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Early Life Stress Changes Concentrations of Neuropeptide Y and Corticotropin-releasing Hormone in Adult Rat Brain. Lithium Treatment Modifies These Changes

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Experiences of early life stress are more prevalent among depressed patients than healthy controls. Neuropeptide Y (NPY) was suggested to play a role in the pathophysiology of depression. Consequently, we investigated in adult rats the effects of maternal deprivation for 3 h/day during postnatal days (PND) 2–14 and of dietary lithium during PND 50–83 on brain levels of NPY-like immunoreactivity (LI). Brain levels of corticotropin-releasing hormone (CRH) and serum corticosterone were also measured. Maternal deprivation reduced NPY-LI levels in the hippocampus and the striatum but increased NPY-LI and CRH-LI levels in the hypothalamus. Lithium treatment counteracted the effect of maternal deprivation in the hippocampus and striatum by increasing NPY-LI levels. In the hypothalamus, lithium tended to decrease CRH-LI but further increased levels of NPY-LI; it also increased serum corticosterone levels. The results suggest that early life stress has long-term effects on brain NPY with implications for the development of depression/vulnerability to stress, and that one therapeutic mechanism of action of lithium is to increase brain NPY.

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KEY WORDS: *Maternal deprivation; NPY; CRH; Corticosterone; Stress; Lithium; Rat*

The etiology and pathophysiology of major depression remain largely unknown. Epidemiological and genetic data indicate that both environmental and genetic factors are operative in the precipitation and development of the disorder. Clinical studies have found a higher prevalence of childhood abuse/neglect or parental loss (i.e. separation from parents or parental death) among depressed patients than healthy control subjects (Agid et al. 1999; Kendler et al. 1992; McCauley et al. 1997; Tennant et al. 1982). Early life stress may cause changes in the central nervous system e.g. hypothalamic-pituitary adrenal (HPA) dysregulation that is associated with an increased risk of adult life depressive psychopathology (Arborelius et al. 1999; Holsboer 2000). Animal models have been developed to investigate these phenomena, and in non-human primates and rats, early life maternal deprivation leads to an increased activity and sensitivity of the HPA axis (Arborelius et al. 1999; Kalin and Carnes 1984; Ladd et al. 1996; Liu et al. 1997; Meaney et al. 1989; Plotsky and Meaney 1993).

Neuropeptide Y (NPY), a 36-amino acid peptide member of the pancreatic polypeptide family (Tatemoto

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et al. 1982), affects a variety of functions in the central nervous system (CNS); for example, it modifies food intake, ethanol consumption, circadian rhythms, memory processing, and anxiety behavior and has also been proposed to play a role in depression (Widerlöv et al. 1986). Thus, most studies found reduced concentrations of NPY in cerebrospinal fluid (CSF) or plasma from depressed patients (Mathé et al. 1996; Westrin et al. 1999; Widerlöv et al. 1986), although contradictory findings have also been reported (Gjerris et al. 1992). In rodents, Flinders Sensitive Line rats, Fawn Hooded rats, and bulbectomized rats, all considered to be models of depression, have reduced levels of NPY in the hippocampus and an exaggerated immobility in the Porsolt swim test (Holmes et al. 1998; Overstreet et al. 2001). Interestingly, central administration of NPY reduces immobility in a manner comparable to that of imipramine (Husum et al. 2000; Stogner and Holmes 2000). Moreover, antidepressants, electroconvulsive stimulations (ECS), and lithium were found to increase hippocampal NPYergic neurotransmission (Husum et al. 2000; Jiménez Vasquez et al. 2000; Mathé et al. 1997; Stenfors et al. 1989; Widdowson and Halaris 1989; Zachrisson et al. 1995a,b). Taken together, these studies suggest an antidepressant-like profile of NPY and support our hypothesis that NPY plays a role in the pathophysiology of depression and that the therapeutic effects of antidepressant and/or mood stabilizing treatment modalities are mediated, at least in part, via the NPY system (Mathé 1999).

