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Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats

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Abstract

Postnatal days (PNDs) 4–14 constitute the stress hyporesponsive period (SHRP) of the rat's pituitary–adrenal axis. The impact of manipulation of the pup–dam relationship during the SHRP on neuroendocrine and behavioural function has been the subject of considerable investigation. A single period of 24-h separation of the litter from the dam (maternal separation, MS) during the SHRP increases pup pituitary–adrenal activity and attenuates the SHRP. The MS manipulation also allows for the age-specific analysis of the chronic effects of early-life stress. Here we report on the effects of MS performed at the beginning of (PND 4), or about midway into (PND 9), or after (PND 18) the SHRP, on basal and stress-related blood corticosterone (CORT) titers in mature (month 5) and old (month 20) adult males. MS at PND 4, 9, or 18 did not affect basal CORT plasma titers. MS at each of these ontogenetic stages led to a similar and significant increase in the CORT response to restraint in adults but not in old adults. Therefore, whereas MS exerts a chronic impact on stress-related pituitary–adrenal activity in adult male rats, the effect of this postnatal experience does not depend upon the ontogenetic/SHRP status of the pup, and nor does it persist into senescence. © 2002 Published by Elsevier Science Inc.

Keywords: Stress hyporesponsive period; 24 h maternal separation; Development; Pituitary–adrenal; Stress reactivity; Corticosterone

1. Introduction

During most of infancy, from approximately postnatal day (PND) 4 to PND 14, the rat displays a stress hyporesponsive period (SHRP) in the form of markedly attenuated adrenocorticotrophin (ACTH) and corticosterone (CORT) responses to environmental stressors that elicit pronounced elevation of ACTH and CORT in pre- and post-SHRP rats (Rosenfeld et al., 1992; Sapolsky and Meaney, 1986). With regards to regulation of the rat SHRP, most attention has focused on intrinsic factors, including the theory that low pituitary levels of CORT-binding-globulin pertain during the SHRP such that pituitary CORT negative feedback is enhanced (Walker et al., 1986). It is now clear, however, that extrinsic factors, specifically in the form of inputs provided by maternal care, are also responsible for maintenance of the SHRP. Removal of the mother for a single and continuous period of 24 h, i.e., maternal deprivation or maternal separation (MS), during the SHRP results in (1) elevated plasma ACTH and CORT values (Avishai-Eliner et al., 1995; Kuhn et al., 1990; Levine et al., 1992; Pihoker et al., 1993; Suchecki et al., 1995), and (2) attenuation of the SHRP to stressors such as novelty or saline injection (Levine et al., 1992; Stanton et al., 1988; Suchecki et al., 1995). There is evidence that the timing of MS relative to the SHRP is important in terms of acute and medium-term effects on the HPA axis; for example, MS at PND 3 does not lead to an acute increase in CORT whereas MS at PND 7 or PND 11 does (Levine et al., 1992); MS at PND 3 leads to an enhanced stress-induced ACTH response at PND 20 whereas MS at PND 11 leads to an attenuated such response (van Oers et al., 1997).

In addition to short-term effects, it is well documented that early-life experience in the rat can lead to permanently altered pituitary–adrenocortical activity and reactivity. The most-studied model in this respect is that of early handling versus early nonhandling: Early handling, which takes the form of repeated 15-min daily pup–dam separations (Levine, 1960), leads to adults with an attenuated stress-induced

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ACTH and CORT response relative to nonhandling, which involves complete nondisturbance of dam and pups (Meaney et al., 1996; Plotsky and Meaney, 1993). A major advantage of the single MS relative to repeated separation paradigms is the opportunity it provides to investigate ontogeny-dependent effects of early-life stress (Lehmann and Feldon, 2000). In the present study, we report our findings on the effects of MS conducted during early, mid–late, or post-SHRP on basal and stress-induced CORT levels in adulthood. MS at PND 3 is reported to lead to increased basal ACTH and CORT levels in adulthood (Rots et al., 1996), whereas effects on adult pituitary–adrenal function of MS conducted later in infancy have not been studied. We previously reported evidence that there are age-dependent effects of MS on behavioural processes, including behaviours that are regulated by or correlated with CORT activity: For example, adult rats exposed to MS at PND 4 demonstrated reduced active avoidance learning, whereas those exposed to MS at PND 9 demonstrated enhanced active avoidance learning and also enhanced spatial learning in the water maze (Lehmann et al., 1999). Therefore, we wanted to investigate the existence of adrenocortical correlates of these ontogeny-dependent behavioural effects.

