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Corticosterone administration to rat pups, but not maternal separation, affects sexual maturation and glucocorticoid receptor immunoreactivity in the testis

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Abstract

Prenatal stress strongly affects sexual dimorphism of male rats. Much less information is instead available on the effects of postnatal stress on sexual maturation during the so-called stress hyporesponsive period (SHRP). For this reason, we compared corticosterone-treated (CS; 10 mg/kg sc, suspended in sesame oil) or maternally separated pups (MS; 5 h/day in the first week of life) with control rats. Control and MS pups also received sesame oil injections. The effects of these procedures on physical development (body weight and eye opening), sexual maturation [anogenital distance, testis weight, 3β-hydroxysteroid dehydrogenase/$^{\Delta 5-4}$ (3βHSD) isomerase activity and time to testis descent] and glucocorticoid receptor (GR) immunoreactivity in the testis were examined. Corticosterone treatment significantly ($P < .05$) advanced testis descent and increased testis weight and 3βHSD activity at puberty. In addition, adult CS rats presented higher levels of GR immunoreactivity in testicular tubules when compared to control and MS rats. No differences were found between control and MS rats. On this basis, we propose that the silencing of adrenocortical function during the SHRP could be finalized to preserve sexual maturation from the influence of glucocorticoid effects. As SHRP is unique to rodents, this phenomenon could be related to their successful reproductive strategy.

Keywords: Corticosterone; Fertility; Glucocorticoid receptor; Maternal separation; Neonatal; Puberty; Reproduction; Sexual behavior; Stress; Testis

1. Introduction

Sexual maturation depends on a series of factors, the most relevant being the amount of androgens present in certain phases of development and the exposure to environmental stressors. To this regard, attention has been focussed mainly on the effects of prenatal stress on male rat development of reproductive function (reviewed in Reznikov et al., 1999). When pregnant female rats were exposed to environmental stressors during the last week of pregnancy, their male offspring developed demasculinization of sexual behavior (Ward, 1972). In addition, they had reduced anogenital distance after birth, reduced testis weight at puberty (Dahlof et al., 1978) and significantly decreased sexually dimorphic nucleus volume in the preoptic area (see Reznikov et al., 1999). These effects were related to changes in testosterone and luteinizing hormone levels in the fetal and newborn blood (Salisbury et al., 1989; Ward and Weisz, 1980), reduced brain catecholamine levels (Moyer et al., 1978) and aromatase activity (Weisz et al., 1982), and to abnormal 3β-hydroxysteroid dehydrogenase/$^{\Delta 5-4}$ (3βHSD) isomerase activity in fetal Leydig cells (Orth et al., 1983).

Similar findings were obtained by treating pregnant females with ethanol (McGivern et al., 1984), nicotine (Segarra and Strand, 1989), cannabinoids (Dalterio et al., 1984), morphine (Gagin et al., 1997), cocaine (Vathy et al., 1993) or dopamine receptor agonists and antagonists (Hull et al., 1984). Each of these procedures is known to affect ACTH and corticosterone release, pointing to a central role of limbic–hypothalamic–pituitary–adrenal (LHPA) axis activation in the mother to produce feminization of male off-

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spring (Rabin et al., 1988). Accordingly, several reports showed that treating the pregnant mother with corticosterone analogues or ACTH had consequences similar to those of prenatal stress, suggesting that glucocorticoid could negatively influence testosterone synthesis during development (Lalau et al., 1990; Reznikov et al., 1999; Rhees and Fleeming, 1981). However, no definite agreement exists on this point, since other authors either did not find comparable changes in sexual dimorphism by administering corticosterone or ACTH (Holson et al., 1995), or instead demonstrated a primary role for β-endorphin in mediating the long-term consequences of prenatal stress (Ward et al., 1986). Moreover, even the hypothesized inhibitory effects of glucocorticoids on testosterone production have been questioned when considering the immature testis (Meidan et al., 1985).

During fetal life, the LHPA axis is generally regarded as immature and unable to respond adequately to stress stimuli (De Kloet et al., 1987; Sapolsky and Meaney, 1986). A blunted LHPA axis response to stress has been described in newborn rats, and this so-called "stress hyporesponsive period" (SHRP) was found to be present up to the second week of life in rodents (for a review, see Hennessy, 1997; Vazquez, 1998). Interestingly, this period is characterized by low basal levels of ACTH, corticosterone and also of testosterone. This last finding indicates that the testes, very active immediately before birth (Orth et al., 1983; Ward and Weisz, 1980), had shifted toward a phase of silence. In rats, testosterone production starts again at puberty, 3–6 weeks after birth, when histochemical 3βHSD activity reappears within Leydig cells (Niemi and Ikonen, 1962). The initial period of life, in which the newborn begins to adapt himself to the environment, is therefore characterized by low circulating steroid levels. However, a growing evidence suggests that stress stimuli delivered during the SHRP could result in behavioral changes that permanently mark the adult animal (Hennessy, 1997; Vazquez, 1998). In particular, we recently showed that rat pups maternally separated during the first week of life respond with abnormally prolonged corticosterone release to mild stressors in the adulthood, an alteration that is related to decreased efficiency of corticosterone feedback regulation in the hippocampus (Biagini et al., 1998).

