

Repeated convulsions induce pseudopregnancy in the intact rat and inhibit steroid-mediated gonadotrophin secretion in the ovariectomized rat

R. Bhanot* and M. Wilkinson*†
Departments of *Physiology and Biophysics, and †Obstetrics and Gynaecology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

(Received 16 December 1981)

SUMMARY

We have investigated the effects of repeated flurothyl-induced seizures on reproductive function in the female rat. This treatment rapidly induced a state of pseudopregnancy in intact cyclic rats. Prolactin is clearly implicated in this response since treatment with bromocriptine readily counteracted the influence of the convulsions. The mechanism of action of repeated seizures was further characterized in experiments on ovariectomized rats. Thus, 11 daily convulsions, but not a single acute seizure, were able to inhibit the positive feedback effect of progesterone on LH and FSH release in oestrogen-primed animals. In this model also the pituitary gland response to gonadotrophin releasing hormone in vitro was significantly reduced. However, the convulsions had no effect on basal serum or basal in-vitro secretion of LH and FSH in ovariectomized or oestrogen-treated ovariectomized rats. Thus, repeated seizures modified the hypothalamic-pituitary axis in such a way as to prevent it from responding to stimulation.

Our results indicate that normal reproductive function in the female rat is very sensitive to repeated seizures and suggest that similar effects may be evident in women subjected to electroconvulsive shock therapy. The successful use of bromocriptine in reversing the influence of seizures in the rat suggests its use in man also.

INTRODUCTION

Convulsive shock therapy, in spite of a lack of understanding of its mechanism of action, remains an empirically useful technique for the treatment of certain refractory mental disorders (Fink, 1979). However, we have described a disruptive influence of flurothyl-induced convulsions on the developing reproductive system of the rat (Wilkinson, Bhanot, Pincock & Donald, 1982). We were able to show that puberty was delayed through an effect of the convulsions on the hypothalamic-pituitary axis, a system regulated by the same neurotransmitters which have been implicated in the effects of convulsions (Van Praag, 1978). In the present paper we describe the influence of repeated seizures on the reproductive system of the mature female rat and demonstrate that cyclicity, pituitary gland responsiveness to stimulation with gonadotrophin releasing hormone (GnRH) and steroid-induced gonadotrophin release are all adversely affected by flurothyl-induced convulsions.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing approximately 150 g were obtained from Canadian Breeding Farm and Laboratories, St Constant, Quebec, Canada. The animals were housed...
four per cage in light- (lights on 07.00–19.00 h) and temperature- (21 °C) controlled rooms and given free access to food and water. Daily vaginal smears were performed after 2 weeks of adjustment to local conditions. Steroid treatment in ovariectomized rats was initiated 2 weeks after surgery.

Convulsions
Convulsions were induced by placing each animal into a 1.2 l screw-top jar containing 20 µl pure flurothyl (Wilkinson et al. 1982). Single daily convulsions were given between 12.00 and 13.00 h for the duration required in a particular experiment. Control subjects were placed briefly in the empty jar.

Deciduoma response
Pseudopregnancy was confirmed by eliciting the deciduoma reaction (de Greef, Dullaart & Zeilmaker, 1977). The right uterine horn of rats undergoing daily convulsions was traumatized using the bevel of a 25-gauge hypodermic needle. The surgery was performed under halothane on day 4 of persistent vaginal dioestrous. The rats were killed 4 days later and the weights of the uterine horns were compared.

Hormone treatment
Pro-oestrous-like gonadotrophin surges were elicited in ovariectomized rats using the method of Caligaris, Astrada & Taleisnik (1968). A single injection of oestradiol benzoate (OB) (20 µg OB per animal s.c. in 0.1 ml sesame oil) was given at 12.00 h on day 1. On day 4, injection of progesterone (1.0 mg progesterone per animal in 0.2 ml oil) at 12.00 h elicited a surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) 5 h later. The effects of convulsions were also studied in ovariectomized, untreated rats as well as in the ovariectomized group given OB (i.e. in rats showing no surge of gonadotrophins).

Pituitary gland incubations
Anterior pituitary glands and trunk blood were collected after decapitation. Pituitary glands were incubated as described previously (Wilkinson & Moger, 1981). Samples (20 µl) were removed after 3 h of stimulation with 8.5 x 10^{-10} mol GnRH/1 and stored at -20 °C before determination of LH and FSH (see below).

Bromocriptine injections
The effect of bromocriptine (Parlodel; Sandoz, Dorval, Quebec, Canada) on convulsion-induced pseudopregnancy was studied by injecting bromocriptine (2.0 mg bromocriptine mesylate per rat, 0.5 ml suspension in 0.9% NaCl (w/v), s.c.) 30 min before each convulsion.

Hormone assays
Serum levels of LH and FSH and samples of pituitary gland incubation medium were determined using reagents supplied by the NIAMDD and expressed in terms of LH/FSH RP-1 standards.

