0014-4754/94/080721-03$1.50 + 0.20 © Birkhäuser Verlag Basel, 1994

Effect of glucocorticoids injected into pregnant female mice and rats on weight of male sexual glands in adult offspring and testosterone level in fetus is genotype-dependent

G. T. Shishkina* and N. N. Dygalo

Institute of Cytology and Genetics, Siberian Branch of the Academy of Science of Russia, 630090 Novosibirsk 90 (Russia)
Received 31 January 1994; accepted 9 March 1994

Abstract. Injection of corticosterone into CBA/Lac, C57BL/6J and BALB/c mice, or hydrocortisone into aggressive and domesticated rats, on days 16 and 18 of pregnancy decreased the weight of sexual glands in adult male offspring of the C57BL/6J and domesticated mothers but increased these values in male offspring of the CBA/Lac and aggressive mothers. When injected into pregnant aggressive and domesticated rats, corticosterone affected testosterone levels in 21-day-old male fetuses. The changes were also genotype-dependent and followed the course of changes in the weight of the accessory sex glands in adults. It is suggested that glucocorticoids given during the prenatal period can affect plasma testosterone levels of male fetuses and the development of the sexual glands in a genotype-dependent manner.
Key words. Glucocorticoids; sex organ weight; testosterone; fetus; mouse; rat; strains.

Pregnant rodents subjected to stress can produce male offspring showing demasculinized development of the reproductive organs and sexual behavior. Decreases in the anogenital distance and the weight of the sex glands in newborns[1], as well as in the weight of these glands[2] and in plasma testosterone level[3] in adults from mothers exposed to stress during gestation, have been reported. Furthermore, prenatally stressed males frequently show altered male sexual behavior[4–10], the demasculinization effect of prenatal stress has been related to a reduction in plasma testosterone that promotes normal sexual development in male fetuses[11,12].

However, prenatally stressed rats did not show any changes in sexual behavior according to Chapman and Stern[13], and even exhibited enhanced male sexual behavior[14]. Moreover, prenatal stress increased the anogenital distance in newborn C57BL/6J but decreased it in DBA/2J male mice[15].

These inconsistencies in the reported changes in the genital system and male sexual behavior in the prenatally stressed adults are thought-provoking. One explanation may be differing effects after exposure to different stressors during pregnancy. Other hypotheses are that different humoral changes may be elicited by the same stressor in pregnant females of different genotypes[16] or, that according to their genotype, fetuses respond differently to hormonal shifts[17].

The maternal humoral changes most plausibly affecting the fetus is an increase in plasma glucocorticoid concentrations. Elevation of plasma glucocorticoids, a predicted consequence of stress, depends on both the size of the stress and the animal's genotype. Glucocorticoids easily pass through the placenta and enter the fetus; when injected into pregnant mice, they prolong diestrus[18] and attenuate male sexual activity[19]. However, it is not known whether the effects of early administration of glucocorticoids on the development of the reproductive system are genotype-dependent.

The aim of the present study was to obtain morphological measures of the sex organs in adult male offspring from mothers (rats and mice of different genotypes) treated with glucocorticoids during pregnancy. We also determined the level of plasma testosterone in fetuses from glucocorticoid-treated rats.

Materials and methods

Animals were mice of three inbred strains, CBA/Lac, C57BL/6J, and BALB/c, and grey rats of two outbred strains derived from wild-caught rats[20]. One rat strain was selected for absence of aggressive response towards man (domesticated rats), and the other was subjected to selection for aggressiveness inherent in feral rats (aggressive rats). All the animals had free access to water and food and bred in our animal facility under natural illumination conditions.

Two females were mated with a male of the same strain, and the day of finding spermatozoa in the vaginal smear was taken as day 1 of pregnancy.

