Zeilmaker 1965; Kisch 1968); in most of these investigations the initial pseudopregnancy was induced by electrical stimulation or by sterile mating, except for some of Zeilmaker's series (see below).

ECO given on day L₁ of EP, induced oestrous which was followed by a new pseudopregnancy in 50–70% of rats (Zeilmaker 1965; Kraicer & Shelesnyak 1964; Kisch 1968 and the present data); however, ECO administration to rats on L₄ of RP caused oestrous which was not followed by pseudopregnancy: It seems therefore that reserpine, while giving all the outward signs of pseudopregnancy (including the capacity of decidual cell reaction), does not activate the central memory. Another possibility is that ECO specifically counteracts this latter effect in RP only. In this respect it should be noted that Zeilmaker (1965) when using reserpine in a dose of 3 mg/kg – but with another method for inducing oestrous during initial pseudopregnancy – was able to obtain a secondary pseudopregnancy. In our series, where ECO was used throughout, this large dose of reserpine failed to do so. It is therefore possible that Zeilmaker achieved his positive results precisely because of the absence of ECO in his protocol. One may speculate that the graded response seen by using reserpine for the induction of pseudopregnancy (i.e. higher incidence of pseudopregnancies and longer average duration, in proportion to dose) can be extended into the realm of the CNS «memory» (as expressed in secondary pseudopregnancy). Yet, this dose of reserpine seems rather excessive for the study of CNS phenomena.

We have shown that the absence of a secondary pseudopregnancy after ECO
interruption of RP is probably not due to the inhibition of such an effect by the reserpine; the administration of ECO to rats in which pseudopregnancy was induced by electrical stimulation and who also received reserpine, was followed by secondary pseudopregnancy in a number of cases smaller, but not significantly different, from the results obtained after ECO administration in EP rats. However, a certain measure of depression by reserpine is reflected in the diminished decidual response during the initial pseudopregnancy; apparently this effect of reserpine has ceased by the time a DCR is induced in secondary pseudopregnancy (see Table 3) and where normal scores for DCR are obtained.

In conclusion, electrical stimulation both induces pseudopregnancy and stimulates the CNS »memory«. reserpine causes pseudopregnancy only. Whether this dissociation of effects is based on a quantitative or a qualitative difference in the mode of action of the two methods for the induction of pseudopregnancy, is not clear.

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