Statistical analyses
Sample means were compared using Student’s t-test. A value of $P < 0.05$ was considered to denote significant differences.

RESULTS
Effect of convulsions on the oestrous cycle
Female rats which had shown two consecutive 4-day oestrous cycles were convulsed once daily for a total of 10 days. The convulsions were begun on dioestrous of the third cycle. The
first three convulsive shocks did not block the subsequent expected vaginal pro-oestrous and oestrous smears (except in one case out of 12). Subsequently, the treated groups displayed a sequence of uninterrupted dioestrous smears lasting for at least 14 days. Most (11 out of 12) of the control animals continued to have 4-day oestrous cycles.

The convulsed group showed a marked net increase in somatic weight over the 10-day period: control animals had an 11% increase in body weight compared with 24% for the treated group (n = 12, P < 0.005). A comparison of the pituitary, adrenal, ovarian and uterine weights of convulsed animals with those of cyclic controls at metoestrus showed a large increase in absolute adrenal weight (P < 0.005) (Table 1).

Table 1. Body and paired organ weights in intact rats after ten convulsions. Values are means ± S.E.M. of six rats per group

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Pituitary gland (mg)</th>
<th>Adrenal (mg)</th>
<th>Ovaries (mg)</th>
<th>Uterus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>216 ± 3</td>
<td>11 ± 1.0</td>
<td>65 ± 3</td>
<td>74 ± 3</td>
<td>258 ± 17</td>
</tr>
<tr>
<td>Convulsions</td>
<td>261 ± 6***</td>
<td>11 ± 0.4</td>
<td>80 ± 1.4***</td>
<td>81 ± 6</td>
<td>245 ± 16</td>
</tr>
</tbody>
</table>

***P < 0.005 compared with controls (Student’s t-test).

A separate but similar group of rats undergoing daily convulsions responded dramatically to uterine traumatization: the affected horn weighed 1484 ± 226 (s.e.m.) mg (n = 5) compared with 221 ± 9 mg for the contralateral horn. This response and its temporal specificity (i.e. uterine manipulation on the fifth dioestrus was ineffective) both reflected the acute oestradiol sensitization of the uterus in pseudopregnancy (Shaikh & Abraham, 1969).

Since prolactin is the luteotrophic ovarian stimulus for progesterone secretion and the maintenance of pseudopregnancy (Neill, 1980), we investigated whether bromocriptine, a drug known to inhibit prolactin secretion and to terminate pseudopregnancy (Smith, McLean & Neill, 1976; Megory & Ishay, 1980), had any effect on seizure-induced pseudopregnancy. We observed that daily injection of bromocriptine 30 min before each of ten convulsions completely prevented the induction of pseudopregnancy (six out of six rats). The rats were autopsied at either pro-oestrous or oestrus. The former rats had bloated fluid-filled uteri whereas the latter group had ova in the oviducts (details not shown).

Effect of convulsions on steroid-induced LH and FSH release in ovariectomized rats

We examined the effects of repeated seizures on both tonic and phasic release of LH and FSH. Table 2 illustrates that an 11-day regimen of convulsions given to ovariectomized, table continued...
untreated rats or to ovariectomized rats treated with OB failed to modify serum levels of LH. However, surge levels of LH in animals given OB and progesterone were significantly reduced, though not blocked. The corresponding values for FSH are also given in Table 2. Unlike pseudopregnant animals, these convulsed rats did not undergo accelerated weight gain (Table 2) although they showed adrenal hypertrophy.

We were further able to show that a single convolution did not suppress gonadotrophin surges in ovariectomized animals given OB and progesterone though four convulsions were inhibitory (Table 3). Note, however, that the reduction in FSH levels was minimal.

Table 3. Effect of one or four convulsions on mean (± S.E.M.) serum levels of LH and FSH in untreated long-term ovariectomized (OVX) rats or ovariectomized rats treated with oestradiol benzoate (OVX-OB) or oestradiol benzoate and progesterone (OVX-OB/P)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Serum LH (µg/l)</th>
<th>Serum FSH (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX</td>
<td>5</td>
<td>624 ± 37</td>
<td>1345 ± 105</td>
</tr>
<tr>
<td>OVX + 4 convulsions</td>
<td>4</td>
<td>599 ± 106</td>
<td>1302 ± 48</td>
</tr>
<tr>
<td>OVX-OB/P</td>
<td>8</td>
<td>1794 ± 156</td>
<td>1831 ± 80</td>
</tr>
<tr>
<td>OVX-OB/P + 1 convolution</td>
<td>7</td>
<td>1673 ± 152</td>
<td>1603 ± 114</td>
</tr>
<tr>
<td>OVX-OB/P + 4 convulsions</td>
<td>7</td>
<td>1109 ± 179**</td>
<td>1615 ± 80*</td>
</tr>
</tbody>
</table>

*P < 0.05. **P < 0.01 compared with OVX-OB/P group (Student’s t-test).