In mammals, the endocrine stress response is associated with an activation of the HPA-axis, primarily controlled by corticotropin-releasing hormone (CRH, CRF), a 41-amino acid hormone (Vale et al. 1981). Synapses between NPY containing neurons and dendrites and cell bodies of CRHergic neurons have been demonstrated in the hypothalamic paraventricular nucleus (PVN) (Liposits et al. 1988) and central administration of NPY stimulates the synthesis and release of CRH in the PVN and increases plasma levels of ACTH and corticosterone (Albers et al. 1990; Haas and George 1987; Leibowitz et al. 1988; Suda et al. 1993; Tsagarakis et al. 1989; Wahlestedt et al. 1987). These experiments indicate that in addition to CRH, hypothalamic NPY is also involved in the endocrine response to stress. In view of the above, the purpose of the present study was to investigate in adult rats the consequence of early life maternal deprivation on concentrations of NPY and CRH in subcortical brain regions and on plasma corticosterone and whether lithium treatment will modify such effects.

MATERIALS AND METHODS

Animals

Timed-pregnant Sprague-Dawley rats (B&K Universal, Sweden) arrived at the animal facility on gestational day 12. The day of delivery was designated postnatal day (PND) 0. On PND 2, the pups were sexed and culled into litters of 8–10 male pups and randomly assigned to maternal deprivation for 15 (MS15) or 180 min/day (MS180). The separation procedures took place from PND 2–14. During the separation, the pups were placed in a neonatal incubator to prevent metabolic changes caused by change of body temperature. Non-handled pups (NH) were left undisturbed until PND 23 when all pups were weaned. From this day onwards, the rats were maintained five per cage at a constant room temperature of 22 ± 1°C in a 12-h light/dark cycle (light on at 6:00 A.M.) with free access to chow (Lactamin R36) and tap water. The experiments met the guidelines approved by Stockholm's Ethical Committee for Protection of Animals and were conducted in accordance with the Karolinska Institutet's Guidelines for the Care and Use of Laboratory Animals.

Treatments

On PND 50, MS15 and MS180 animals were randomly assigned to 33-day dietary treatments where either Li_2SO_4 (17.5 mmol/kg chow for the first week, 25 mmol/kg chow during the rest of the experiment) or vehicle had been admixed to the rat chow. The NH-group received the vehicle diet. The lithium treated rats had in addition free access to 0.9% NaCl solution. Numbers of animals in the study were: NH: vehicle (n = 7); MS15: vehicle (n = 9), lithium (n = 11); MS180: vehicle (n = 9), lithium (n = 10).

Sample Processing

On PND 83, rats were decapitated and trunk blood collected for serum analysis. The brains were quickly removed and hippocampus, hypothalamus and striatum were dissected on ice and immediately stored at −80°C. Later, the brain tissues were homogenized, ultrasonicated and twice extracted by boiling in 1 M acetic acid and water, respectively. Following centrifugation, the supernatants were lyophilized and reconstituted in phosphate-buffer prior to radioimmunoassay analysis. The concentrations of NPY-like immunoreactivity (LI) were analyzed using Bolton-Hunter labeled ^{125}I-NPY (4000 Ci/mmol, Amersham) and NPY antiserum (a generous gift from Dr. R. Ekman and Dr. M. Heilig). This antibody shows no cross-reactivity with pancreatic polypeptide or peptide YY. It cross-reacts 100% with $NPY_{2\text{-}36}$, 5% with $NPY_{5\text{-}36}$ and 0.5% or less with shorter C-terminal NPY fragments (Heilig and Ekman 1995). CRH-LI was analyzed using ^{125}I-CRH (2000 Ci/mmol, Amersham) and CRH antiserum (Linton and Lowry 1986), a generous gift from Dr. P. Lowry. Samples were analyzed for peptide-LI in triplicates and all samples from one brain region were assayed in the same RIA

run. The lower detection limit of both RIAs was 3.9 pmol/L and the intra-assay coefficients of variation were 5% and 7%, respectively.

Serum concentrations of corticosterone were analyzed in duplicate using a commercially available kit (Diagnostic Products Corporation, Los Angeles, USA). Serum concentrations of lithium were determined by atomic flame photometry.

Data Presentation and Statistical Analysis

NPY/CRH ratios were calculated for each animal as concentration of NPY-LI divided by concentration of CRH-LI. Differences in baseline levels of measured variables among vehicle treated NH, MS15 and MS180 groups were evaluated by 1-way analysis of variance (ANOVA). Overall group effects (MS15 vs. MS180) and treatment effects (vehicle vs. lithium) on measured parameters were analyzed by 2-factor ANOVA. In case of a significant interaction, this has been stated in the results section. When appropriate, Tukey's test was used for post hoc analysis. Values of p less than .05 were considered significant. All data are presented as mean ± SD.