2. Materials and methods

2.1. Subjects

The experiments were carried out with male Wistar rats, and in accordance with the regulations of the Swiss Federal Veterinary Office. Dams and subjects were bred in-house [Zur:WIST(HanIbm) Animal Services, Swiss Federal Institute of Technology Zürich, Schwerzenbach]. Breeding and experimental animals were maintained under constant husbandry conditions of reversed cycle lighting (lights on: 19:00–07:00 h) in a temperature (21 ± 1 °C) and humidity (55 ± 5%) controlled animal facility. Subjects were derived from 64 different litters to 64 different dams. Dams were checked twice daily for birth, and the day of birth was assigned as PND 0. All litters were born within a 5-day period. Within 24 h of birth, all litters from which a subject was derived were culled to the same litter size and composition of four males and four females. Only one pup per litter was used as a subject in the present study; the littermates were the subjects of related studies (e.g., Lehmann et al., 1999). In order to minimize disturbances to litters that might confound effects of the MS procedure (see below), cage cleaning was carried out once only, at PND 11, for all litters between birth and weaning at PND 21. At weaning, subjects were weighed and placed in group-cages (Perspex Macrolon type IV, 59.0 × 38.5 × 20.0 cm), with four same-sex animals per cage, with each group of four animals derived from different litters and belonging to the same treatment group. Food (Kliba 3430, Klibamühlen, Kaiseraugst, CH) and water were available ad libitum.

2.2. MS procedure

From the 64 litters, three experimental treatments and one control group were formed, $N = 16$ per group. MS subjects were separated from their mothers for 24 h, 18:00–18:00 h, at PND 4 (MS4), or PND 9 (MS9), or PND 18 (MS18). To carry out MS, the dam was removed to another cage while the pups remained together with the litter in their home cage. The home cage was then transferred to a separate room and placed on a heat pad set at 33 °C. After 24 h, the pups were returned to the colony room and the mother was returned to the home cage. Control animals (CON), apart from culling and the single cage cleaning, remained with their mothers without human contact from birth until weaning; CON litters were not held in a separate room with restricted personnel entry and, therefore, although disturbance was minimal, the control condition was not nonhandling.

2.3. Restraint stress and blood sampling

The effect of MS on CORT basal activity and stress reactivity was studied in one cohort of naïve subjects aged 5 months (adult) and, 15 months later, in a second cohort of naïve subjects aged 20 months (old adult). Eight subjects per treatment group were included in each experiment, and only one subject was included from any one litter such that statistical analysis was not confounded by litter effect. Beginning 3 days prior to restraint and blood sampling, subjects were habituated daily to the experimental room and to the handling required for tail-vein blood sampling. In an attempt to ensure that basal trough levels were obtained, blood sampling and restraint were performed between 1300 and 2000 h, i.e., mid- to late-dark phase. Basal blood sampling and restraint, followed by serial blood sampling, were performed on rats in counterbalanced squads of four. The subject was removed from the home cage situated in a room adjacent to the experimental room, and was placed immediately into a restraint tube. The restraint tube was 5 cm in diameter, equipped with air holes, closed at one end by a wall of the same material, and adjustable at the open end with an O-shaped fixture that allowed the length of the tube to be set to the exact size of the animal and provided access to the tail for blood sampling. The first, i.e., basal, blood sample was taken immediately after placing the animal into the restraint tube: 100–200 μl of blood were obtained from the tail vein and collected into a prechilled heparinized tube (Microvette, Sarstedt, Sevelen, Switzerland), as described and validated by Fluttert et al. (2000). The subject in the restraint tube was then placed in a well-illuminated area and remained there for 30 min. After this time (t0) a second blood sample, i.e., peak stress response sample, was collected from the same incision site, and the subject was returned to its home cage. Further, poststress blood samples were collected rapidly at t30, t60, and t120 min, to monitor

poststress recovery. Blood tubes were kept on ice between collection and centrifugation, and plasma was removed and stored at −25 °C prior to assay. All plasma samples from the adult males were determined in a single radioimmunoassay (RIA) and the same was the case for all the old adult males. The RIA system used was a ^{3}H Corticosterone kit (07-120016, ICN Biomedicals, Costa Mesa, CA, USA), validated as described in Pryce et al. (2001).