Further evidence pointing to a hypothalamic-pituitary derangement in the cyclic mechanism controlling gonadotrophin release was afforded by the depressed in-vitro pituitary responses to GnRH in the convulsed ovariectomized rats given OB and progesterone but not in the ovariectomized, untreated group (Table 4).

Table 4. Effect of convulsions on in-vitro pituitary responsiveness to gonadotrophin-releasing hormone in ovariectomized (OVX) rats and ovariectomized rats treated with oestradiol benzoate (OVX-OB) or oestradiol benzoate and progesterone (OVX-OB/P). Data are expressed as mg gonadotrophin released/l per mg pituitary gland weight every 3 h ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>LH (µg/l)</th>
<th>FSH (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX</td>
<td>4.5 ± 0.6</td>
<td>11.2 ± 1.2</td>
</tr>
<tr>
<td>OVX + 4 convulsions</td>
<td>5.5 ± 1.1</td>
<td>12.0 ± 1.1</td>
</tr>
<tr>
<td>OVX-OB/P</td>
<td>15.4 ± 2.3</td>
<td>11.0 ± 1.1</td>
</tr>
<tr>
<td>OVX-OB/P + 1 convolution</td>
<td>17.3 ± 1.8</td>
<td>11.4 ± 0.7</td>
</tr>
<tr>
<td>OVX-OB/P + 4 convulsions</td>
<td>9.0 ± 1.1**</td>
<td>8.4 ± 0.6*</td>
</tr>
</tbody>
</table>

*P < 0.05. **P < 0.01 compared with OVX-OB/P group (Student’s t-test).

**DISCUSSION**

Electroconvulsive shock therapy has been shown to induce amenorrhoea in mentally ill patients who were menstruating normally at the commencement of treatment (Kalinowski, 1948; Michael, 1954). However, characterization of the neuroendocrine abnormalities accompanying electrotherapy has not been forthcoming. Previous work on laboratory animals has shown that repeated electroshocks given to female rats disrupt oestrous cycles (Milner & Green, 1980). Our observations in the rat confirm and extend previous reports. We have clearly demonstrated that multiple convulsions elicit a state of pseudopregnancy. Experiments with ovariectomized rats showed that repeated seizures disrupt the positive
feedback effect of oestrogen-progesterone treatment on LH and FSH release (Table 2). Anterior pituitary response to stimulation with GnRH in vitro was also adversely affected (Table 4). Thus, our data point to a direct effect of the convulsions on the hypothalamo-pituitary axis. Interestingly, there appeared to be no influence exerted upon the high gonadectomy-induced levels of LH and FSH (ovariectomized group) or on the low titres seen in the ovariectomized rats treated with OB (Table 2). In man the picture is less clear; large changes in LH and FSH have been reported but even within treatment groups this effect is not consistently seen (Beuret & Swanson, 1969; Ryan, Swanson, Faiman, Mayberry & Spadoni, 1970; Ylikorkala, Kauppila, Haapalahti & Karppanen, 1976; Delitala, Masala, Rosati, Aiello & Agnetti, 1977). Consistent with our studies in the immature rat (Wilkinson et al. 1982), it thus appears that repeated seizures induce within the hypothalamo-pituitary unit an inability to respond to stimulation. The finding that bromocriptine was able to reverse the anovulatory influence of convulsions firmly implicates a critical role for prolactin. Electroconvulsive treatment rapidly stimulates prolactin secretion in men and women (O'Dea, Gould, Hallberg & Wieland, 1978). Moreover, our data show significant increases in adrenal weights following repeated convulsions indicating activation of the pituitary-adrenal axis. In the rat stress is known to increase prolactin and disrupt the oestrous cycle (Megory & Ishay, 1980; Neill, 1980). Consequently, the well-described (Smith, 1980) antigoandotrophic effect of hyperprolactinaemia could account for the present results in the rat. That is, hyperprolactinaemia reduces pituitary gland responsiveness to GnRH (Vasquez, Ellegood, Nazian & Mahesh, 1980) and profoundly inhibits the positive feedback effect of progesterone in oestrogen-primed rats (Carter & Whitehead, 1981).

In conclusion, the reproductive systems of rats and possibly also of women appear to be susceptible to repeated convulsions. This may be only one aspect of hypothalamic function which is seizure-sensitive. The evidence that bromocriptine successfully prevents the adverse effects of convulsions on cyclicity in the rat recommends the use of this drug in women.

We are grateful for the radioiodinations performed by Mrs Alice Giles. Thanks are also extended to Dr D. Nance for his helpful suggestions concerning the deciduoma response, to Dr J. A. Pincock and L. Donald for the flurothyl and to Beverley Theriault for excellent secretarial assistance. Financial support was obtained from the Canadian Medical Research Council, Grant number MA-7131 (to M.W.).

REFERENCES

Megory, E. & Ishay, J. S. (1980). Hypergravity induced prolonged diestrus in the rat can be prevented by bromoergocryptine or by previous exposure to the same conditions—a ‘memory’ effect. Life Sciences 27, 1503–1507.


