Life Sciences, Vol. 48, pp. 2341-2348 Pergamon Press
Printed in the U.S.A.

ENDOTOXIN INDUCED CHANGES IN SERUM ESTROGEN IN MALE RATS: INFLUENCE OF TESTICULAR MATURATION.

Névéna Christeff, Marie-Claude Auclair*, Nicole Thobie, Claudine Benassayag and Emmanuel A. Nunez (§)

U.224, INSERM affiliée au CNRS - 16, rue Henri Huchard - 75018 Paris
* U.228, INSERM - 15, rue de l'Ecole de Médecine - 75006 Paris

(Received in final form April 10, 1991)

Summary

The influence of acute endotoxin (Endo) administration on adrenal and testicular serum hormones, corticosterone (B), progesterone (P4), 17α OH progesterone (17α OH P4), androstenedione (Δ4), testosterone (T), estrone (E1) and estradiol (E2) was studied in male rats aged 8, 12 and 15 weeks. The present study confirms that the concentrations of circulating steroid hormones in male rats vary with age, and indicate that the adrenal glands mature before the testes. The steroid response to Endo is age-dependent. B, P4, 17α OH P4 was increased and T decreased in all animals. But, there was a very significant increase in estrogens (E1 and E2) and a decrease in Δ4 only in male rats aged 12 weeks and over. The lack of an estrogen response to Endo injection in 8 week-old rats may indicate that the reduced sensitivity (refractory period) to Endo (which has been reported to last until 21 days of age) continues longer. The reduced sensitivity to Endo which occurs in young rats could be due in part to the absence of adrenal-testicular cooperation as a result of partial testicular immaturity.

During septic shock, the circulating concentrations of estrogens increase dramatically, while that of serum testosterone decreases in human males (1, 2).These changes in sex hormone concentrations have been confirmed in experimental studies on male rats (12-week old) acutely treated with non-lethal, non-hypotensive doses of Escherichia coli endotoxin (Endo), a constituant of the bacterial cell wall released during septic shock (3). The steroid hormone responses are " rapid ", being significantly different from control at 1 h., maximal at 2 h. and near normal values at 4 h. (3) following injection of endotoxin. Endotoxin administrated in similar conditions to adrenalectomized or orchidectomized male rats do not induce these hormonal changes (3). Such results suggest that there is an adrenal-testicular cooperation in the hormonal response to acute endotoxin administration.

(§) Corresponding author : Pr. E.A. Nunez, U.224, INSERM - Faculté de Médecine Xavier Bichat - 16, rue Henri Huchard - 75018 Paris, France

0024-3205/91 $3.00 + .00

There is good evidence that testicular and adrenal steroid hormone secretions change with age (4, 5). Any change in either testicular or adrenal steroidogenesis may consequently influence the level of steroid hormones after endotoxin administration.We have therefore analysed the changes in the hormonal response to non-lethal doses of Escherichia coli endotoxin (2 mg/kg) in male rats aged 8,12 and 15 weeks. Short-term changes (2 and 4 hrs) were also assessed to detect any age-dependent change in the rate of hormonal response.

The adrenal or testicular steroid hormones studied were : progesterone (P4) 17αhydroxyprogesterone (17αOHP4), corticosterone (B), testosterone (T), Δ4 androstenedione (Δ4), estrone (E1) and estradiol (E2).

Methods

One hundred and forty eight intact male Wistar rats were used in this study and consisted of : 51 rats 8-weeks old (weighing 230 ± 10 g) ; 64 rats 12-weeks old (weighing 350 ±10 g) and 33 rats 15-weeks old (weighing 380 ±10 g).

Endotoxin administration

Rats were intervenaously injected with 2 mg/kg Escherichia coli endotoxin (0.127 B8, Sigma) at 10 am. This dose was selected as it induces major steroid hormone changes. None of the rats died or developed the shock syndrome of systemic hypotension within the 4 hours of the experiment when this dose of Endo was used (3). Control rats were given vehicle alone (0.9% saline, 1.0 ml/kg).

