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The postnatal development of the hypothalamic–pituitary–adrenal axis in the mouse

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Abstract

The main characteristic of the postnatal development of the stress system in the rat is the so-called stress hypo-responsive period (SHRP). Lasting from postnatal day (pnd) 4 to pnd 14, this period is characterized by very low basal corticosterone levels and an inability of mild stressors to induce an enhanced ACTH and corticosterone release. During the last years, the mouse has become a generally used animal in stress research, also due to the wide availability of genetically modified mouse strains. However, very few data are available on the ontogeny of the stress system in the mouse. This study therefore describes the postnatal ontogeny of peripheral and central aspects of the hypothalamic–pituitary–adrenal (HPA) axis in the mouse. We measured ACTH and corticosterone in blood and CRH, urocortin 3 (UCN3), mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) transcripts in the brain at postnatal days 1, 2, 4, 6, 9, 12, 14 and 16. Our results show that we can subdivide the postnatal development of the HPA axis in the mouse in two phases. The first phase corresponds to the SHRP in the rat and lasts from right after birth (pnd 1) until pnd 12. Basal corticosterone levels were low and novelty exposure did not enhance corticosterone or ACTH levels. This period is further characterized by a high expression of CRH in the paraventricular nucleus (PVN) of the hypothalamus. Expression levels of GR in the hippocampus and UCN3 in the perifornical area are low at birth but increase significantly during the SHRP, both reaching the highest expression level at pnd 12. In the second phase, the mice have developed past the SHRP and were now exhibiting enhanced corticosterone basal levels and a response of ACTH and corticosterone to mild novelty stress. CRH expression was decreased significantly, while expression of UCN3 and GR remained high, with a small decrease at pnd 16. The expression of MR in the hippocampus was very dynamic throughout the postnatal development of the HPA axis and changed in a time and subregion specific manner. These results demonstrate for the first time the correlation between the postnatal endocrine development of the mouse and gene expression changes of central regulators of HPA axis function.

Keywords: Postnatal development; Hypothalamic–pituitary–adrenal axis; Adrenocorticotropin releasing hormone

1. Introduction

An undisturbed development of the hypothalamic–pituitary–adrenal (HPA) axis is essential for the normal function of the organism during adulthood. Several studies in humans demonstrated, that an early life time trauma, such as childhood abuse or maltreatment, has lasting effects on pituitary–adrenal and autonomic function and represents a major risk factor for the development of mood and anxiety disorders (Heim and Nemeroff, 2001; Heim et al., 2000a,b, 2001; Agid et al., 1999; Welberg and Seckl, 2001). To understand the underlying mechanism of vulnerability to stress related diseases it is therefore essential, to study the development of the stress system.

Until recently, the rat has been the most commonly used laboratory animal in developmental stress research. On the other hand, readily available genetically modified mice have become a powerful tool to study the function of specific components of the stress system in more detail (Muller and Keck, 2002). Yet, few studies focused on the influences of the genetic modification on the development of the animal, including possible compensatory effects (Gross et al., 2002).

Abbreviations: ACTH, adrenocorticotropin releasing hormone; ANOVA, analysis of variance; CRH, corticotropin releasing hormone; GR, glucocorticoid receptor; HPA axis, hypothalamic–pituitary–adrenal axis; MR, mineralocorticoid receptor; PVN, paraventricular nucleus of the hypothalamus; SHRP, stress hypo-responsive period; UCN3, urocortin 3

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The use of genetically modified mice therefore requires a greater understanding of the development of the stress system in the mouse.

In the rat, the postnatal development of the stress system is characterized by a so-called stress hypo-responsive period (SHRP) (Schapiro et al., 1962). Lasting from about postnatal day (pnd) 4 to pnd 14, rat pups during the SHRP exhibit low basal concentration of corticosterone and an inability of mild stressors to induce a corticosterone response (Levine, 1994). A number of studies also demonstrated the existence of a SHRP in the mouse (D'Amato et al., 1992; Cirulli et al., 1994, 1997; del Rey et al., 1996; Spinedi et al., 1997; Schmidt et al., 2002). Still, the exact time course of the SHRP in the mouse was not known.