By analogy with prenatally stressed rats that are hyperresponsive to stress stimuli (reviewed in Weinstock, 1997) and present changes in sexual dimorphism, maternally separated rats could have important alterations in the maturation of their sexual phenotype. The behavioral changes consequent to maternal separation could be related to increased glucocorticoid secretion. In fact, rat pups treated with corticosterone in the first week of life presented long-lasting changes in hippocampal glucocorticoid receptor (GR) expression (Zoli et al., 1990) that were similar to those reported in maternally separated rats (Biagini et al., 1998). The dysregulation of LHPA axis could profoundly influence testis activity, as GR were shown to be widely expressed in the interstitial (Evain et al., 1976; Stalker et al., 1989) and tubular compartments (Biagini et al., 1995; Levy et al., 1989). Here we present evidence for a role of increased corticosterone serum levels during the SHRP in regulating pubertal maturation, 3βHSD activity in peripubertal Leydig cells and GR immunoreactivity in the testis of mature animals.

2. Materials and methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies (EEC Council Directive 86/609; November 24, 1986; NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985).

2.1. Animals and treatments

A total of 18 pregnant, specific pathogen-free Sprague–Dawley rats were used in two different experiments. They were given food and water ad libitum and kept under standard temperature (23 ± 1 °C), humidity (60%) and lighting conditions (lights on at 8:00 a.m. and off at 8:00 p.m.). At the beginning of the experiments, the Postnatal Day 2 (P2; setting the day of delivery as P0) litters were culled to 8–10 animals, preferentially by excluding female pups. Male pups were randomly assigned to every different treatment group by tattooing them subcutaneously with black ink in different body regions. Starting from P10, pups were examined every morning to assess eye opening and, subsequently, testis descent. In the first experiment, the male offspring were treated subcutaneously with: (i) corticosterone (Sigma-Aldrich, Milan, Italy; 10 mg/kg/day, suspended in sesame oil; CS group) on P2, P4, P6 and P8 as previously illustrated (Zoli et al., 1990, 1991); (ii) sesame oil subcutaneously (controls, vehicle-treated group); and (iii) sesame oil subcutaneously and maternal separation (MS group), according to a previously specified protocol (Biagini et al., 1998). Briefly, maternal separation consisted of 5 h/day complete isolation (from mother and siblings) in a preheated incubator (31 ± 1 °C) from P2 to P6 (on P8, MS pups were vehicle-treated only). The animals were either sacrificed during the first 2 weeks of life (at ages P2, P6, P8, P11) to measure corticosterone serum concentrations, or at P45 to study GR immunoreactivity in the testis after determination of testis descent time. In the second experiment, CS and vehicle-treated animals were killed at ages P30, P32, P34 and P38 to investigate 3βHSD activity in the testis. In these animals, we also assessed testis descent preceding killing and at autopsy.

2.2. Corticosterone assay

In rat pups, trunk blood samples were obtained by decapitation 2 h after treatments. In adult rats, blood samples were taken by intracardiac puncture of anesthesized rats, as already described (Biagini et al., 1993, 1998; Marzatico

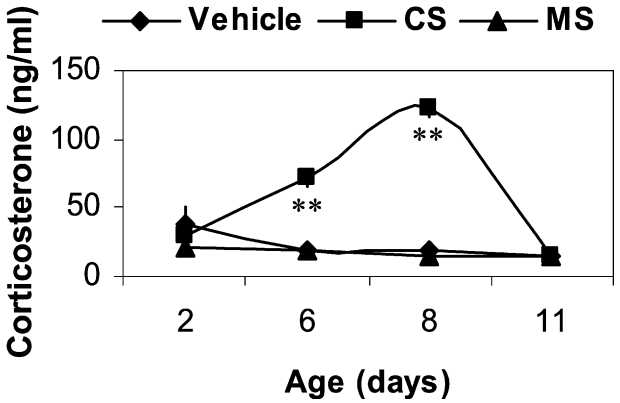


Fig. 1. Corticosterone serum levels measured in control (vehicle, sesame oil-injected), corticosterone-treated (CS) or maternally separated and vehicle-injected (MS) rat pups 2 h after treatment (apart P11, see Materials and Methods for details). Data are presented as mean ± S.E. ($n = 4$/group/time interval) of the postnatal day in which corticosterone was measured. ** $P < .01$, Tukey's test.

et al., 1998). Blood was collected in test tubes, kept at 37 °C for 30 min and then centrifuged. Serum was rapidly frozen with dry ice and kept at − 80 °C until assayed. A commercial radioimmunoassay kit (ICN Biomedicals, Milan, Italy) was used to measure corticosterone levels. Intraassay variability was 6–8%. To avoid interassay variations, all samples were assayed at once.