Glucocorticoid was injected subcutaneously into each member of a strain group consisting of 8–10 females, on days 16 and 18 of pregnancy. The mice received corticosterone (Sigma, USA) at a dose of 5 mg/100 g of b. wt in distilled water supplemented with 2% Tween-80 (Ferak, Germany), and the control females received a vehicle, an equivalent volume of 2% aqueous solution of Tween-80, or no treatment. The pregnant rats were injected with either hydrocortisone (Gedeon Richter, Hungary) or corticosterone (Sigma, USA) at the same

doses as the mice. Control pregnant rats received the same amount of saline (0.9% NaCl) or no treatment.
Adult (3-month-old) male offspring of these groups of mothers (1–2 from each litter) were decapitated, and the testes, seminal vesicles, and preputial glands removed and weighed. We also studied 21-day-old rat fetuses from intact and corticosterone-treated mothers. Blood was collected in heparinized capillary tubes from individual fetuses and centrifuged. After matching by sex, plasma from several fetuses was pooled to a volume of 0.30 ml per sample. Testosterone was measured in plasma samples by radioimmunoassay (Standard Kit sets, Cea-Ire-Sorin, France.
The data were analyzed by two-way ANOVA and Student's t test.

Results

Morphological measures. Prenatal hormonal manipulations failed to affect b. wt in CBA/Lac and BALB/c males, but significantly reduced that of C57BL/6J mice (table 1). The relation between hormonal effects and genotype is confirmed by the significance of treatment × strain interaction ($F_{4,93} = 7.35$, $p < 0.001$). No significant strain or treatment effects were found for rat b. wt (table 1).
Two-way analysis of variance gave evidence of significant strain differences in the weights of the testes ($F_{2,90} = 77.4$, $p < 0.001$) and accessory glands ($F_{2,92} = 56.0$, $p < 0.001$ for the seminal vesicles; $F_{2,93} = 10.4$, $p < 0.001$ for the preputial glands) in the control male mice (fig. 1). The values for all these sex organs were the lowest for CBA/Lac and the highest for C57BL/6J. Corticosterone treatment differentially affected the morphological measures for the sex organs of the male offspring of different strains (fig. 1). Significant corticosterone treatment × strain interaction effects were found for testes ($F_{4,90} = 2.56$, $p < 0.05$), seminal vesicles ($F_{2,92} = 4.17$, $p < 0.01$), and preputial glands ($F_{2,93} = 1.46$, $p < 0.05$). The effects of corticosterone on the weights of the sex glands in the two extreme groups C57BL/6J and CBA/Lac, were clearly opposite. Injection of the vehicle into pregnant mice had, as a rule, an effect similar to corticosterone or was without effect on the development of sex organs (fig. 1).

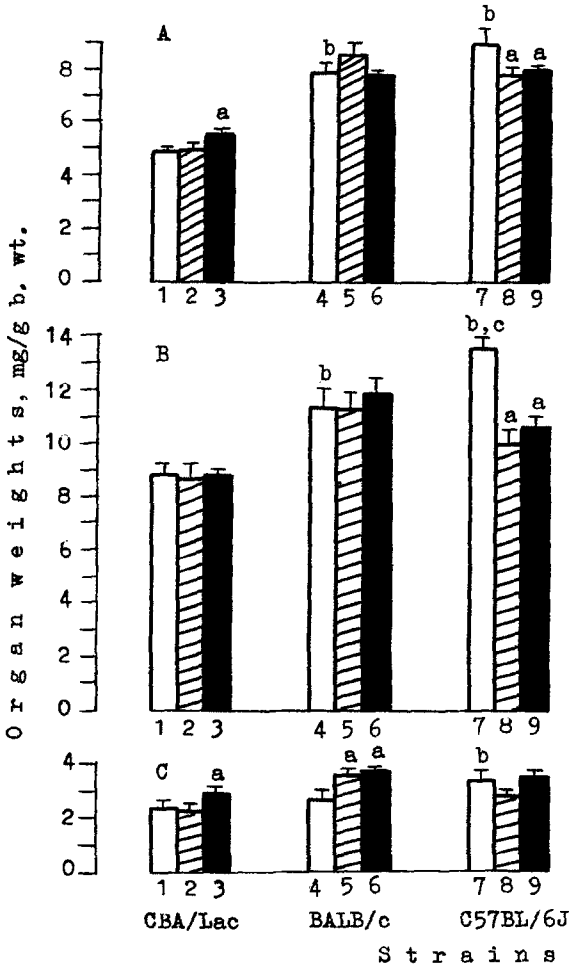

Figure 1. Relative weights (M ± S.E.M.; mg/g b.wt.) of testes (A) seminal vesicles (B) and preputial glands (C) in adult male mice from CBA/Lac, BALB/c and C57BL/6J mothers, treated with corticosterone (filled column) or vehicle (hatched column) on days 16 and 18 of pregnancy, or not treated (open column)
a, b and c show statistically significant values ($p < 0.05$): a vs untreated group; b vs 1; c vs 4.

