INDIRECT EVIDENCES OF PROLACTIN INVOLVEMENT IN PRECOCIOUS PUBERTY INDUCED BY HYPOTHALAMIC LESIONS IN FEMALE RATS

By

E. O. Alvarez, J. L. Hancke and J. P. Advis

ABSTRACT

The effects of 2-bromo-α-ergocryptine (CB-154) administration in maturing female rats with precocious puberty induced by hypothalamic lesions, as well as the milk secretion rate in these animals after their first parturition were studied. Treatment with CB-154 inhibited precocious vaginal opening induced by hypothalamic lesions. After the treatment with the derivative was terminated, the ovulatory response was not different from that of intact control rats. After their first parturition lesioned rats accumulate milk at a faster rate, following an initial depletion period, than their non-operated controls. These results suggest that an alteration in prolactin release may be involved in determining the precocious puberty induced by lesions of the anterior hypothalamic area.

It is well known that lesions or transverse cuts in the anterior hypothalamic area (AHA) of the rat, advance the time of vaginal opening (Donovan & Van der Werff ten Bosch 1959; Critchlow & Bar-Sela 1967; Ramaley & Gorski 1967; Meijs-Roelofs & Moll 1972; Sherwood & Timiras 1974). On the basis

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of these studies it is generally believed that some fibers transversing the rostral hypothalamus inhibited pituitary gonadotrophin secretion before the onset of puberty. Previous work in our laboratory (Advis & Ramirez, unpublished results) has shown that maturing female rats with precocious puberty due to hypothalamic lesions, present advanced vaginal opening (VO), ovulation and the first oestrus without any apparent alterations of pre-puberal and puberal plasma FSH levels as measured by RIA. Taking into account that chronic interruption between the preoptic area and the hypothalamic tuberal region induces a prolactin (PRL) hypersecretion (Blake et al. 1973; McCann 1974; Neill 1974; Ojeda, in press), it is possible that this hormone could be altered by our radio frequency lesions of the AHA. Moreover, it has been reported that prolactin administration induces precocious puberty (Clemens et al. 1969). In view of these observations it appeared of interest to study the effect of an ergot derivative drug 2-bromo-α-ergocryptine (CB-154), on the precocious puberty induced by our hypothalamic lesions. Furthermore the effect on the milk secretion rate of these animals, after their first parturition, was also examined.

MATERIALS AND METHODS

Female albino rats belonging to a Wistar–Holtzman mixed strain inbred for at least 12 years were used in these experiments. The rats were housed under controlled conditions of temperature (24–26°C) and light (14 h on, from 05.00 to 19.00). Tap water and pelleted food (Cooperative Agrícola de Purranque) were supplied ad libitum.

All the animals were weaned at 20 days of age and littermates were divided into two groups. On the same day lesions were produced in one group in the anterior hypothalamic area using a high frequency current; the other group served as intact non-operated control.

Hypothalamic lesions

Animals were positioned in a rat stereotaxic apparatus (David Kopf Instruments) and bilateral lesions were produced by a radio-frequency (r-f) lesion maker (Grass, model LM5) through a unipolar (26 gauge) stainless steel electrode (anode) completely insulated with epoxilite resin except for 1 mm at the tip. Voltage was monitored throughout the process with a multitester (Simpson, Model 260, 5Ω/volt) connected in parallel with the lesion maker. The cathode was connected to the stereotaxic apparatus.

The lesions were placed 1.2 mm anterior to bregma, 0.5 mm lateral to the mid-sagittal plane and 7.8 mm below the cerebral cortex surface plane.

Experiment 1

At 20 days, after the lesions had been made, the lesioned animals were divided into two groups:

Group 1. – These rats were injected ip once a day with 0.5 mg/100 g body weight of 2-bromo-α-ergocryptine methanesulphonate (CB-154, Sandoz AG., Basel, Switzer-
land) which was prepared in 10% ethanol-saline with added tartaric acid in the same amount as that of CB-154. Treatment was started when the lesioned rats were 20 days old and continued for 12 days.

**Group 2.** – The rats were injected with 10% ethanol-tartaric acid-saline (1 ml/100 g body weight) under the same conditions as Group 1. From the day of the operation all the animals were checked daily for vaginal opening (VO). All the lesioned rats, treated with CB-154 or solvent were sacrificed on the day of VO. At sacrifice (between 10.00 and 12.00 h) the body weights as well as uterine and ovarian weights were recorded. The presence of ovulation was determined by dissection of the Fallopian tubes and direct microscopic observation of the ova.

**Experiment 2**

Control and lesioned littermates were caged with males of proven fertility on the day of vaginal opening. Each female rat (control or lesioned) remained housed with the male up to its first dioestrus. Males were caged first with a lesioned rat and after 3 days of rest, with control littermates. The day on which spermatozoa were detected in the vagina was considered as the day of conception. From that day, each rat was kept separately until delivery.

On the day of delivery, all mothers lesioned or controls, were left with 10 pups in order to standardize the suckling stimulus. The 10 pups per mother were divided randomly into two subgroups of 5 pups per rat, called from here on the “initial” and the “later depletors”. Experiments were started 2 days after delivery and lasted for 14 days. During these 2 weeks both subgroups of 5 pups were separated daily from their mothers for a period of 8 h. After this period, “initial depletors” were allowed to suckle during 30 min. Thirty and 60 min after “the initial depletor subgroups” had finished their 30 min suckling period parallel “later depletor subgroups” (one for point 30 and one for point 60) were also allowed to suckle during 30 min. This procedure was applied both to lesioned and to control mothers. The amount of milk ingested was calculated by weighing each “5 pups” subgroups before and after the 30 min suckling period. These daily increments of each 5 pups subgroup, “initial” and “later depletors”, were added up to complete the 14 day study period for each rat. This method was reliable up to ± 0.5 g and is a slight modification of one reported elsewhere (Bruce & Ramírez 1970). The results were expressed as the percentage of body weight increment of the “later depletors” with respect to the “initial depletors”, which was considered as 0%.