Blood sampling

A single (8-12 ml) blood sample was taken from each rat. Each rats was anesthetised with urethane (1.2 g/kg i.p.) and bled at 2h or 4 h. after injection. This anesthetic is without effect on the response to ENDO (6). The blood samples were allowed to coagulate and the serum was separated by centrifugation (1500 g for 10 min. at 4°C) and stored at -20°C until assayed.

Steroid extraction and chromatographic fractionation

1. Serum samples (1 ml) were extracted for 30 min. with 5 ml of organic solvent (ethyl acetate/cyclohexane, 1/1) and the aqueous phase was removed by freezing (-20°C). The organic phase was evaporated to dryness, taken up in 1 ml of solvent system I (benzene/ethanol, 95/5) and placed on a Sephadex LH20 microcolumn (0.5x6 cm). Free fatty acids, progesterone (P4) and androstenedione (Δ4) were first eluted with 2.6 ml of solvent I. Estrone (E1) and corticosterone (B) were then eluted with 3.5 ml of solvent I followed by 1.0 ml of solvent II (benzene/ethanol, 90/10). Finally, estradiol (E2) was eluted with 6 ml of solvent II.
2. Serum samples (0.5 ml) were extracted as above and placed on a Sephadex LH20 microcolumn : testosterone (T) and 17α hydroxyprogesterone (17α OHP4) were eluted with 5.5 ml of solvent I.
3. The fractions were evaporated to dryness and dissolved in RIA buffer for steroid hormone assays.
The yields from these extraction and purification steps were between 70 and 95%.

Radioimmunoassay (RIA) of steroids

Samples of E1, E2, P4, 17α OH P4, B, T, and Δ4 were assayed using rabbit antisera from Miles, Yeda Ltd, Israel (anti E1 6-thyroglobulin serum, anti-17β -E2-6-BSA serum, anti-17α-hydroxyprogesterone-7-BSA serum, anti-B-21 thyroglobulin serum, anti-T-7-α-BSA serum and anti-androstenedione 7-α-BSA serum) and rabbit antisera from Biosys, France (anti P4-11HS-BSA). The detection limit was 18 pmol/L in all cases.
The tritiated steroids 2,4,6,7 ^{3}H E2 (100 Ci/mmol) ; 2,4,6,7 ^{3}H E1 (91 Ci/mmol) ; 1,2,6,7 ^{3}H T (81 Ci/mmol) ; 1,2,6,7 ^{3}H P4 (82 Ci/mmol) ; 1,2,6,7 ^{3}H B (93 Ci/mmol), 1,2,6,7 ^{3}H androstenedione (90 Ci/mmol), 1,2,6,7 ^{3}H 17α OH P4 (56 Ci/mmol) were purchased from the Radiochemical Center, Amersham. All were 99% pure ; purity was checked by thin layer chromatography. Radioactivity was determined on samples dissolved in 4 ml PCSII (Amersham) by counting in an Packard 1500 liquid scintillation analyzer using the internal standard for quench correction.

Statistics

All data were analyzed by analysis of variance (ANOVA) followed by the Duncan Kramer test (7, 8). Result were considered significant when the probabilities were : * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Results

I - Steroid hormone levels in control male rats

The serum steroid hormone levels of control rats 2h after saline injection are shown in fig. 1 and 2 (open columns). The serum B level of 8-week old rats (n=18) was higher (about 2 fold, $p<0.001$) than those of the 12 (n=29) and 15-week (n=9) old rats. By contrast, the androgen levels (Δ4 and T) of the 8-week old rats were lower : i.e. the mean serum T level of the 8-week old rats was 68% ($p<0.01$) of the 12-week old rats value and 57% ($p<0.001$) of the 15-week old rats value ; the mean serum Δ4 concentrations of the 8-week old rats was 33% ($p<0.001$) of the 12-week old rats and 28% ($p<0.001$) of that of the 15-week old rats.