Table 1. Body weight (M ± S.E.M.; g) in adult males of different mouse and rat strains from mothers treated on days 16 and 18 of pregnancy, or not treated. Pregnant mice were injected with corticosterone or distilled water with 2% Tween-80, pregnant rats were injected with hydrocortisone or saline.

Strain	Prenatal treatment		
	Untreated	Saline	Glucocorticoid
Mice			
CBA/Lac	24.5 ± 0.4 (10)	25.7 ± 1.6 (5)	24.0 ± 1.3 (7)
BALB/c	25.4 ± 1.1 (9)	23.9 ± 0.6 (11)	24.6 ± 0.5 (23)
C57BL/6J	29.6 ± 1.1 (14)	21.3 ± 0.4 (7)[a]	25.1 ± 0.6 (16)[a]
Rats			
Aggressive	172.5 ± 12.3 (8)	198.8 ± 13.9 (12)	193.1 ± 5.6 (13)
Domesticated	173.9 ± 6.3 (9)	207.1 ± 8.5 (12)	193.6 ± 9.6 (7)

[a]Statistically significant values ($p < 0.05$) vs untreated group.
Number of animals in brackets.

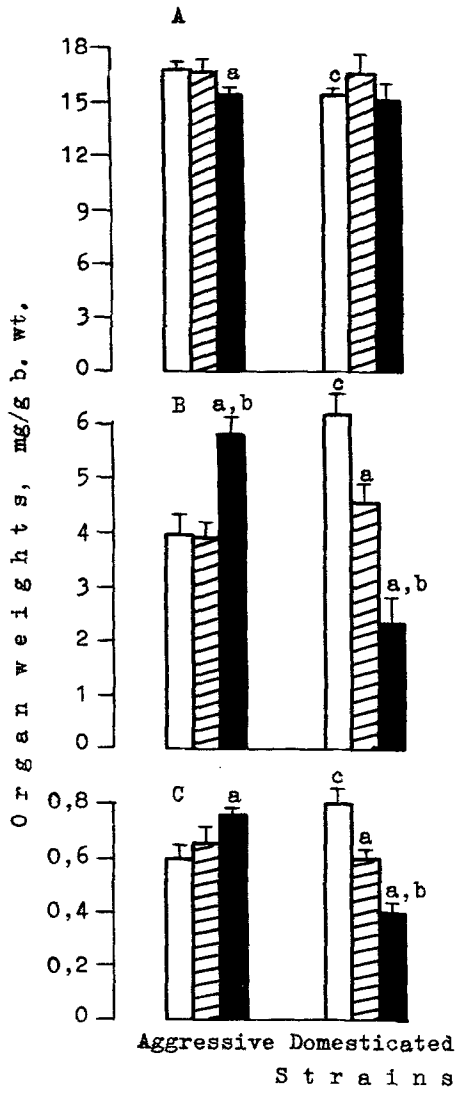

Figure 2. Relative weights (M ± S.E.M.; mg/g b.wt.) of testes (A) seminal vesicles (B) and preputial glands (C) in adult male rats from aggressive and domesticated mothers treated with hydrocortisone (filled column) or saline (hatched column) on days 16 and 18 of pregnancy, or not treated (open column)
a, b and c show statistically significant values ($p < 0.05$): a vs untreated group; b vs saline group; c vs aggressive group.

Two-way analysis of the effects of selection and hormonal treatment on the morphological measures of the sex organs in grey rats showed that these factors had no significant effects on the relative weights of the testes. The analysis, however, indicated that there is a highly significant selection × prenatal hydrocortisone treatment interaction, confirming the relation of genotype-dependent glucocorticoid effect on the relative weight of the seminal vesicles ($F_{2,55} = 27.4$, $p < 0.001$) and preputial glands ($F_{2,55} = 18.3$, $p < 0.001$). There was an increase in the relative weight of the accessory glands in the offspring of aggressive females, and a decrease in that of domesticated females given glucocorticoid during pregnancy (fig. 2B, C). Likewise, saline produced a significant, although less pronounced decrease in the relative weights of the seminal vesicles and preputial glands in offspring of domesticated mothers. In contrast, saline administered in the same amounts was without effects on aggressive rats.