2.3. Enzymohistochemistry

For enzymohistochemical studies, rats were anesthesized (100 mg/kg ketamine hydrochloride) and transcardially perfused with 100 ml of ice-cold saline. Before sacrifice, each rat was weighed and assessed for testis descent. Testes were dissected out, weighed, frozen on dry ice and stored at − 80 °C. Cryostat sections (14 μm) were processed for 3βHSD following indications of Robertson (1979). For all 3βHSD assays, the medium contained 0.4 mg/ml dehydroepiandrosterone (Fluka, Milan, Italy) dissolved in dimethylformamide 5% vol/vol and dispersed in a solution containing polyvinyl alcohol 8% wt/vol and phosphate buffer 0.1 M (pH = 7.5). The chromogen nitroblue tetrazolium (1.5 mg/ml; Sigma-Aldrich) and the cofactor NAD^+ (0.4 mg/ml; DBH, Milan, Italy) were added and the reaction proceeded for 30 min at 37 °C. According to Robertson (1979), the substrate, cofactor and chromogen concentrations as well as the experimental conditions were optimal to study the enzymatic activity within the linear range of the reaction. Control sections were included by omitting the substrate or the cofactor. After a rinse in cold water (4 °C), sections were fixed in Clarke solution, dehydrated in ethanol, cleared in xylene and mounted under a coverslip for image analysis.

2.4. Immunohistochemistry

For immunohistochemistry, animals were anesthesized as previously illustrated and sacrificed in the morning (between 9 and 11 a.m.) by intracardiac perfusion with 100 ml of warm saline followed by 100 ml of ice-cold 2% paraformaldehyde in 0.1 M acetate buffer (pH 6.4) and then 2% paraformaldehyde and 0.01% glutaraldehyde in 0.1 M borate buffer (pH 8.2) (see, for details, Biagini et al., 1995). After cryoprotection (sucrose 10% in 0.2 M phosphate buffer, pH 7.2), rat testes were cut as described in the Enzymohistochemistry section. A previously characterized (Biagini et al., 1993, 1995, 1998; Zoli et al., 1990, 1991) monoclonal antibody against rat liver GR (kindly provided by Dr. K. Fuxe, Karolinska Institute, Stockholm, Sweden) was used after 1:1000 dilution. This antibody was repeatedly shown to bind preferentially GR translocated into cell nuclei (Biagini et al., 1993, 1995, 1998; Zoli et al., 1990, 1991). In control sections, the primary antibody was omitted. Testis sections were processed according to the indirect immunoperoxidase procedure by the ABC technique as already illustrated (Biagini et al., 1995), using diaminobenzidine (Sigma-Aldrich) as chromogen.

2.5. Microdensitometric analysis

The semiquantitative evaluation of the intensity of the staining in rat testis was performed by means of an automatic image analyser (IBAS I–II; Zeiss Kontron, Munich, Germany) according to Biagini et al. (1995). This analysis was based on the identification of specific profiles from the background, obtaining three parameters: (i) mean grey tone value (G) of specific profiles; (ii) field area (FA), i.e., the area covered by specific profiles (for further details, see Biagini et al., 1993, 1995, 1998; Zoli et al., 1990, 1991); and (iii) sampled area.

The analysis of the enzymohistochemical preparations was carried out by setting the microscope magnification at 6.5 × and measuring G and FA in at least five fields per section in three different sections per rat. Discrimination of specific profiles was attained by setting as threshold the value obtained by subtracting five standard deviations (S.D.) from the mean G of the background as measured in testicular tubules. Multiplying matched G and FA values of specific profiles, we obtained a summing-up parameter of the total enzymatic activity that was divided by the number of stained Leydig cell clusters. Finally, the values obtained from different animal sections were averaged.

Table 1
Effects of maternal separation and corticosterone treatment on the physical development of preweaning rat pups

Treatments	Eye opening (days)	Body weight (g)	Anogenital distance (mm)
Vehicle	14.2 ± 0.2	22.8 ± 0.4	1.14 ± 0.03
CS	14.4 ± 0.1	23.2 ± 0.3	1.21 ± 0.03
MS	13.9 ± 0.1	22.3 ± 0.3	1.21 ± 0.05

All data (mean ± S.E., $n = 18–20$/group) refer to eye opening time. No differences were found between the various treatment groups. CS = corticosterone-treated pups; MS = maternally separated and vehicle-treated pups.

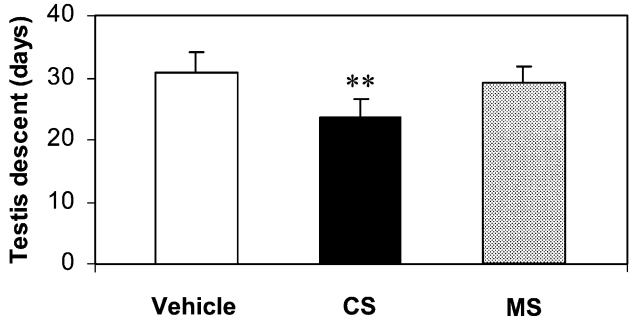


Fig. 2. Time to testis descent in control (vehicle), corticosterone-treated (CS) or maternally separated and vehicle-injected (MS) rats. Data are presented as mean ± S.E. ($n = 10–12$/group) of the postnatal day in which the event was recorded. ** $P < .01$, Dunnett's test.