The mean E2 level of the 8-week old rats was significantly higher (2 to 3 fold) than that of both the 12-week old rats ($p<0.01$) and the 15-week old rats ($p<0.05$). The serum concentrations of P4, 17αOHP4 and E1 were in the same range in all three groups.
The steroid hormone levels of control rats 4h. after saline injection were not significantly different from the levels at 2h.

II - Time course of the hormonal response to Endotoxin

The serum hormone levels of male rats aged 8, 12 and 15 weeks were measured 2 h. and 4 h. after administration of a single (2 mg/kg) non lethal dose of ENDO.

Hormone levels 2 h. after ENDO injection.

There were significant increases in P4, B serum levels (2-3 fold, $p<0.001$) and 17αOHP4 (30-40%, $p<0.01$, $p<0.001$) in the male rats of all ages (Fig. 1), while the serum T concentration decreased (2 fold, $p<0.001$, $p<0.05$) in all three groups compared to their respective controls (Fig. 2).

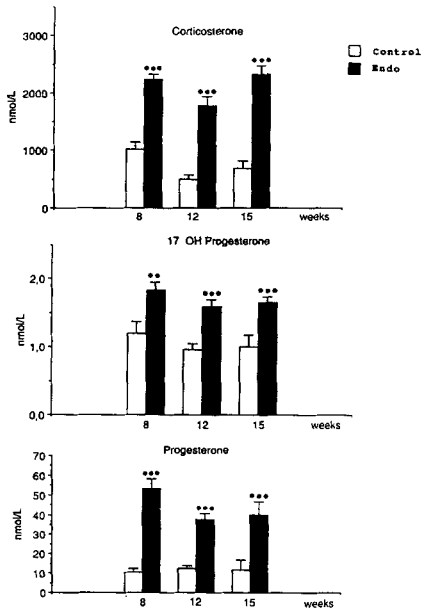

Fig. 1.

Corticosterone, progesterone and 17α OH progesterone levels 2 h. after injection of a non-lethal (2 mg/kg) dose of Endo to intact male rats. Mean (±SEM) serum concentrations were determined by RIA. ** p<0.01, *** p<0.001

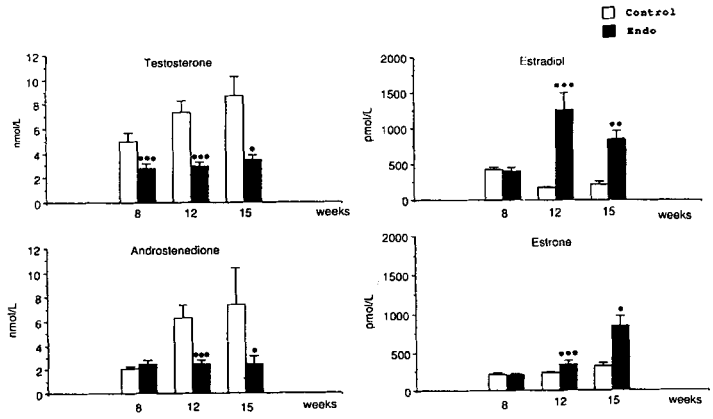

Fig. 2.

Androgen and estrogen levels 2h. after injection of a non-lethal (2 mg/kg) dose of Endo to intact male rats. Mean (±SEM) serum concentrations were determined by RIA. * p<0.05, ** p<0.01, *** p<0.001

The serum Δ4 concentration (Fig. 2) dropped (50%, $p<0.001$) only in the male rats aged 12 weeks (n=25) and 15 weeks (n=14) ; it was not different from that of the controls in the 8-week old rats (n=23).

Only the 12-week old and 15-week old rats showed a large increase in serum estrogens after Endo injection (Fig. 2). The serum E1 levels increased 1.5 fold ($p<0.001$) and 2 fold ($p<0.01$). The serum E2 response was 3-8 fold ($p<0.001$, $p<0.01$) above that of control animals. The serum E1 and E2 concentration of 8-week old rats did not change significantly.