2.4. Data analysis

Because restraint and CORT determination were performed separately in adults and old adults using different subjects in the two experiments and with an interval of 15 months between these two experiments, it was essential that data analysis be performed separately for adult and old adult subjects. Basal plasma CORT titers were analyzed by a one-way ANOVA with a main factor of treatment (MS4/MS9/MS18/CON). Plasma CORT stress response and poststress recovery were analyzed by a 4×4 ANOVA with the main factor of treatment (MS4/MS9/MS18/CON) and a repeated measures factor of time (t0, t30, t60, t120). Post hoc pairwise comparisons were performed using *t* tests based on the error term derived from the overall ANOVA.

3. Results

3.1. Adults

3.1.1. Basal CORT titers

The ANOVA did not yield a significant effect of treatment ($P > .25$), indicating that there was no difference between the four groups in basal CORT concentrations (Fig. 1a).

3.1.2. CORT titer response to restraint

The 4×4 ANOVA revealed a significant effect of the repeated measure of time ($F = 271.95$, $df = 3/81$, $P < .001$), indicating increased plasma CORT titers as a consequence of restraint and a subsequent decrease back towards basal levels (Fig. 1a). The factor of treatment reached significance ($F = 3.81$, $df = 2/27$, $P < .03$), indicating group differences in stress-related plasma CORT levels between t0 and t120. Post hoc analysis revealed that during this time period plasma CORT titers were significantly greater in MS4 vs. CON ($P < .05$), MS9 vs. CON ($P < .007$), and MS18 vs. CON ($P < .007$), whereas all three MS groups displayed very similar CORT levels to each other. Referring to individual time points, MS9 ($P < .05$) and MS18 ($P < .01$) demonstrated significantly greater CORT levels than CON at t0, MS4 ($P < .05$), MS9 ($P < .01$), and MS18 ($P < .01$) demonstrated significantly greater CORT levels than CON at t30, and there were no further significant differences beyond this time point (Fig. 1a).

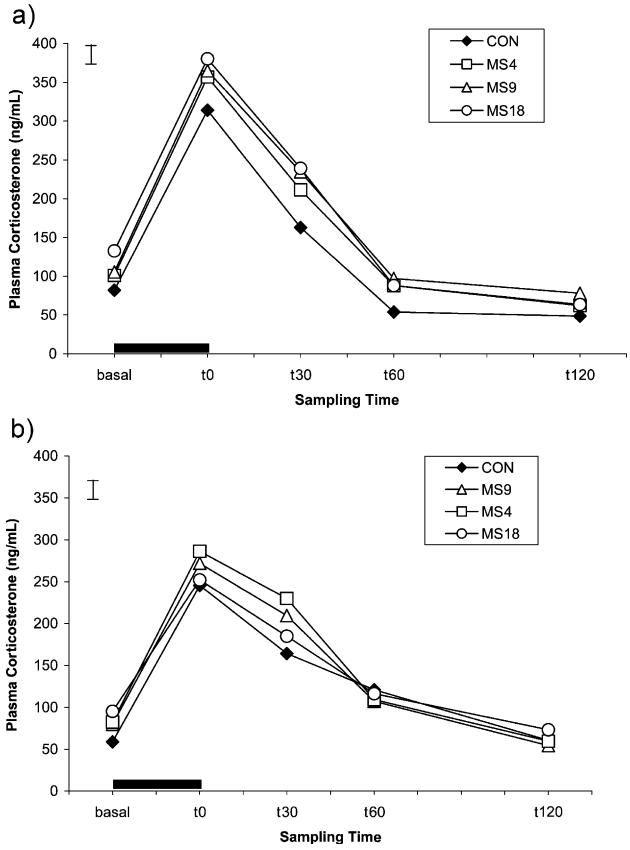

Fig. 1. Effects of 24-h MS at different stages of the SHRP on mean plasma CORT values at rest and following restraint stress in male rats aged (a) 5 months, or (b) 20 months. Values are the mean of eight unrelated subjects per treatment and age. The estimate of standard error is denoted by the vertical bar and was calculated using the mean square of the analysis of variance error term. The black horizontal bar denotes the period of 30-min restraint stress, beginning immediately after collection of the basal blood sample. t0 denotes the sample taken immediately after restraint, and t30, t60 and t120 denote the samples taken 30, 60, and 120 min following poststress return to the home cage. In (a) there was a significant main effect of treatment between t0 and t120, with post hoc analysis revealing that plasma CORT values were significantly greater in MS9 and MS18 than in CON at t0 and significantly greater in all MS groups than in CON at t30.