For immunohistochemistry, we analyzed the GR staining in 139,879 μm^2 square fields. Measurements were done separately for tubules and interstitium, selecting background staining in areas close to GR-positive cells. After having interactively selected the specific profiles by choosing a threshold corresponding to background mean *G* value minus three S.D. (see, for details, Biagini et al., 1995), we calculated *G* values of specific profiles and FA in percent of the sampled area (FA%) as summing-up parameter. At least five fields per section and two sections per animal were analyzed.

2.6. Statistical analysis

All data were analyzed with parametrical tests. Data on corticosterone serum levels in pups were analyzed with a 3 (Groups: vehicle, CS, MS) × 4 (Time Intervals: P2, P6, P8, P11) analysis of variance (ANOVA). Two-way ANOVA was used also for data on testis weight and body weight, with two groups (vehicle and CS) and four time intervals (ages P30, P32, P34, P38), as well as for data on 3βHSD activity (2 Groups × 2 Time Intervals). Post hoc comparisons, when appropriated, were done using Tukey's test. Body development, time to testis descent and GR immunoreactivity in

Table 2
Effects of neonatal corticosterone treatment (CS) on testis and body weight in juvenile rats

		Age (Postnatal Days, P)			
	Treatments	P30	P32	P34	P38
Testis weight	Vehicle	0.37 ± 0.02	0.41 ± 0.02	0.45 ± 0.02	0.56 ± 0.01
(TW; g)	CS*	0.41 ± 0.02	0.46 ± 0.04	0.47 ± 0.01	0.60 ± 0.01
Body weight	Vehicle	61.0 ± 2.72	63.8 ± 3.47	75.8 ± 3.20	88.0 ± 2.38
(BW; kg)	CS	64.5 ± 2.47	63.5 ± 1.50	78.0 ± 2.38	97.8 ± 2.66
TW/BW	Vehicle	6.15 ± 0.06	6.43 ± 0.43	5.94 ± 0.35	6.36 ± 0.07
	CS	6.28 ± 0.20	7.26 ± 0.61	6.07 ± 0.26	6.14 ± 0.04

Sesame oil-treated rats (vehicle) represent the control group. Measurements (reported as mean ± S.E.) were obtained around puberty. Statistically significant effects in testis weight and body weight values were found for the age ($n = 8$/group of age) and treatment ($n = 16$/group of treatment) factors (see text for details). No significant differences were instead found in testis and body weight ratios.

*$P < .05$, two-way ANOVA.

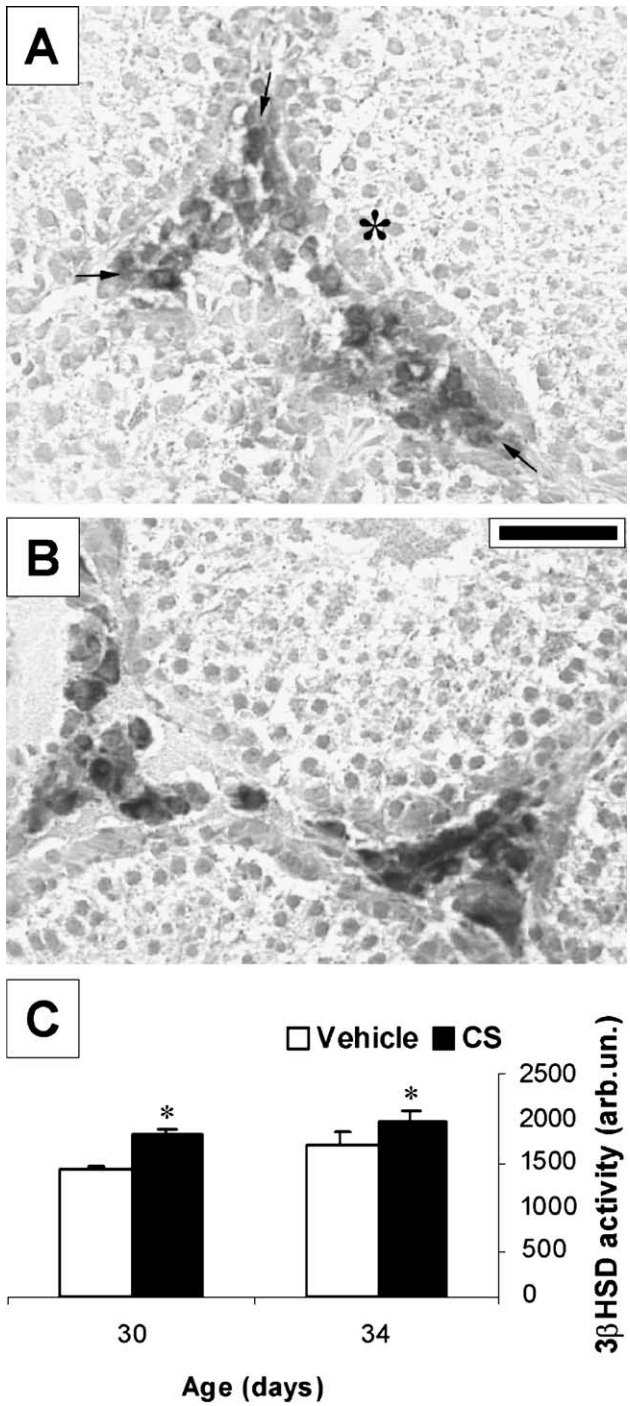


Fig. 3. Photomicrographs of 3βHSD isomerase activity in Leydig cell clusters (delimited by arrows in panel A) of rats treated with vehicle (sesame oil, $n = 8$, panel A) or corticosterone (CS, $n = 8$, panel B) during the first week of life. In panel C, microdensitometric values of 3βHSD activity measured after discrimination of specific profiles from the background (indicated by an asterisk in panel A) are expressed as mean ± S.E. (arbitrary units, arb. un.; see Materials and Methods) . * $P < .05$, two-way ANOVA. Scale bar = 50 μm.