Additionally, very few data were available on the ontogeny of central components of the stress system in the mouse. In a recent study from our laboratory, we examined the expression levels of corticotropin releasing hormone (CRH), mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in mouse pups at pnds 9 and 18 (Schmidt et al., 2002). These data indicated a change of CRH and MR expression during development. However, no conclusions could be drawn regarding the time course of these changes.

In the current study, we describe the postnatal ontogeny of both peripheral and central key players of the HPA system in the mouse. We investigated the basal postnatal ontogeny of corticosterone and ACTH in the blood as well as CRH, urocortin 3 (UCN3), MR and GR expression in the brain at eight different postnatal ages (pnds 1, 2, 4, 6, 9, 12, 14 and 16). Secondly, we characterized the time frame of the SHRP in the mouse, based on corticosterone and ACTH activation following novelty exposure at these 8 postnatal days.

2. Methods

2.1. Animals

The offspring of CD1 mice (obtained from Charles River, NL) were used in this study. After a habituation period of 1 week four females were mated with one male in type 3 polycarbonate boxes (820 cm^3) containing sawdust bedding. Pregnant females were transferred to clean type 3 polycarbonate cages containing sawdust and two sheets of paper towels for nest material during the last week of gestation. Pregnant females were checked for litters daily at 09:00 a.m. If litters were found, the day of birth was defined as Day 0 for that litter. On the day after parturition, Day 1, each litter was culled to eight healthy pups (four males and four females) and remained undisturbed until used in the experiment. A total of 40 litters was used in the study. All animals were housed under a 12L:12D cycle (lights on at 07:00 a.m.) and constant temperature (23 ± 2 °C) and humidity (55 ± 5%) conditions. Food (SRM-A; Hope Farms, NL) and water were provided ad libitum.

The experiment was carried out in accordance with European Communities Council Directive 86/609/EEC. All efforts were made to minimize animal suffering during the experiments. The protocols were approved by the Animal Care Committee of the Faculty of Medicine, University of Leiden (Leiden, The Netherlands).

2.2. Experimental design

We studied the basal and the novelty stress induced activity of peripheral and central HPA related parameters during the first two weeks after gestation. We tested mice at 8 different postnatal days (pnds 1, 2, 4, 6, 9, 12, 14 and 16). For each testing day, animals were subdivided in two different conditions, a basal condition and a stress condition. As mild stimulus, we used 30 min of isolated novelty exposure, which has been shown to elicit a pronounced ACTH and corticosterone response in mice outside the SHRP (Schmidt et al., 2002).

2.3. Testing procedure

Testing took place between 09:00 and 11:00 a.m. At the beginning of testing, the mother was removed from the home cage. One male and one female pup were immediately removed from the home cage and sacrificed by decapitation (basal condition). The remaining litter was also removed from the home cage and placed individually in novel cages containing sawdust bedding. The cages were placed on a heating pad (30–34 °C) to maintain the body temperature of the pups. After 30 min in the novel environment, one male and one female of the litter were sacrificed. The remaining littermates were used for another study. For each age and each condition a total of eight male and eight female mouse pups were used.

Trunk blood from all pups was collected individually in labeled 1.5 ml EDTA-coated microcentrifuge tubes. All blood samples were kept on ice and later centrifuged for 10 min at 13,000 rpm at 5 °C. Plasma was transferred to clean, labeled 1.5 ml microcentrifuge tubes. All plasma samples were stored frozen at −20 °C until the determination of ACTH and CORT. ACTH and CORT were measured by RIA (ICN Biomedicals Inc., CA; sensitivity 10 pg/ml and 12.5 ng/ml, respectively). For ACTH, due to the small volume, always two blood plasma samples of 1-, 2-, 4- or 6-day-old pups of the same sex and time point were pooled. Whole heads (without skin and jaw) were removed, frozen in isopentane at −40 °C and stored at −80 °C for in situ hybridization.

