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Prolactin Response to Stress in Neonatally Estrogenized Male Rats

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Male Wistar rats were injected either 500 µg of estradiol benzoate or olive oil on their first day of life. Blood samples were obtained from the adult by decapitation, by decapitation after 15 min of restraint, by decapitation 10 min after a 5 min period of ether exposure or by jugular venipuncture after 60 s of ether exposure. Prolactin (PRL) plasma levels were measured by RIA. The PRL levels in samples obtained by decapitation were similar to control and estrogenized groups. A similar response to restraint was also found in both groups. Sixty s of exposure to ether stress stimulates PRL secretion only in the estrogenized males, this effect being blocked by treatment with Normifensine (5 mg/kg two hours prior to blood sampling). These results suggested that estrogenized male rats show greater sensitivity to acute ether stress than the controls. and that changes in the dopaminergic system could be involved in this response.

Key words: Prolactin secretion, Neonatally estrogenized rats.

The control of prolactin (PRL) secretion in adult rats could be modified by neonatal administration of steroids. Administration of estradiol or testosterone to females induced anovulation with hyperprolactinemia (1, 3, 13, 14). Neonatal estrogen administration to male rats induced hyperprolactinemia before puberty attachment (4), the results obtained being contradictory in the adult. When blood samples were obtained by jugular venipuncture after light ether anesthesia, the estrogenized animals showed higher prolactin levels than the controls (2, 16). Therefore if blood samples were obtained by decapitation, the PRL levels were similar in control and estrogenized animals, suggesting that neonatal estrogenization could modify the PRL response to ether stress (12). To analyze this question, blood samples from control and estrogenized males were obtained in the adult in different experimental conditions. The results obtained confirm that neonatal estrogenization modifies the PRL response to acute

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ether exposure, but not to restraint. The possible participation of the dopaminergic system in this response is analyzed.

Materials and Methods

Animals. Male Wistar rats were raised in our laboratory under controlled light (12 h light: 12 h darkness) and temperature (22 ± 2°C) conditions. On day one of life male pups were s.c. injected either with olive oil (0.1 ml) or with 500 µg of estradiol benzoate (Sigma) dissolved in 0.1 ml of olive oil. After weaning, rats were housed 4-5 per cage. Approximately 24 h before each experiment, all rats were placed in individual cages in the room on which the samples were to be collected. None of the rats were exposed to ether prior to the day of the experiment. Experiments were performed between 10.00 and 12.00 h in adulthood (three months). After the experiment, testicular and seminal atrophy in estrogenized males were analyzed to confirm the effectiveness of neonatal estrogenization (12).

In experiment 1, blood samples were obtained as follows: a) by decapitation; b) by decapitation after 15 min of restraint; c) by decapitation 10 min after exposure to ether vapour for 5 min; d) by jugular venipuncture after ether anesthesia. This procedure rarely exceeded 60 s.

In experiment 2, the animals were injected ip with Nomifensine (5 mg/kg) or vehicle (ClNa 0.9 %) two hours before sampling. Blood samples were obtained by jugular venipuncture after 60 s of ether anesthesia.

PRL-assay. Plasma was separated by low speed centrifugation and frozen at −20°C until analyzed. PRL levels were measured using a kit from NIAMDD (Maryland, Bethesda, USA). Rat-PRL-I-4 was labelled with I-125 by the Chloramine T method (8). PRL values are expressed in ng/ml of the reference preparation Rat-PRL-RP-3. All samples were run in the same assay, 9 % being the intraassay variability and 0.1 ng/tube the sensitivity.

Statistical analysis. Comparison among sample means was made with Duncans's new multiple range test.

Results

In control and estrogenized animals, 15 min of restraint significantly increased

Table I. *Effect of sampling procedure on PRL levels (ng/ml) in control and neonatally estrogenized male rats.* Values are given as means ± SEM. The number of animals per group are shown in parentheses.

Sampling procedure	Controls	Estrogenized
Decapitation	9.39 ± 2.5 (11)	13.23 ± 3.3 (8)
Decapitation after 15 min of restraint	40.77 ± 11.5 (10)[a]	46.35 ± 5.0 (7)[a]
Decapitation 10 min after 5 min of ether exposure	13.01 ± 3.6 (10)	18.84 ± 4.8 (7)
Jugular venipuncture after 60 s of ether exposure	17.11 ± 2.8 (22)	47.94 ± 3.6 (20)[a,b]

[a] $p < 0.01$ vs animals decapitated. [b] $p < 0.01$ vs control animals.