testis were studied with one-way ANOVA followed by Dunnett's test when required. All values are presented as mean ± standard error (S.E.), with $P < .05$ having been chosen as the significant difference level. Statistical analysis was accomplished using the SPSS software (Statistical Package for the Social Sciences, version 8.0, Chicago, IL, 1998).

3. Results

3.1. Physical development

Neonatal corticosterone treatment induced a many-fold increase in corticosterone serum levels (up to fourfold the basal levels on the last day of treatment) that rapidly recovered basal values within the third day after treatment interruption (Fig. 1). Thus, the increase in corticosterone levels was well within the first 12 days of life, the "core" of SHRP (Sapolsky and Meaney, 1986). By contrast, MS pups did not manifest changes in corticosterone serum levels, suggesting that 5 h/day of maternal separation was not able to stimulate corticosterone secretion during the SHRP. During the first 10 days of life, MS pups had a delay in body weight gain (data not shown), as previously reported (Biagini et al., 1998). However, at age P13, the weight of MS pups was comparable to that of the other groups of animals (Table 1). As regards body development, no differences were found for eye opening (around 14 days) and

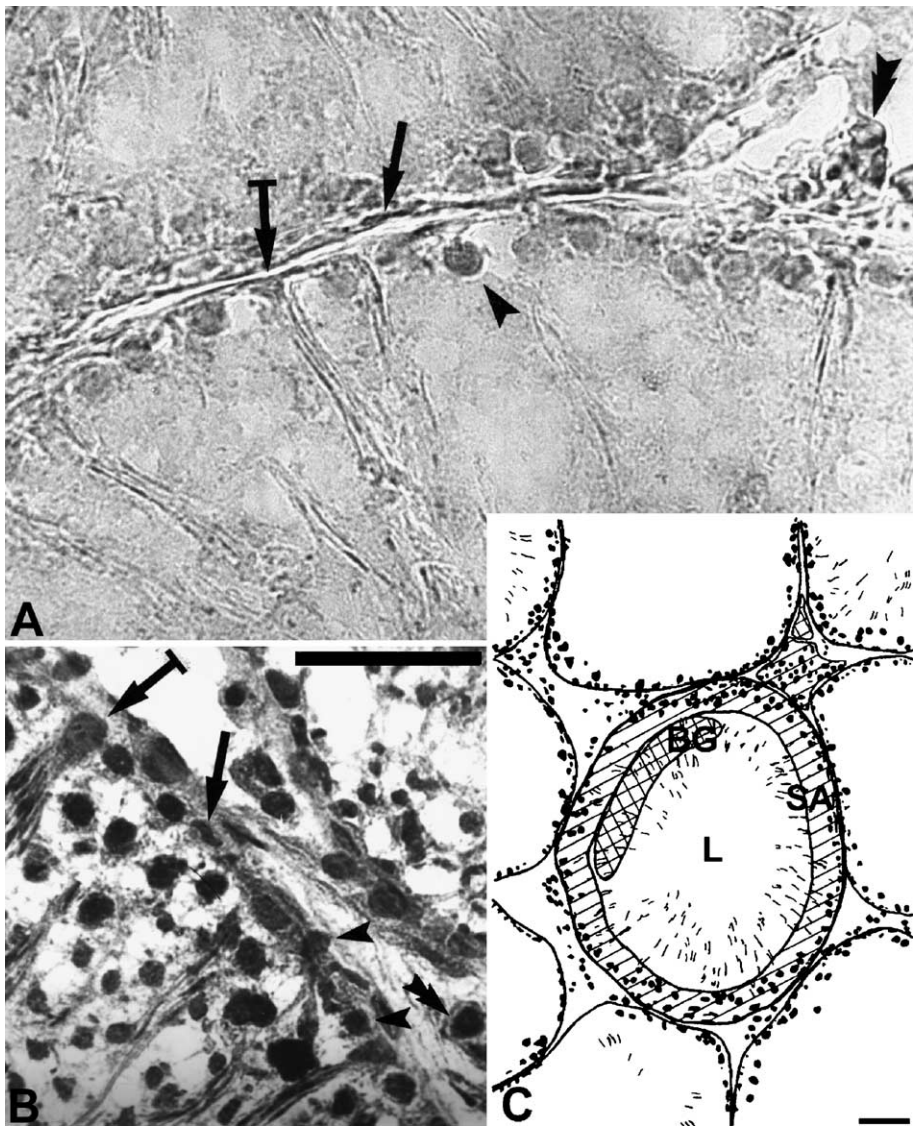
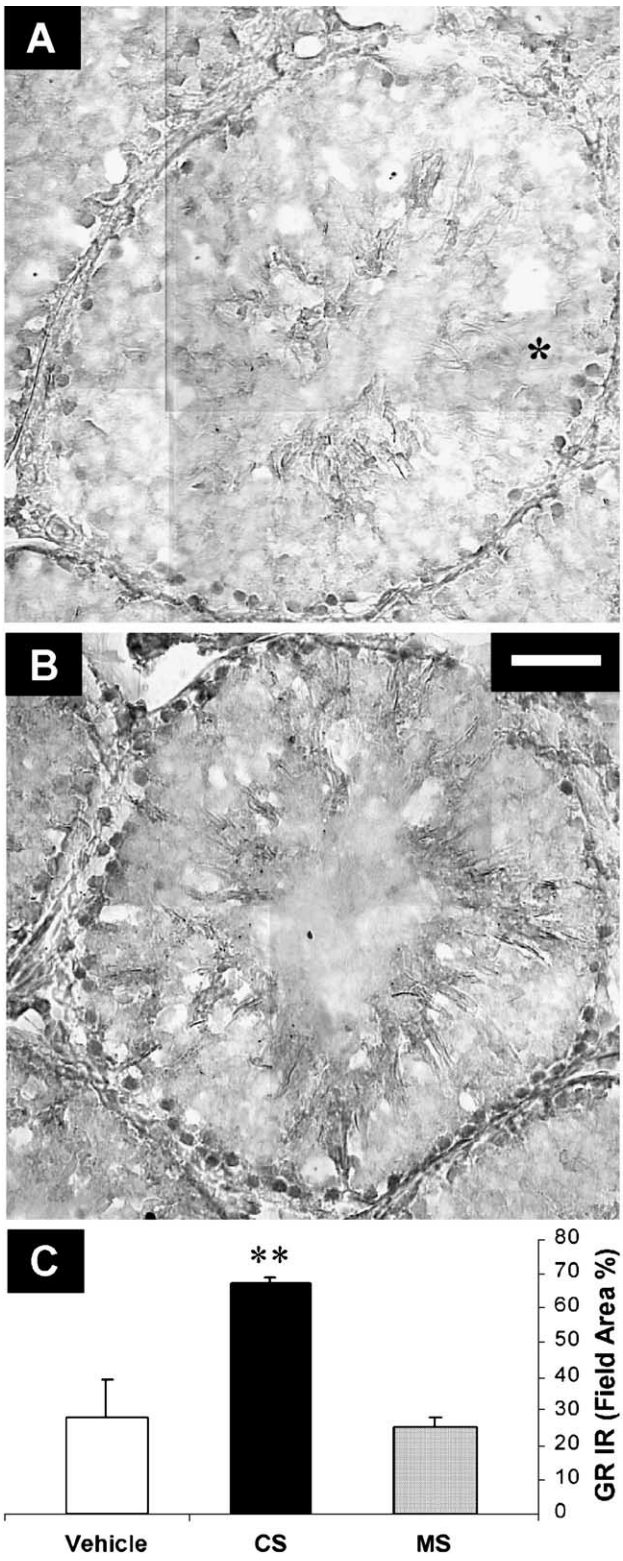


Fig. 4. Cellular distribution of GR immunoreactivity in a vehicle-treated control rat at P45 (panel A). In panel B, hematoxylin–eosin staining of rat testis is shown to facilitate the identification of GR-positive cell types. Sertoli cells are indicated by crossarrows, spermatogenic cells by arrowheads, peritubular cells by arrows and Leydig cells by double arrowheads. In panel C, the sampling procedure used to quantify GR immunoreactivity is shown (see Materials and Methods for details). Abbreviations: BG, background staining; L, lumen; SA, sampled area. Scale bars: 40 μm in panel B; 50 μm in panel C.