2.4. In situ hybridization

Only animals from the basal condition were used for in situ hybridization. Frozen brains were sectioned at −20 °C in a cryostat microtome at 16 μm in the coronal plane through the level of the hypothalamic paraventricular nucleus (PVN) and dorsal hypothalamus. The sections were thaw-mounted

on poly-L-lysine coated slides, dried and kept at −80 °C. In situ hybridizations using ^{35}S UTP labeled ribonucleotide probes (CRH, UCN3, GR, MR) were performed as described previously (Makino et al., 1995) with some adaptations. Briefly, sections were fixed in 4% paraformaldehyde/0.5% glutaraldehyde and acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine/HCl. Subsequently, brain sections were dehydrated in increasing concentrations of ethanol. The cRNA probes contained the full length coding regions of CRH (rat), GR, MR and UCN3 (mouse), respectively. The antisense cRNA probes were transcribed from a linearized plasmid. Tissue sections (two brain sections per slide) were saturated with 100 μl of hybridization buffer (20 mM Tris–HCl (pH 7.4), 50% formamide, 300 mM NaCl, 1 mM EDTA (pH 8), 1X Deinhardt's, 250 μg/ml yeast transfer RNA, 250 μl/ml total RNA, 10 mg/ml salmon sperm DNA, 10% dextran sulfate, 100 mM dithiothreitol, 0.1% SDS and 0.1% sodium thiosulfate) containing approximately 1.5×10^6 cpm ^{35}S labeled riboprobe. Brain sections were coverslipped and incubated overnight at 55 °C. The following day, the sections were rinsed in 2X SSC (standard saline citrate), treated with RNAse A (20 mg/l) and washed in increasingly stringent SSC solutions at room temperature. Finally, sections were washed in 0.1X SSC for 30 min at 65 °C and dehydrated through increasing concentrations of alcohol. The slides were apposed to Kodak Biomax MR film (Eastman Kodak Co., Rochester, NY) and developed.

2.5. Data analysis

Data were analyzed by analysis of variance (ANOVA) with the level of significance set at $P < 0.05$. When appropriate, tests of simple main and interaction effects and subsequent post hoc comparisons were made by Newman–Keuls procedures. The initial analysis included sex as a factor; once it was determined that sex was not a significant factor, the data were collapsed across this variable. Autoradiographs were digitized, and relative expression of CRH, UCN3, MR and GR mRNA was determined by computer-assisted optical densitometry (analySIS 3.1, Soft Imaging System GmbH). The mean of four to eight measurements was calculated from each animal.

3. Results

3.1. Hormones: developmental pattern and response to novelty

3.1.1. Corticosterone (Fig. 1)

ANOVA revealed a main effect of age ($F_{7,138} = 51.146$, $P < 0.001$) and treatment ($F_{1,138} = 98.245$, $P < 0.001$), and an interaction between age and treatment ($F_{7,138} = 27.853$, $P < 0.001$). Under basal conditions, animals from pnd 1 to pnd 12 showed very low corticosterone concentrations. At pnd 14 and pnd 16 basal corticosterone concentrations increased. From pnd 1 to pnd 9, novelty stress had no significant effect on plasma corticosterone. After pnd 9 (pnds 12, 14 and 16), 30 min of novelty stress did elicit a significant corticosterone response, which was also increasing with age.

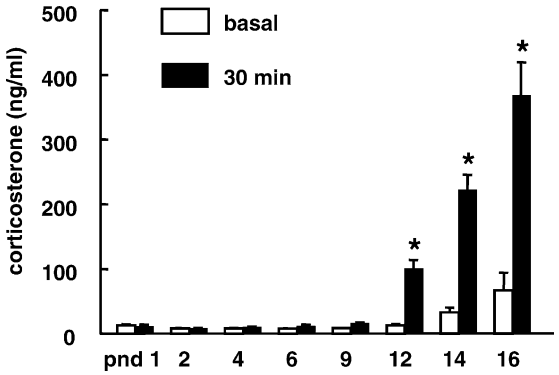

Fig. 1. Basal and stress induced (novelty) plasma corticosterone levels in mouse pups at eight different postnatal ages. Basal corticosterone levels are low between pnd 1 and pnd 12 and are elevated at pnd 14 and pnd 16. Between pnd 1 and pnd 9, there is no increase of corticosterone release following novelty stress. In contrast, pups at pnds 12, 14 and 16 do respond to novelty stress with an elevation of plasma corticosterone. Data represent mean ± S.E.M., $^*P < 0.05$ (significant from basal).