Table 2. Plasma testosterone levels (M ± S.E.M.; ng/ml in 21-day-old male and female rat fetuses from aggressive and domesticated mothers treated with corticosterone on days 16 and 18 of pregnancy or from untreated mothers.

Strain	Treatment	
	Untreated	Corticosterone
Male fetuses		
Aggressive	0.504 ± 0.020 (5)	0.735 ± 0.043 (5)[a]
Domesticated	0.879 ± 0.078 (10)[b]	0.516 ± 0.068 (6)[a,b]
Female fetuses		
Aggressive	0.252 ± 0.025 (5)[c]	0.255 ± 0.045 (4)[c]
Domesticated	0.339 ± 0.049 (10)[c]	0.267 ± 0.042 (7)[c]

[a,b] and [c] show statistically significant values ($p < 0.05$): [a] vs untreated group; [b] vs aggressive males; [c] vs corresponding male group. Number of determinations in brackets.

Testosterone. Plasma testosterone levels were significantly higher in 21-day-old male fetuses from untreated domesticated mothers than in their counterparts from aggressive mothers (table 2). When injected into pregnant domesticated or aggressive mothers, corticosterone altered testosterone levels in male fetuses, but the effect was not uniform: plasma testosterone levels decreased in fetuses from domesticated mothers, and increased in those from aggressive ones. A two-way analysis demonstrated that the effect of injected corticosterone on plasma testosterone in male fetuses is significantly related to the genotype ($F_{1,18} = 9.69$, $p < 0.01$). As seen in table 2, the concentration of testosterone in plasma from female fetuses was lower than that in male fetuses, and injections of corticosterone did not affect the testosterone level.

Discussion

The present data demonstrate that the change in weight of sex glands in male offspring following an increase in plasma glucocorticoid concentration of pregnant mice or rats depends on the genotype. Treatment of pregnant females with a vehicle or saline is a stressor that produces an elevation in plasma corticosteroids[21]. This treatment can also have either a genotype-dependent effect on the development of the sex glands, similar in pattern to the one for glucocorticoids, or no effect. It is pertinent to note that the same pattern for the interaction between stress during pregnancy and the genotypic values of a trait has been observed for the anogenital distance in two mouse strains[15].

The inhibition of the development of the gonadal system due to prenatal stress has been attributed to a deficiency in androgens during sexual differentiation[11,12]. Diametrically opposed changes in sex organ weight in adults of different strains treated prenatally

with glucocorticoids may also be related to genotype-dependent changes in testosterone levels in fetuses. Treatment of aggressive and domesticated rats during pregnancy altered testosterone levels in plasma from male fetuses, following the course of changes in the weight of the accessory glands in adults. The testosterone level declined in male fetuses from domesticated mothers, whereas it rose in those from aggressive mothers.

The results from domesticated rats agree with those reported for offspring of stressed pregnant laboratory rats of Sprague-Dawley and Wistar strains. There was a 20% decrease in the percentage of Leydig cells[3] and a marked decrease in the activities of testicular steroidogenic enzymes[3,22] in 18-day-old male fetuses recovered from stressed mothers. The content of testosterone in plasma was found to be decreased in fetuses from stressed mothers[11,12]. The testosterone-dependent anogenital distance and testes weight also indicated a deficiency in androgens in such fetuses[23]. Wistar male fetuses whose mothers received dexamethasone in the drinking water during days 15–21 of pregnancy[24] showed decreased plasma testosterone. These data, and ours obtained with domesticated rats, together support the idea that glucocorticoids are responsible for the suppressive action of prenatal stress on sexual development.