anogenital distance (Table 1). Although no substantial differences were found between rat pups of different groups, after weaning, a significant ($P<.01$) advance of time to testis descent was evident in CS rats compared with control animals (Fig. 2).



3.2. Enzymohistochemistry

To investigate this finding, in our second experiment, we examined body weight, testis weight and 3βHSD activity in the testis of CS and control vehicle-treated rats during the pubertal period (Table 2 and Fig. 3). As expected, we found a significant correlation between body weight and testis weight in both CS ($r=.77$, $P<.05$) and vehicle-treated ($r=.65$, $P<.05$) groups (not illustrated). For testis weight, two-way ANOVA revealed main effects of Time [$F(3,24)=31.58$, $P<.01$] and Treatment Factors [$F(1,24)=6.49$, $P<.05$], with higher values found in CS rats. Increase in body weight was found to be time-dependent [$F(3,24)=56.50$, $P<.01$] but not significantly affected by treatment [$F(3,24)=4.11$, $P=.06$]. However, considering testis weight and body weight ratio (TW/BW), only a trend for higher TW/BW was found in CS rats on P32 (Table 2). The distribution pattern of interstitial clusters of Leydig cells presenting 3βHSD activity in the rat testis is shown in Fig. 3. No differences were found between CS and control rats as regards Leydig cell cluster number (control = 35.0 ± 3.3, CS = 39.2 ± 4.5 at P30; control = 45.2 ± 3.4, CS = 44.0 ± 3.3 at P34). However, the analysis of microdensitometric measurements of 3βHSD activity in testis sections, obtained by multiplying G and FA values, indicated significantly [$F(1,9)=8.64$, $P<.05$; two-way ANOVA] higher levels in CS than in vehicle-treated rats. By contrast, time-related changes did not reach a statistically significant level [$F(1,9)=4.25$, $P=.07$], although a progressive recruitment of active Leydig cells by time was found by other authors (Mack et al., 2000).