3.1.2. ACTH (Fig. 2)

ANOVA revealed a main effect of age ($F_{7,140} = 8.121$, $P < 0.001$) and treatment ($F_{1,140} = 18.266$, $P < 0.001$), as well as an interaction between age and treatment ($F_{7,140} = 3.967$, $P < 0.001$). Adult-like basal ACTH levels were already present at pnd 1 and did not change significantly at any of the other ages. From pnd 1 to pnd 9 novelty did not result in a change in plasma ACTH. However, at pnds 12, 14 and 16 novelty exposure did significantly increase plasma concentrations of ACTH.

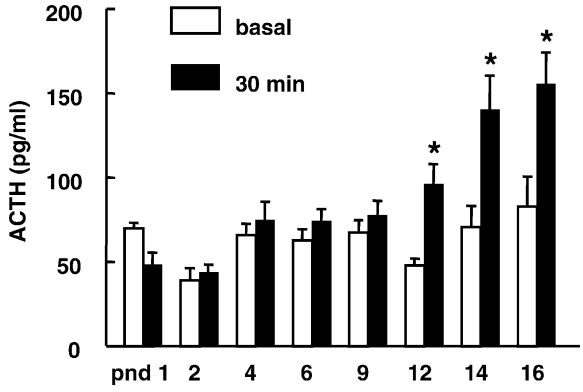

Fig. 2. Basal and stress induced (novelty) plasma ACTH levels in mouse pups at eight different postnatal ages. Basal ACTH levels do not differ between ages. Between pnd 1 and pnd 9, there is no increase of ACTH release following novelty stress. In contrast, pups at pnds 12, 14 and 16 do respond to novelty stress with an elevation of plasma ACTH. Data represent mean ± S.E.M., $^*P < 0.05$ (significant from basal).

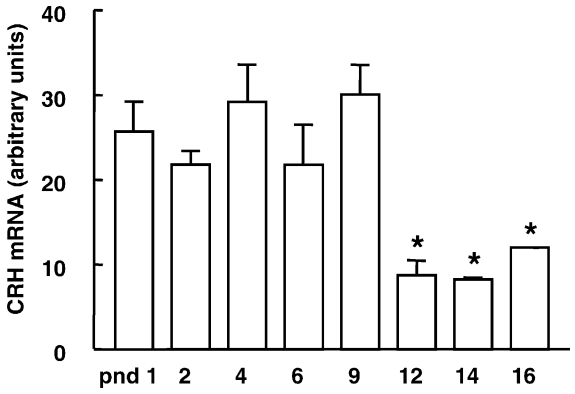

Fig. 3. Basal expression levels of CRH mRNA in the PVN in mouse pups at eight different postnatal ages. Between pnd 1 and pnd 9, CRH expression is higher compared to pnds 12, 14 and 16. Data represent mean ± S.E.M., $^{*}P < 0.05$ (significant from pnd 1 to pnd 9).

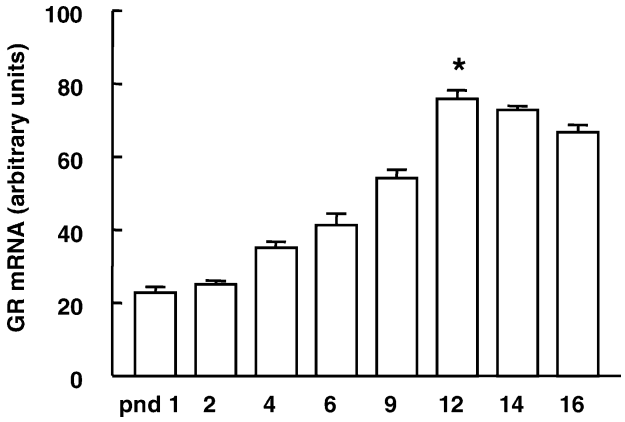

Fig. 4. Basal expression levels of GR mRNA in the CA1 subfield of the hippocampus in mouse pups at eight different postnatal ages. Expression of GR is low after birth, starts to increase at pnd 4 and reaches the highest expression at pnd 12, after which it declines again slightly. Data represent mean ± S.E.M., $^{*}P < 0.05$ (significant from pnd 1 to pnd 9 and pnd 16).