**RESULTS**

The treatment of 0.5 mg/100 g body weight of CB-154 for 12 days once a day completely inhibited the precocious VO observed in the lesioned group injected with solvent (Table 1). Age of VO in the CB-154 treated lesioned rats and intact control animals were similar (Table 1). Uterine weight at VO was higher in the ergot drug treated lesioned rats than in the intact control (Table 2). Both the ovarian weight and the ovulatory response (percentage and number of ova found) were similar for both groups (Table 2).

Thirty min after the initial depletion the increment in body weight of late
Table 1.
Age of vaginal opening and body weight of lesioned rats treated with 2-bromo-α-ergocryptine.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaginal opening</th>
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<tbody>
<tr>
<td></td>
<td>Age (days)</td>
<td>Body weight (g)</td>
<td></td>
</tr>
<tr>
<td>Lesioned rats treated with CB-154</td>
<td>34.9 ± 0.4a</td>
<td>83.0 ± 2.0d</td>
<td></td>
</tr>
<tr>
<td>0.5 mg/100 g body weight</td>
<td>(18)</td>
<td>(18)</td>
<td></td>
</tr>
<tr>
<td>Lesioned rats treated with solvent</td>
<td>29.2 ± 0.3b</td>
<td>69.5 ± 1.9e</td>
<td></td>
</tr>
<tr>
<td>1.0 ml/100 g body weight</td>
<td>(6)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Intact control rats</td>
<td>35.6 ± 0.7c</td>
<td>91.6 ± 2.6f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(21)</td>
<td></td>
</tr>
</tbody>
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Results: expressed as mean ± sem. In brackets: number of animals used.

a vs b: P < 0.0005.
a vs c: n.s.
d vs e: P < 0.0025.
d vs f: P < 0.01.

ip injection started at 20 days and lasted for 12 days.

Table 2.
Some parameters of puberty at vaginal opening in lesioned CB-154 treated and control rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Uterine weight mg/100 g body weight</th>
<th>Ovarian weight mg/100 g body weight</th>
<th>Ovulatory response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No. of ova</td>
<td></td>
</tr>
<tr>
<td>Lesioned rats treated with CB-154</td>
<td>179.7 ± 12.7a (18)</td>
<td>37.6 ± 1.5 (18)</td>
<td>50.0</td>
</tr>
<tr>
<td>0.5 mg/100 g body weight</td>
<td></td>
<td></td>
<td>10.3 ± 0.4 (9)</td>
</tr>
<tr>
<td>Intact control rats</td>
<td>156.2 ± 7.1b (10)</td>
<td>35.0 ± 2.6 (10)</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.3 ± 0.9 (10)</td>
</tr>
</tbody>
</table>

See Fig. 1 and Table 1 for details a vs b: P < 0.0025.
Lactation response in lesioned and control mother rats 30 and 60 min following mammary glands depletion. Results are expressed as percentage of the initial depletor body weight increment. For details, see Materials and Methods. Number in brackets, total number of animals used. Vertical line, standard error of the mean (SEM).

*, $P < 0.05$; **, $P < 0.025$.

Depleters pups in control mothers was $27.2 \pm 5.9$ (mean % ± SEM) and the percentage observed in lesioned rats was $42.5 \pm 4.6$ (Fig. 1). This difference was statistically significant ($P < 0.05$). Sixty min after the initial depletion body weight increment of pups in control rats was $32.7 \pm 5.6\%$ and in lesioned rats $72.4 \pm 18.3\%$. The difference was also statistically significant ($P < 0.025$).

**DISCUSSION**

The precocious VO induced by the hypothalamic lesions was completely inhibited by the treatment with CB-154 (Table 1). This compound like other ergot derivatives has been reported to inhibit PRL secretion both in rat and sheep (Lu et al. 1971; Clemens et al. 1974; Niswender 1974). Treatment with 2-bromo-α-ergocryptine did not impair reproductive capacity since our lesioned animals, after treatment was suspended, opened vagina and ovulated to the same extent as normal control (Table 2).

It is interesting to consider that in agreement with evidence that chronic interruption between the preoptic area and the hypothalamic tuberal region provoke a PRL hypersecretion (Blake et al. 1973; McCann 1974; Neill 1974;
Ojeda, in press), our results with CB-154 suggest a causal relationship between PRL and precocious puberty induced by the lesions.

Prolactin is one of the most important regulators in the secretion of milk by the mammary glands (Neill 1974; Nicoll 1974). It was expected that if PRL was involved in the effects produced in our lesioned rats, then a lactation study might provide a useful additional parameter, though we are well aware that it is an indirect method which does not take into account differences in sensitivity in target tissues to PRL, participation of other hormones or quantitation of PRL levels (Neill 1974).

It is interesting to note that after initial depletion, ingestion of milk was higher in pups of lesioned mothers than of control rats. Assuming that pups suckled about the same and that milk remaining in the glands was similar, this result could be interpreted as showing that the mammary glands milk replenishment in lesioned mother rats is faster than in control rats. It has been reported that the PRL mechanism of action in mammary tissue is to provoke an increased synthesis of galactosyl transferase and milk proteins (Topper 1969, 1970; Turkington 1972; Nicoll 1974).

Our preliminary results are far from being definite in regard to the mechanism of action of hypothalamic lesions on precocious puberty. However, the CB-154 data, in agreement with the lactation studies suggest that PRL secretion can be altered.

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REFERENCES


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