3.3. GR immunoreactivity and corticosterone serum levels

Changes in LHPA axis activity due to stress response or neonatal treatments could predict parallel changes in GR immunoreactivity in the brain (Biagini et al., 1993, 1998; Zoli et al., 1990) or in the testis (Biagini et al., 1995). Thus, we studied the distribution of GR immunoreactivity in the interstitium and basal zone of testicular tubules in P45 vehicle-treated, CS and MS rats. Distribution of GR immunoreactivity did not differ from that described previously in older rats (Biagini et al., 1995). GR-positive cells were found in the basal zone of tubules, where Sertoli cells, spermatogonia and primary spermatocytes, as well as peritubular cells showed stained nuclei (Fig. 4). In the interstitium, the immunostaining was generally less intense. Semiquantitative

Fig. 5. Photomicrographs illustrating the intensity of GR immunostaining in 45-day-old rats neonatally treated with sesame oil (vehicle, panel A) or corticosterone (CS, panel B). Following discrimination of specific profiles from the background, indicated by an asterisk in panel A, GR immunoreactivity was quantified (panel C) with a semiquantitative microdensitometric procedure (described in Materials and Methods and illustrated in Fig. 4) in vehicle (controls), CS and maternally separated and vehicle-treated rats (MS). Values are reported as mean ± S.E. ($n=5-7$/group). ** $P<.01$ vs. controls, Dunnett's test. Scale bar = 50 μm.

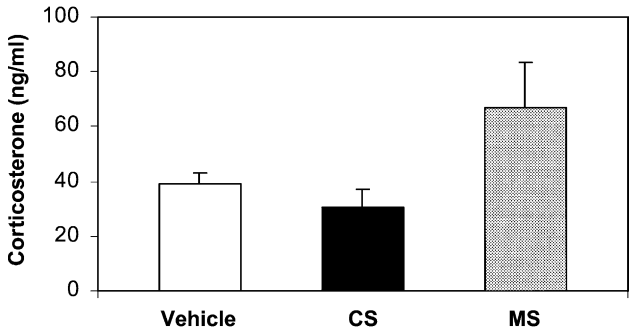


Fig. 6. Corticosterone serum levels in 45-day-old rats treated with sesame oil (vehicle), corticosterone (CS) or maternal separation (MS). Data are presented as mean ± S.E. ($n = 4-6$/group).

image analysis revealed a significantly ($P < .01$) higher intensity of GR immunoreactivity in the basal zone of CS rat tubules in comparison with values observed in the control group (Fig. 5). Conversely, the intensity of immunostaining in the interstitium was not significantly different between CS and control groups (not shown). Maternal separation was not effective in changing GR immunoreactivity both in tubules and interstitium. As GR nuclear immunoreactivity is related to agonist circulating levels also in the testis (Biagini et al., 1995), we evaluated corticosterone in serum samples taken at killing time from the animals studied with immunohistochemistry. No significant ($P = .09$) differences were found in basal corticosterone serum levels between the various treatment groups, although the highest levels were noticed in MS rats (Fig. 6).

4. Discussion

Our findings suggest that maternal separation during the first week of life is not sufficient stress to induce alterations in sexual maturation of the male rat. This procedure is known to produce an array of biological effects in mammals, such as decreased heart rate, growth hormone secretion and ornithine decarboxylase activity (reviewed in Kuhn and Schanberg, 1998). However, maternally separated rat pups do not present increased corticosterone secretion until P12 (reviewed in Hennessy, 1997; Vazquez, 1998). In the same period, the effects of maternal separation cannot be antagonized by suppressing adrenocortical function with dexamethasone injection (Van Oers et al., 1998). In line with previous findings (Levine et al., 1992; Rosenfeld et al., 1992; Vazquez, 1998), our procedure did not alter corticosterone secretion in MS pups, as their values were comparable to those found in vehicle-treated pups at least until P11. These observations do not exclude that other stress-activated central pathways could be stimulated early in development, as maternally separated rats were found to present stress-induced analgesia in response to mother absence during the SHRP (Kehoe and Blass, 1986). We previously characterized well-defined alterations in the LHPA axis activity of adult maternally separated rats, consisting of increased corticosterone basal levels, decreased GR immunoreactivity in the CA1 hippocampal field and thus hampered corticosterone feedback regulation with prolonged adrenocortical response to stress stimuli (Biagini et al., 1998). Accordingly, our present findings show that MS rats at P45 presented elevated (although statistically not different) corticosterone serum levels in basal conditions.