3.2. Ontogeny of central regulators of HPA activity

3.2.1. CRH mRNA in the PVN (Fig. 3)

ANOVA revealed a main effect of age ($F_{7,38} = 8.067$, $P < 0.001$). From pnd 1 to pnd 9 CRH expression levels were relatively high. No change of expression was observed during these days. However, CRH expression at pnd 12 was significantly decreased compared to the earlier ages and remained at this level at pnd 14 and pnd 16.

3.2.2. GR mRNA in the CA1 area of the hippocampus (Fig. 4)

In the CA3 and the dentate gyrus of the hippocampus, GR expression was very low and remained below detection limit until pnd 16. We were therefore only able to measure GR expression in the CA1 subfield of the hippocampus. For the CA1 area, ANOVA revealed a main effect of age ($F_{7,59} = 99.776$, $P < 0.0001$). GR expression in the CA1 subfield was very low at pnd 1 and pnd 2, but started to increase at pnd 4. The highest expression of GR in the CA1 was detected at pnd 12. After pnd 12, expression of the GR remained high, but decreased slightly at pnd 16 ($P < 0.01$ compared to pnd 12).

3.2.3. MR mRNA expression in the hippocampus (Fig. 5)

ANOVA revealed a main effect of age in all hippocampal subfields (CA1: $F_{7,59} = 7.763$, $P < 0.0001$; CA2: $F_{7,61} = 3.236$, $P < 0.01$; CA3: $F_{7,61} = 13.538$, $P < 0.0001$; DG: $F_{7,58} = 15.214$, $P < 0.0001$). In contrast to the GR data, MR expression was detected at high levels in all areas of the hippocampus throughout postnatal development. Interestingly, the ontogeny of MR expression showed a different

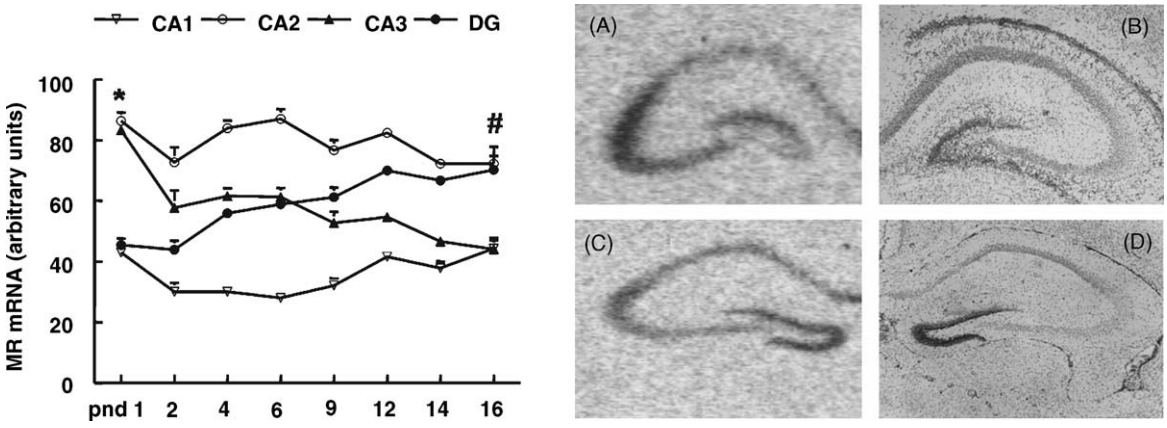

Fig. 5. (Left): Basal expression levels of MR mRNA in the CA1, CA2, CA3 and dentate gyrus of the hippocampus in mouse pups at eight different postnatal ages. At pnd 1, MR expression in the CA2 and CA3 are higher compared to CA1 and dentate gyrus. During postnatal development, the expression of MR in the CA1 and CA2 area remain around the same level, while the expression levels in the CA3 and the dentate gyrus reverse. Data represent mean ± S.E.M., *CA2 and CA3 significantly different from CA1 and dentate gyrus, # CA2 and dentate gyrus significantly different from CA1 and CA3, $P < 0.05$. (Right): Sample pictures of autoradiograms of hippocampal sections at pnd 1 (A) and pnd 16 (C) with their corresponding tissue sections stained with cresyl violet (B and D).