RESULTS

Serum Lithium

The lithium treated rats showed no overt symptoms of toxicity; normal grooming and sleeping behavior was observed. There was no significant difference in serum concentrations of lithium between the MS15 and MS180 groups ($F_{1,17)} = 1.92$, $p = .18$), which were 0.48 ± 0.10 and 0.40 ± 0.07 mM, respectively.

Serum Corticosterone

No difference in baseline serum corticosterone levels among vehicle treated NH, MS15, and MS180 groups was observed ($F_{2,18}$) = 1.47, $p = .26$), Table 1. Two-way ANOVA revealed no difference in corticosterone concentrations among MS15 rats and MS180 rats ($F_{1,33} = 0.02$, $p = .90$). However, lithium treatment significantly increased serum corticosterone ($F_{1,33} = 8.43$, $p < .01$).

NPY-like Immunoreactivity

In the hippocampus, significant differences in baseline NPY-LI levels between vehicle treated NH, MS15 and MS180 animals were detected ($F_{2,21} = 31.8$, $p < .001$), Figure 1. Post hoc analysis showed that NPY-LI levels were lower in both MS15 and MS180 compared with NH animals ($p < .001$). Two-way ANOVA showed that NPY-LI levels were lower in the MS180 group than the MS15 group ($F_{1,32} = 31.5$, $p < .001$) and that lithium treatment markedly increased NPY-LI ($F_{1,32} = 81.9$, $p < .001$). There was a significant Group X Treatment interaction ($F_{1,32} = 6.0$, $p < .05$) and post hoc analysis showed that NPY-LI levels were reduced in vehicle treated MS180 animals compared with vehicle treated MS15 animals ($p < .05$) and that the effect of lithium on NPY-LI was larger in the MS15 group ($p < .0001$) than the MS180 group ($p < .001$).

In the striatum, in parallel to the hippocampus, significant differences in baseline NPY-LI levels between vehicle treated NH, MS15, and MS180 animals were detected ($F_{2,21} = 17.9$, $p < .001$). Post hoc analysis showed that NPY-LI levels were lower in both MS15 and MS180 compared with NH animals ($p < .001$). Two-way ANOVA showed that NPY-LI was lower in the MS180 group compared with the MS15 group ($F_{1,33} = 13.6$, $p < .001$) and that lithium treatment significantly increased NPY-LI ($F_{1,33} = 25.1$, $p < .001$).

In the hypothalamus, baseline levels of NPY-LI were different among vehicle treated NH, MS15, and M180 animals ($F_{2,22} = 6.35$, $p < .01$). Post hoc analysis showed that NPY-LI levels were significantly higher in MS180 ($p < .01$) but not MS15 ($p = .19$), compared with NH animals. Levels of NPY-LI were higher in the MS180 compared with the MS15 group ($F_{1,34} = 4.98$, $p < .05$) and that lithium treatment significantly increased NPY-LI levels in this region ($F_{1,34} = 7.21$, $p < .01$).

Table 1. Effects of Maternal Deprivation and Dietary Lithium Treatment on Serum Corticosterone.

NH	MS15		MS180	
Vehicle	Vehicle	Lithium	Vehicle	Lithium
378 ± 78	273 ± 136	397 ± 121[a]	282 ± 112	398 ± 129[a]

Effects of maternal deprivation (PND2-14) and dietary lithium treatment (PND50-83) on serum concentrations of corticosterone. Data are presented as mean ng/ml ± SD. There was no significant difference in corticosterone levels among vehicle treated NH, MS15 and MS180 animals. Lithium significantly increased corticosterone concentrations (a P < 0.01). For details on overall group and treatment effects, see the results section.

CRH-like Immunoreactivity

In the hippocampus, no significant difference in baseline CRH-LI among vehicle-treated NH, MS15, and MS180 animals was found ($F_{2,21} = 1.177$, $p = .33$) (Table 2). Two-way ANOVA showed a slight reduction of CRH-LI in the MS180 group compared with the MS15 group ($F_{1,33} = 5.89$, $p < .05$) and lithium treatment led to a modest increase in CRH-LI ($F_{1,33} = 4.69$, $p < .05$. Interestingly, there was a highly significant interaction between group and treatment ($F_{1,33} = 20.9$, $p < .001$). Post hoc analysis showed that lithium treatment increased CRH-LI levels in the MS15 group only ($p < .001$).