3.2. Old adults

3.2.1. Basal CORT titers

The ANOVA did not yield a significant effect of treatment ($P > .48$), indicating that there was no difference between the four groups in basal CORT concentrations (Fig. 1b).

3.2.2. CORT titer response to restraint

The 4×4 ANOVA revealed a significant effect of the repeated measure of time ($F = 119.94$, $df = 3/69$, $P < .001$), indicating increased plasma CORT titers as a consequence of restraint stress and a subsequent decrease back toward basal levels (Fig. 1b). The factor of treatment did not reach significance ($P > .83$), indicating no group differences in stress-related plasma CORT titers in aged male rats.

4. Discussion

The present study has demonstrated that a single 24-h MS performed at PNDs 4–5, 9–10, or 18–19 leads to an increased CORT response to restraint in adult male Wistar rats aged 5 months, and not in aged (20-month-old) male Wistar rats. Although the three ages at which MS was performed correspond to very different ontogenetic phases relative to the SHRP, there were no consistent differences between the three MS groups at either age in terms of their stress-related CORT response.

MS did not lead to differences in basal levels of plasma CORT in the present study, which is somewhat in contrast to the finding of Rots et al. (1996), the latter reporting increased basal CORT and ACTH levels in male Wistar rats following a very similar MS procedure to that used here, performed however at PND 3, i.e., possibly immediately prior to the SHRP. That 24-h MS leads consistently to an increased CORT stress response in adult rats is important in several respects. It demonstrates that the endocrine effects of MS persist into mature adulthood; that is, well beyond the effects reported to pertain at Day 20 of life by van Oers et al. (1997). It also demonstrates that the age-specific effects of MS observed at PND 20, namely, MS at PND 3 leading to an increased pituitary–endocrine stress response and MS at PND 11 leading to an attenuated such response are apparently not maintained in mature rats. In fact, MS performed at early-, mid-, or even post-SHRP resulted in a consistent increase in the stress-induced CORT response. This finding was unexpected with respect to the existing neuroendocrine studies of MS effects, and also with respect to the behavioural evidence that we have obtained—indeed with littermates of the animals used in the present study—demonstrating that the effects of MS on learning and memory in aversive situations are dependent on the age at which MS occurred (Lehmann et al., 1999). When MS was performed at PND 3, Rots et al. demonstrated significantly reduced adulthood glucocorticoid receptor (GR) mRNA levels in the hypothalamic paraventricular nucleus and the anterior pituitary, the two principal sites of CORT tonic feedback inhibition of the HPA axis (De Kloet et al., 1998), but both hippocampal MR and GR expression were unaffected. In contrast to the Rots et al. study, basal CORT values were not affected by MS at any postnatal stage in the present study, and extrapolation from Rots et al. would therefore suggest that altered GR gene expression is not a candidate to explain the present MS-related increase in the CORT stress response. One other potential candidate is altered hypothalamic CRF gene expression: Avishai-Eliner et al. (2001) have demonstrated that early handling leads to persistent down-regulation of hypothalamic CRH-mRNA levels and does so independently of up-regulated GR-mRNA expression; MS might lead to persistent changes in the opposite direction.

The generalized MS-related CORT stress hyperactivity we observed in mature adults did not extend into senescence. The study design was not appropriate for a statistical comparison of the CORT titers of the two age cohorts. There are several reports of basal CORT levels increasing with the onset of senescence (Sapolsky et al., 1986; Stöhr et al., 2000), but we did not obtain any evidence for this phenomenon in the present study. This negative outcome for the effect of 24-h MS on CORT stress response in aged rats contrasts with previous reports that early handling reduces stress-related CORT levels in aged rats (Lehmann et al., 2002; Meaney et al., 1988, 1991). It is, however, consistent with our recent finding that repeated MS for 6 h per day on PNDs 12, 14, 16, and 18 also fails to exert a significant impact on CORT responsiveness to restraint stress (Lehmann et al., 2002).

The 24-h MS paradigm provides a tool via which the interrelationships between significant early-life experience and stage of ontogeny can be investigated. In the present study, we have provided evidence for male adult Wistar rats that this experience leads to increased stress-related CORT levels in mature adulthood and regardless of whether it occurs at the beginning, about midway, or after the SHRP. We also provide evidence that this effect is not maintained throughout the life span; old adult males that experienced MS did not differ from their age-matched controls in terms of stress-related CORT activity.

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