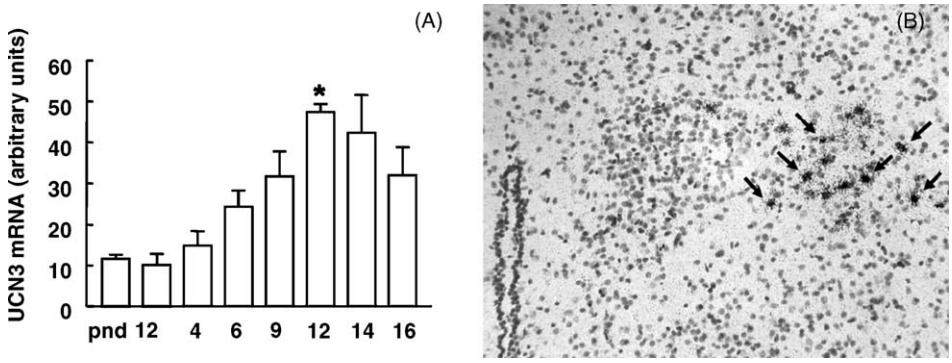

Fig. 6. (A) Basal expression of UCN3 mRNA in the perifornical area of the hypothalamus in mouse pups at eight different postnatal ages. Expression of UCN3 is low after birth, starts to increase at pnd 6 and reaches the highest expression at pnd 12, after which it declines again. Data represent mean ± S.E.M., $^{*}P < 0.05$ (significant from pnd 1 to 9 and pnd 16). (B) Sample picture of a hypothalamic section with UCN3 labeling at pnd 12. Arrows indicate UCN3 positive cells.

pattern in each hippocampal subfield. Right after birth, MR expression in the CA1 area and the dentate gyrus was lower compared to the CA2 and CA3 area. However, while MR expression intensity in the CA1 and CA2 areas oscillated around the same level throughout the postnatal period, the expression intensity of the MR in the CA3 area and the dentate gyrus gradually reversed from pnd 1 until pnd 16. As a result, the CA2 and the dentate gyrus now showed a higher expression of MR compared to the CA1 and the CA3 area at pnd 16.

3.2.4. UCN3 mRNA expression in the perifornical area of the hypothalamus (Fig. 6)

ANOVA revealed a main effect of age ($F_{7,52} = 9.044$, $P < 0.0001$). After birth, the expression of UCN3 was very low. Starting at pnd 6, expression of UCN3 increased, reaching the highest expression at pnd 12. At pnd 16, expression of UCN3 decreased again to levels equal to pnd 9 ($P < 0.05$ compared to pnd 12).

4. Discussion

In this study, we examined the postnatal ontogeny of a number of peripheral and central HPA related factors in the mouse. Our data suggest a division of the postnatal stress system development in the mouse in two main phases. In the first phase, which lasts from right after birth (pnd 1) to about pnd 12, the stress system of the mouse is hypo-responsive (SHRP phase). In the second phase of development, which takes place from pnd 12 onwards, the HPA system of the mouse has matured past the SHRP. Some, but not all, central HPA markers in the brain showed a developmental pattern that reflected these two phases.

4.1. Phase 1—the SHRP

This first developmental stage of the HPA axis after birth in the mouse is characterized by a so-called stress hypo-responsiveness. During this time, mice showed very low basal corticosterone concentrations, while basal ACTH was already present in adult-like concentrations. A mild stressor, such as exposure to novelty, did not elicit an elevation of either ACTH or corticosterone. This phenomenon has first been described in the rat as stress hypo-responsive period (SHRP) (Schapiro et al., 1962; Levine, 1994). Now, we clearly demonstrated this period in the mouse. Interestingly, the time frame of the SHRP is different in both species. In the rat, the SHRP has been described to last from about pnd 4 to pnd 14 (Schapiro et al., 1962; Levine, 1994; Walker et al., 1986; Levine, 2001). Our data demonstrate, that in the mouse the SHRP occurs already at pnd 1, thus earlier than in the rat. On the other hand, we observed a corticosterone response to novelty already at pnd 12, which became even more pronounced at pnd 14 and pnd 16. We can therefore conclude that in the mouse, the SHRP starts right after birth (pnd 1) and lasts until about pnd 12.