Table II. *Effect of Nomifensine pretreatment (5 mg/kg) on Prl levels (ng/ml) in control and estrogenized male rats.*

Values are given as means ± SEM. The number of animals per group are shown in parenthese. Nomifensine or vehicle was administered 2 hours prior to blood sampling. Samples were obtained by jugular venipuncture after 60 s of ether anesthesia.

Treatment	Controls	Estrogenized
Vehicle	12.33±3.1 (11)	37.96±3.4 (13)[a]
Nomifensine	13.20±4.5 (11)	15.35±4.1 (12)[b]

[a] $p < 0.01$ vs control animals injected with vehicle.
[b] $p < 0.01$ vs estrogenized animals injected with vehicle.

($p < 0.01$) PRL plasma values (with respect to the values obtained after decapitation without stress). When blood samples were obtained by decapitation 10 min after exposure during 5 min to ether vapour, no differences were observed with data obtained after decapitation without stress. In estrogenized animals, but not in the controls, high PRL levels were observed when samples were obtained by jugular venipuncture after ether anesthesia (table I). Nomifensine pretreatment did not modify the PRL levels in the control group, but significantly reduced ($p < 0.01$) the high levels obtained in estrogenized males when blood samples were obtained by jugular venipuncture (table II).

Discussion

Different procedures such as immovilization (9, 11), ether exposure (5, 10), cold (11), forced running or footshock (9) increase PRL levels in male rats. In the present work the PRL response to different kinds of stress was studied in neonatal estrogenized males and their respective oil-injected controls.

Present results showed that when blood samples were carefully obtained by decapitation without stress, the PRL levels in control and estrogenized males were similar, in agreement with previous data (12). To analyze if neonatal estrogenization changes the PRL response to stress in the adult, immovilization and two types of ether exposure were used. Only estrogenized males increased their PRL levels after 60 s of ether exposure. The hyperprolactinemia described in these animals, when blood samples were obtained by jugular venipuncture after ether anesthesia (5, 16), could reflect the differences in the response to acute etherization now reported. Further experiments including sampling at different times after ether exposure are necessary to clarify whether these differences in the response are due to changes in the amplitude or in the speed of prolactin secretion.

Control and estrogenized animals responded in a similar fashion to restraint, indicating that this type of stress acts at the time studied in a similar mode in both groups of animals. PRL levels remain unchanged ten min after a five min period of ether exposure. This lack of effect could be explained by the short duration of ether action on PRL levels (15).

It has been suggested that acute stress involves the liberation of a PRL-releasing factor (15). It may be that neonatally estrogenized males have an increased capacity to produce or release this factor after acute ether exposure. On the other hand, our data using Nomifensine suggest that the dopaminergic system may be involved in the response to acute etherization observed in estrogenized animals. Nomifensine increased dopaminergic neurotransmission and by this mechanism prolactin secretion decreased (6). Pretreatment with this drug abolished the PRL response to acute etherization in estrogenized males. One may speculate that the dopaminergic system has been affected in male rats by neonatal estrogenization, as has been reported in neonatally androgenized female rats (7), and that these changes could be re-

sponsible for the greater sensitivity to acute etherization present in the estrogenized males.

In conclusion this work evidences that neonatal estrogenized male rats showed a different response to acute ether stress and suggests that changes in the dopaminergic system could be involved in this response.

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Resumen

Se emplean ratas macho Wistar inyectadas en el día 1 de vida con 500 µg de benzoato de estradiol o aceite de oliva. En la edad adulta se obtienen muestras de sangre por decapitación, por decapitación tras 15 min de inmovilización, por decapitación 10 min después de exposición a éter durante 5 min o por punción yugular tras anestesia con éter durante 60 s. Los niveles de prolactina en muestras obtenidas por decapitación y los incrementos obtenidos tras la inmovilización son semejantes en los animales controles y los estrogenizados. La exposición durante 60 s a éter eleva significativamente los niveles de prolactina plasmática solamente en los animales estrogenizados, siendo el efecto bloqueado por el pretratamiento con Nomifensina (5 mg/kg dos horas antes de la punción yugular). Estos resultados indican que modificaciones en el sistema dopaminérgico pueden estar involucradas en la mayor sensibilidad al estrés agudo con éter que presentan los machos estrogenizados neonatales.

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