Neonatal treatment with repeated administration of relatively low doses of corticosterone induced a steady elevation of corticosterone serum levels that reached concentrations similar to those observed in rat pups when exposed to stressors after the second week of life (Levine et al., 1992; Rosenfeld et al., 1992; Sapolsky and Meaney, 1986). We previously found that this treatment induces a long-lasting decrease in GR immunostaining in the CA1 hippocampal field (Zoli et al., 1990). Contrary to the findings on increased corticosterone secretion in adult maternally separated rats (Biagini et al., 1998), basal corticosterone levels in the CS group were very similar to those found in control animals. Although we did not relate the changes in hippocampal GR immunoreactivity to behavior of CS rats, other authors showed that corticosterone administration to rat pups ameliorates their coping response studied after maturation (Casolini et al., 1997). Interestingly, in CS animals, we observed advanced testis descent with respect to control and MS rats. In addition, a significant increase in testis weight was found in CS rats studied around puberty, between P30 and P38. These findings were accompanied by the stimulation of 3βHSD activity in the interstitium, a marker of steroidogenically competent Leydig cells (reviewed in Chemes, 2001), suggesting that neonatal corticosterone treatment significantly affected the development of peripubertal testes. Since control vehicle-treated and MS rats were also injected, we ascribe these findings directly to the elevation of corticosterone levels in rat pups rather than to injection-induced stress.

In rat neonates, GR nuclear immunostaining is present in the testis already at P0 and declines after P4 until P14, when a brisk increase in GR immunoreactivity to reach adult levels is detected in the tubular compartment (Weber et al., 2000). Thus, postnatal GR immunoreactivity in the testis follows quite precisely the timing of SHRP, and our treatment, by increasing significantly corticosterone levels from P4 to P8, stimulated GR in neonatal testis during the phase of desensitization to corticosterone effects. In consequence of this treatment, we found that 45-day-old rats present greatly increased GR immunoreactivity in testicular tubules. These findings could be related to more than one factor, such as increased number of GR-immunoreactive cells, enhanced GR expression per cell, modified affinity to corticosterone or augmented corticosterone availability in the testicular tubules of CS rats. The last possibility was not confirmed by corticosterone serum concentration measurements at age P45. The recruitment of GR-positive cells was also excluded by normalising GR immunoreactivity with the sampled area values. Thus, corticosterone injections to rat pups probably

affected the total number or the affinity of GR in tubular cells in a stable manner, since these changes were detected a month after the treatment. Regulation of hormone binding sites and affinity by the agonist is not a surprising phenomenon, and it was also recently confirmed in the case of dexamethasone and GR in human blood cells (Vedder et al., 1999).

The present findings open the possibility that LHPA axis activity is suppressed during the SHRP to preserve sexual maturation from the influence of environmental factors able to elevate corticosterone levels. The hypoactivity of the adrenal gland may be highly deleterious for rat pups that in such a way could not adequately control their homeostasis. As we have previously shown, rat pups during the SHRP are sensitized to the toxic effects of monosodium glutamate that could otherwise be prevented by elevating corticosterone levels (Zoli et al., 1991). Until now, the SHRP has been viewed as protective against the possible teratogenic effects of glucocorticoids on the developing nervous system (De Kloet et al., 1987; Sapolsky and Meaney, 1986). Among mammals, the SHRP is unique to rodents, which are distinctively marked by their high rate of reproduction when compared to other species. Thus, the SHRP could be functional to the adequate development of rodent sexual potential. As shown by Weber et al. (2000), during the SHRP, GR expression is downregulated in the tubular but not in the interstitial compartment, in which Leydig cells express GR immunoreactivity at levels comparable with those of the adult animal. In particular, in the basal zone of developing testicular tubules, peritubular cells present a stable GR expression, GR-positive Sertoli cells progressively decrease and spermatogenic cells, mainly spermatogonia and primary spermatocytes, dramatically increase their GR immunoreactivity after P14. Our antibody, by clearly staining peritubular, Sertoli and spermatogenic cell nuclei of 45-day-old rats, demonstrated an upregulation of GR binding in the proliferative compartment of spermatogenic cells. Such findings point to a role of corticosterone in altering some basic developmental mechanisms operative immediately after birth that could set in the long-term period the level of response to glucocorticoids in the basal zone of testicular tubules.

The elevation of circulating glucocorticoids has been generally considered detrimental to Leydig cell function and, by lowering testosterone production, to spermatogenic cell production (reviewed in Rabin et al., 1988). Prolonged stress was shown to suppress the reproductive functions (Orr and Mann, 1990), and male rats immobilized for a short period presented a complete disappearance of spermatogenic cells (Meitner, 1976). These effects could be the consequence of apoptosis directly triggered by glucocorticoids in testicular tubules (Yazawa et al., 1999, 2000). Apoptosis induced by glucocorticoids has been localized in the basal zone of the tubule, where we found increased GR immunoreactivity after corticosterone administration to rat pups. Such findings encourage further experiments to establish the role of corticosterone as a putative determinant of male fertility during sexual maturation.

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