While the glucocorticoid related activity of the stress system during the SHRP was very low in the periphery, HPA markers in the brain showed a more diverse picture. Expression of CRH in the PVN was already high at pnd 1 and did not change until pnd 12. Previous data from our laboratory already indicated a higher CRH expression at pnd 9 compared to pnd 18 (Schmidt et al., 2002). Also in the rat, a relatively high expression of CRH during the first postnatal weeks has been observed (Dent et al., 2000a,b). A recent study of Dent et al. (1999) in the rat also indicated that, in contrast to the adult, a mild stressor could rapidly activate CRH expression during the SHRP. The overall picture emerges that during the SHRP corticosterone and ACTH do not respond to a mild stressor, while CRH is produced at high levels and does (at least in the rat) respond to mild stress. Why CRH during development is unable to induce an ACTH response remains unclear.

Also the MR was highly expressed in all subfields during the SHRP. Fascinatingly, all hippocampal subfields exhibited their own age-dependent ontogeny of MR expression. The highest expression of MR during the SHRP was observed

in the CA2 area, while the lowest expression was observed in the CA1 area. In both subfields, the expression of MR remained at about the same level. Interestingly, the expression level of the MR in the CA3 area was also relatively high at pnd 1, but decreased by 34% till pnd 12. In contrast, MR expression in the dentate gyrus was low at pnd 1, but increased to 154% during the SHRP (pnd 1–pnd 12). Thus, the balance of MR expression between the dentate gyrus and the CA3 region changes dramatically during this phase of development. It can be hypothesized, that this striking change differentially influences behavior (Oitzl et al., 1997) and HPA activation in response to (limbic) stressors. There are different possibilities for the underlying reason of these changes. The low expression of MR in the dentate gyrus may at least partly be due to the later postnatal development of this structure. Additionally, differences in cell density at the developmental stages may contribute to the distinct expression patterns. In any case, the functional significance of the regulation of the MR in the different subfields is very intriguing. Further studies will have to clarify this question.

In contrast to CRH and MR, which were already highly expressed following birth, the expression of GR in the hippocampus and UCN3 in the perifornical area of the hypothalamus was low at birth. However, both transcripts increased during development, reaching their highest expression at the end of the SHRP at pnd 12. Earlier reports on the ontogeny of the GR in the hippocampus also observed a gradual increase with age (Meaney et al., 1985; Sarrieau et al., 1988; Rosenfeld et al., 1988; Bohn et al., 1994), but none of these studies examined the regional ontogeny of GR expression in such detail. Our study also demonstrates for the first time the postnatal ontogeny of the expression of UCN3. In the adult, it has been shown that UCN3 selectively activates the CRH receptor 2 (CRHr2) (Lewis et al., 2001; Hsu and Hsueh, 2001). This indicates, that UCN3 in the perifornical area might be a modulator the activity of the HPA axis via CRHr2 receptors in the PVN. Indeed our data suggest, that UCN3 is involved in the control of the HPA axis activation at the end of the SHRP. Given the surprisingly similar postnatal expression pattern of the GR and UCN3 it is furthermore likely, that both peptides are regulated via the same mechanism. Alternatively, the expression of UCN3 could be directly regulated by corticosterone via the GR. Further studies will have to address this question in detail.

4.2. Phase 2—outside the SHRP

Starting at pnd 12, we observed a pronounced change of HPA axis activity. The stress system of the mouse pup now responded in a more or less adult-like fashion. Basal ACTH and corticosterone concentrations resembled those of adult mice and a mild stressor elicited a marked elevation of both hormones. Additionally, the sensitivity of the adrenal to ACTH seemed to be enhanced, because the same ACTH concentrations as observed during the SHRP were paralleled by higher corticosterone concentrations. However, even though the hormonal response to a stressor appears mature at this stage, the development of central aspects of the HPA axis continues. We found that some of the brain markers paralleled the hormonal changes, while others remained unaffected.