Hormone levels 4h. after ENDO injection (Fig. 3 and 4)

The serum B level of all three groups remained as high as at 2 h., twice that of controls ($p<0.001$). The serum P4 concentrations of the 8-week old (n=10), 12-week old (n=10) and 15 week-old (n=10) rats remained high (5 fold, 2.5 fold, $p<0.001$), while the serum T levels remained low (2.5 fold, $p<0.001$) in all three groups. The mean serum 17αOHP4, Δ4 and estrogen concentrations of the 8-week old, 12-week old and 15-week old rats were not significantly different from controls.

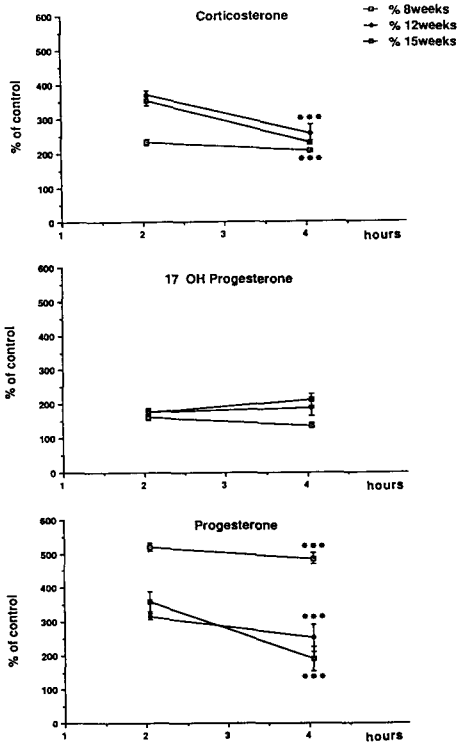

Fig. 3.

Corticosterone, progesterone and 17α OH progesterone levels (% of control) 2h. and 4h. after injection of non-lethal (2 mg/kg) dose of Endo to intact male rats. Serum concentrations (mean ± SEM) were determined by RIA. *** $p<0.001$.

Discussion

The present study confirms that the circulating steroid hormone concentrations of male rats vary with age and suggest that the adrenal glands mature before the testes.
The serum concentrations of the adrenal hormones (P4 and 17αOHP4) of 8-week old rats were similar to those of older rats (12 and 15 weeks) and the particularly high serum B concentrations of the 8 week-old rats reflect the increased adrenal activity of rats at this age. These results are in agreement with previous studies showing that immature rats have active adrenal glands (4, 9).

By contrast, the serum androgen levels (T and Δ4) of the younger rats (8 weeks) were low and increased progressively during development, in agreement with previous reports (5, 10, 11). Changes in several biological parameters have been used as