However, there is a difference between these earlier results and ours from aggressive rats retaining the aggressive response inherent in wild animals. Male fetuses from aggressive rats whose mothers received corticosterone on days 16 and 18 of pregnancy had an elevated plasma testosterone level compared with the controls. It cannot be excluded that the diversity in changes in plasma testosterone results from different responses to stress by mothers during pregnancy. This appears plausible when recalling that a weak, in contrast to a strong stressor increases rather than decreases plasma testosterone levels[25]. Differences in sensitivity to treatment between mouse and rat strains may be a reason why the change in sex glands weight was diverse in these strains. The testosterone level in blood of male fetuses may reflect the interaction between genetic and glucocorticoid-mediated influences on the development of the gonadal system. The data suggest that genetic factors specific for the strain determine the testosterone concentration in plasma during the critical period of sexual differentiations. The strain-specific concentration of the sex hormone provides strain-specific development of the gonadal system. Testosterone levels are higher in the control fetuses of the domesticated strain than in the aggressive one, and domesticated rats have an accelerated rate of sexual maturation, elevated weight of sexual glands and increased sexual activity in comparison with aggressive males[20]. Glucocorticoid-induced increases or decreases in the strain-specic levels of testosterone in the blood of male fetuses were followed by the corresponding changes in the weight of the accessory sex glands in adults.

Glucocorticoid treatment decreased the weight of sex glands in strains in which these weights were already high. The treatment had the opposite effect in strains with relatively low-weight sex glands. Glucocorticoid effects minimized the genetic differences between untreated animals. The different susceptibility to glucocorticoid-mediated environmental effects may be an important 'turning' mechanism of the developing organism anticipating a stressful situation. This mechanism, buffering the genotypic differences at the level of the phenotype, may preserve the genetic variability and potential adaptability of the population

* Corresponding author.

1 Dahlöf, R., Hård, E., and Larsson, K., Physiol. Behav. *20* (1978) 193.
2 Meisel, R. L., Dohanich, G. P., and Ward, I. L., Physiol. Behav. *22* (1979) 527.
3 Pollard, I., and Dyer, S. L., J. Endocr. *107* (1985) 241.
4 Ward, I. L., Science *175* (1972) 82.
5 Herrenkohl, L. R., and Whitney, J. B., Physiol. Behav. *17* (1976) 1019.
6 Whitney, J. B., and Herrenkohl, L. R., Physiol. Behav. *19* (1977) 167.
7 Dunlap, J., Zadina, J. E., and Gougic, G., Physiol. Behav. *21* (1978) 873.
8 Götz, F., Dörner, G., Endokrinologie *76* (1980) 115.
9 Rhees, R. W., and Fleming, D. E., Physiol. Behav. *27* (1981) 879.
10 Harvey, P. W., and Chevins, P. F. D., Horm. Behav. *18* (1984) 101.
11 Ward, I. L., and Weisz, J., Science *207* (1980) 328.
12 Ward, I. L., and Weisz, J., Endocrinology *114* (1984) 1635.
13 Chapman, R. H., and Stern, J. M., J. comp. physiol. Psychol. *92* (1978) 1074.
14 Rojo, M., Marin, B., and Menendez-Patterson, A., Physiol. Behav. *34* (1985) 895.
15 Kinsley, C., and Svare, B., Behav. neural Biol. *47* (1987) 138.
16 Sharks, N., Griffiths, J., Zalcman, S., Zacharko, R. M., and Anisman, H., Pharmac. Biochem. Behav. *36* (1990) 515.
17 Dygalo, N. N., Yudin, N. S., and Naumenko, E. V., in: Stress: Neurochemical and Humoral Mechanisms, vol. 1, pp. 277–284. Eds G. R. VanLoon, R., Kvetnansky, R. McCarty and J. Axelrod. Gordon and Breach Science Publishers S.A., New York 1989.
18 Harvey, P. W., and Chevins, P. F. D., Experientia *41* (1985) 492.
19 Politch, J. A., and Herrenkohl, L. R., Physiol. Behav. *32* (1984) 135.
20 Shishkina, G. T., Borodin, P. M., and Naumenko, E. V., Physiol. Behav. *53* (1993) 389.
21 Grota, L. J., and Ader, R., Physiol. Behav. *5* (1970) 739.
22 Orth, J. M., Weisz, J., Ward, O. B., and Ward, I. L., Biol. Reprod. *28* (1983) 625.
23 Wilke, D. L., Tseu, S. R., Rhees, R. W., and Fleming, D. F., Horm. Behav. *16* (1982) 293.
24 Lalau, J.-D., Aubert, M. L., Carmignac, D. F., Gregoire, I., and Dupuy, J.-P., Neuroendocrinology *51* (1990) 289.
25 Francic, K. T., Experientia *37* (1981) 1296.