A marked down regulation of CRH expression in the PVN was observed at pnd 12. At first glance, this seems to be a paradox, as at the same time ACTH and corticosterone

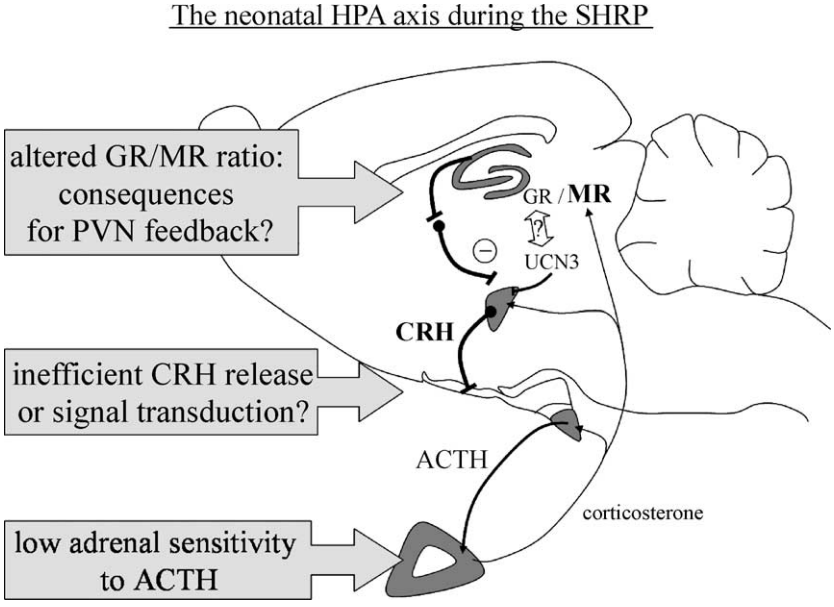

Fig. 7. Schematic illustration of the hypothalamic–pituitary–adrenal (HPA) axis during the stress hypo-responsive period. The state and function of the HPA system in the neonatal mouse is significantly altered at different levels of organization compared to the adult. As a consequence, basal and stress induced corticosterone release is greatly diminished.

become more reactive. In fact, an opposite regulation of CRH and ACTH has already been observed previously (Dent et al., 1999; Schmidt et al., 2002). Also following maternal deprivation, which activates the HPA system during the SHRP, the expression of CRH is down regulated, while ACTH and corticosterone levels are enhanced (Smith et al., 1997; Schmidt et al., 2002). There are at least two possible explanations for this phenomenon.

First, the down regulation of CRH may be a result of elevated circulating corticosterone concentrations. An enhanced activation of MR by elevated corticosterone could therefore down regulate CRH expression in the PVN, since blockade of hippocampal MR in the adult rat increases HPA activity (Ratka et al., 1989; van Haarst et al., 1997). Additionally we showed, that the balance of MR expression between the CA3 region and the dentate gyrus changes dramatically during development. Consequently, MR mediated control of CRH expression by corticosterone could be stronger at this age. Also a role of the GR in mediating CRH expression at this age can not be excluded, as GR expression reaches its peak at pnd 12.

An alternative or additional hypothesis would be a regulation of CRH via the CRHr1. A more active or more responsive CRHr1 around pnd 12 would activate ACTH release following a mild stressor. Recently, the expression of the CRHr1 has also been shown in the adrenal (Muller et al., 2001), where it might function to enhance adrenal sensitivity. On the other hand, a more responsive CRHr1 in the brain could directly down regulate CRH expression in the PVN. This hypothesis is supported by studies using CRHr1 deficient mice, which show a greatly enhanced CRH expression (Timpl et al., 1998; Smith et al., 1998). Further studies of the function of the CRHr1 during the postnatal development of the HPA axis will be able to clarify the role of this receptor.

In summary, our data show that the postnatal development of the HPA axis in the mouse can be subdivided into two developmental phases: the SHRP (pnd 1–pnd 12) and outside the SHRP (from pnd 12 onwards). During the SHRP, the HPA axis of the neonate is altered in its function on a variety of levels compared to the adult, resulting in a general inhibition of the peripheral stress response (Fig. 7). The question arises, which of the characteristic changes in the brain around pnd 12 cause the activation of the HPA axis, and which are consequences. Our challenge for the coming year will be to identify the primary cause of the developmental HPA activation around pnd 12.

Acknowledgements

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