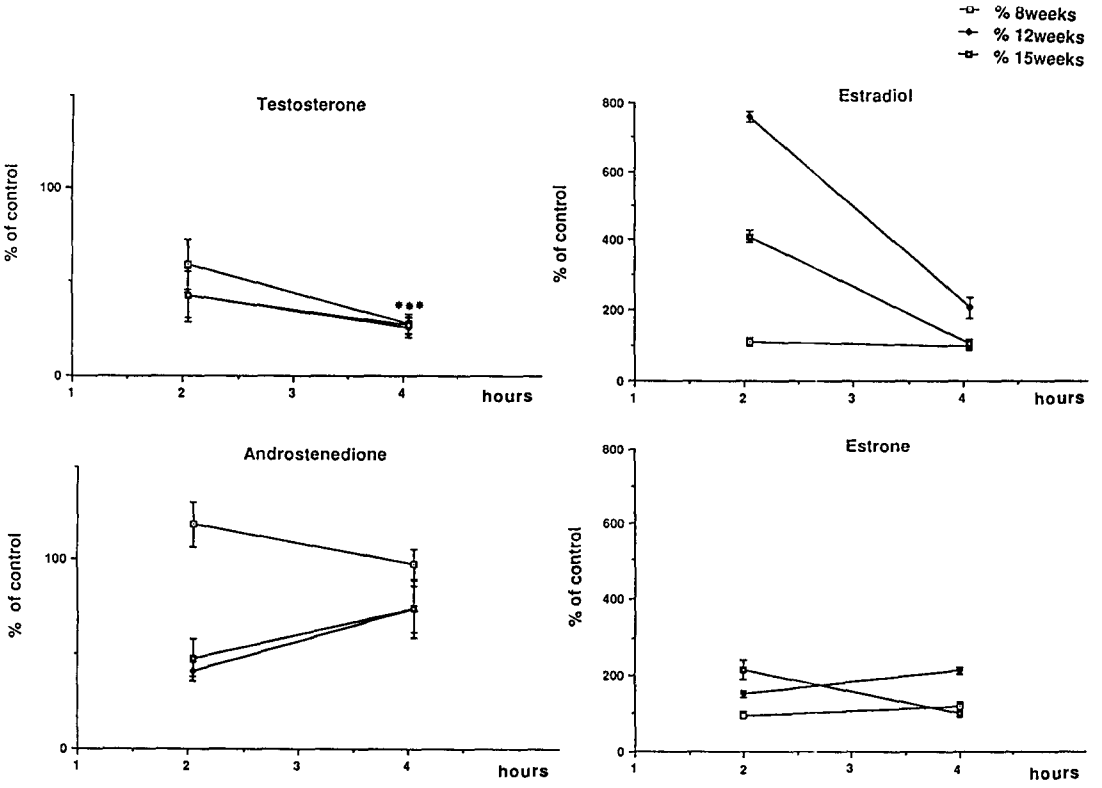

Fig. 4.

Androgen and estrogen levels (% of control) 2h. and 4h. after injection of non-lethal (2 mg/kg) dose of Endo to intact male rats. Serum concentrations (mean ± SEM) were determined by RIA. *** $p<0.001$.

indications of testicular maturation. The intratesticular levels of two key enzymes of androgen biosynthesis, mitochondrial cholesterol mono-oxygenase and microsomal steroid 17α mono-oxygenase, and the circulating concentration of androgen-binding protein (ABP), increase with age (11) and parallel the change in serum testosterone concentration, evidenced in this study. The changes in serum T during puberty are

associated with increases in the numbers and maturation of testicular Leydig and Sertoli cells (12, 13) and hence with the T response to LH stimulation (14).
The elevated E2 levels of young rats may also inhibit intratesticular T biosynthesis (15, 16).

The present study shows that the time-courses of the steroid hormone responses to Endo are different. Changes in P4, T, B and 17αOHP4 lasted for at least 4 hrs (Fig. 3 and 4), regardless of the age of the rats. The changes in Δ4 tended to disappear by the 4th hour. The E1 and E2 responses were more transient (less than 4 hours) than those of the other hormones. There is then a short-term change in estrogen levels which seems not to be influenced by age.

The steroid response to Endo is age dependent in that there were significant increases in serum estrogens (E1 and E2) and a decrease in serum Δ4 only in the male rats aged 12 weeks or older while T decreases at all ages. Previous studies have shown that this hormonal response to endotoxin is due to adrenal-testicular cooperation and stimulation of testicular aromatase activity (3). The lack of change in serum estrogen and Δ4 serum concentrations in young 8-week-old rats in response to Endo indicates that adrenal-testicular interaction is less efficient at this stage of development. This may be due to the relative gonadal immaturity of the 8-week-old rats, as reflected in the low androgen concentrations and lower aromatase testicular activity (17, 18).

The absence of an estrogen response to Endo in 8-week old rats indicates that the "refractory phase" to Endo previously described in younger rats (21 days) (9, 19, 20) as a resistance to the lethal effect of Endo may in fact, extend to at least 8 weeks of age. This refractory period to exogenous Endo may be due to the effect of the relatively high basal concentrations of E2 associated with low T levels in young animals. The way in by which these changes in steroid hormones are correlated with the " refractory phase " need(s) further investigations. However, the immune system may well be implicated, as estrogens are immunostimulators and androgens are known to be immunosuppresive (21, 22).

In conclusion, this work shows that the steroid hormone responses to endotoxin depend on the ages of the rats. Our results show that the most significant age-dependent changes in hormone levels in response to Endo appear to be the increase in E2 at 12 weeks of age or older. The preceding refractory phase seems to be due to the absence of adrenal-testicular interaction as a result of partial testicular immaturity.

Acknowledgments

This work was supported by grants from UER Xavier Bichat (University Paris VII). The skiful technical assistance of Laititia Micheli is acknowledged. The authors are grateful to Dr. Owen Parkes for help in the preparation of the manuscript and to Mrs. Maryvonne Kéréver for secretarial assistance.

Reference

1. C. BENASSAYAG, N. CHRISTEFF, M.C. AUCLAIR, C. VERNIMMEN, C. CARLI-VIELLE, E.A. NUNEZ and A. CARLI, Eur. J. Clin. Invest. 14 288-294 (1984)

2. N. CHRISTEFF, C. BENASSAYAG, C. CARLI-VIELLE, A. CARLI and E.A. NUNEZ, J. Steroid. Biochem. 29, 435-440 (1988)
3. N. CHRISTEFF, M.C. AUCLAIR, C. BENASSAYAG, A. CARLI and E.A. NUNEZ, J. Steroid. Biochem. 26 67-71 (1987)
4. G. VALLETTE, J. DELORME, C. BENASSAYAG, L. SAVU, E.A. NUNEZ, H.M.A. MEIJ-ROELOFS and P. KRAMER, Acta Endocrinol. 101 442-451 (1982)
5. C. CORPECHOT, E.E. BAULIEU and P. ROBEL, Acta Endocrinol. 96 127-135 (1981)
6. A. CARLI, M.C. AUCLAIR, C. BENASSAYAG and E.A. NUNEZ, Circ. Shock 8 301-312 (1981)
7. D.B. DUNCAN, Biometrics, 11 1-42 (1955)
8. C.Y. KRAMER, Biometrics 12 307-310 (1956)
9. L. WITEK-JANUSEK, Am. J. Physiol. 255 E525-E530 (1988)
10. E.J. PODESTA and M.A. RIVAROLA, Endocrinology 95 455-461 (1974)
11. N. KÜHN-VELTEN, D. BOS, R. SCHERMER and W. STAIB, Acta Endocrinol. 115 275-281 (1987)
12. V.G. PAHNKE, F.A. LEIDENBERGER AND H.Y. KÜNZIG, Acta Endocrinol. 79 610-618 (1975)
13. B.M. SANBORN, J.R. WAGLE, A. STEINBERGER and D.G. EMMERT, Endocrinology 118 1700-1709 (1986)
14. W.D. ODELL, R.S. SWERDLOFF, J. BAIN, F. WOLLESEN and P.K. GROVER, Endocrinology 95 1380-1384 (1974)
15. R. D'AGATA, S. GULISIA, S. ANDO, G. VITALE and P. POLOSA, Clin. Endocr. 5 393-398 (1976)
16. T. YANAIHARA and P. TROEN, J. Clin. Endocr. Metab. 34 968-973 (1972)
17. F.W. GEORGE and S.R. OJEDA, Endocrinology 111 522-529 (1982)
18. S. CARREAU, V. PAPADOPOULOS and M.A. DROSDOWSKY, Path. Biol. 36 1002-1006 (1988)
19. B. KOCH, C. MIALHE-VOLOSS and M. FREED STUTINSKY, C.R. Acad. Sc. Paris 266 808-810 série D (1968)
20. C.D. WALKER, M. PERRIN, W. VALE and C. RIVIER, Endocrinology 118 1445-1451 (1986)
21. A.H.W.M. SCHNURS and H.A.M. VERHEUL, J. Steroid. Biochem. 35 157-172 (1990)
22. Z.M. STHOEGER, N. CHIORAZZI and R.G. LAHITA, The J. of Immunol. 141 91-98 (1988)