Table 2. Effects of Maternal Deprivation and Dietary Lithium Treatment on Brain Regional Concentrations of CRH-LI.

	NH	MS15		MS180	
	Vehicle	Vehicle	Lithium	Vehicle	Lithium
Hippocampus	0.69 ± 0.0	0.56 ± 0.1	0.89 ± 0.1^{+++}	0.66 ± 0.2	0.55 ± 0.1
Hypothalamus	5.38 ± 4.8	5.44 ± 2.4	4.85 ± 3.4	11.7 ± 10^{a}	7.78 ± 4.8^{a}

Effects of maternal deprivation (PND2-14) and dietary lithium treatment (PND 50-83) on CRH-LI concentrations in the hippocampus and the hypothalamus. Data are presented as mean ± SD pmol/g wet weight. In the hippocampus, lithium treatment significantly increased CRH-LI in the MS15 (+ + + $P < 0.001$) but not the MS180. In the hypothalamus, CRH-LI levels were increased in the MS180 compared to the MS15 group (a $P < 0.05$). For details on overall group and treatment effects, see the results section.

In the hypothalamus, no significant difference in baseline CRH-LI levels between vehicle treated NH, MS15, and MS180 animals was found ($F_{2,22} = 2.20$, $p = .14$). However, 2-way ANOVA showed that CRH-LI levels were increased in the MS180 group compared with the MS15 group ($F_{1,33} = 4.79$, $p < .05$). Lithium treatment did not significantly affect CRH-LI levels in this region ($F_{1,33} = 1.14$, $p = .29$).

Striatal concentrations of CRH-LI were at the limit of detection and could not be reliably quantified.

NPY-LI/CRH-LI Ratios in the Hippocampus

One-way ANOVA showed a significant difference in the NPY/CRH ratio among vehicle treated NH, MS15, and MS180 animals ($F_{2,19} = 8.34$, $p < .01$), Figure 2. Post hoc analysis showed that the NPY/CRH ratio was lower in MS180 ($p < .01$) and MS15 though not quite reaching the level of statistical significance ($p = .08$) compared with NH animals. Two-way ANOVA revealed no overall difference in the NPY/CRH ratio between MS15 and MS180 rats ($F_{1,30} = 1.01$, $p = .33$). Lithium significantly increased the NPY/CRH ratio ($F_{1,30} = 13.1$, $p = .001$). There was a significant Treatment X Group interaction ($F_{1,30} = 7.29$, $p = .01$). Interestingly, post hoc analysis showed that the NPY-LI/CRH-LI ratio was significantly reduced in the vehicle treated MS180 group compared with the vehicle treated MS15 group ($p < .05$) and that lithium increased the NPY/CRH ratio in the MS180 ($p < .001$) but not the MS15 group ($p = .5$).

DISCUSSION

This study shows that repeated episodes of maternal deprivation, a model of depression/vulnerability to stress, markedly reduce concentrations of NPY-LI in the hippocampus and the striatum of adult rats. Lithium treatment "normalized" NPY-LI levels in these two regions. In addition, the computed ratio of hippocampal NPY/CRH was significantly reduced in the MS180 animals. This measure was also "normalized" by lithium treatment. In the hypothalamus, NPY-LI and CRH-LI levels were increased in MS180 animals. Lithium further increased the NPY-LI level in this region. These changes coincided with findings of increased serum corticosterone following lithium.

We have previously found that Fawn-Hooded and FSL rats, both characterized as genetic models of depression that exhibit higher degrees of immobility in the forced swim test (Overstreet et al. 2001), have reduced levels of NPY in the hippocampus (Husum et al. 2001a; Jiménez Vasquez et al. 2000; Mathé et al. 1998). In this context it is interesting that centrally administered NPY reduces immobility in the rat, indicative of an antidepressant-like property of NPY (Husum et al. 2000; Stogner and Holmes 2000). In conjunction with clinical findings of reduced plasma or CSF levels of NPY in depressed patients, these results indicate that a dysregulated NPY system may play a role in the pathophysiology of depression (Mathé et al. 1996; Westrin et al. 1999; Widerlöv et al. 1986).

Recently, NPY was shown to promote proliferation of neuronal precursor cells in the rat olfactory system (Hansel et al. 2001). Whether NPY has similar action in the hippocampus, one of the few other regions with regenerating neuronal populations in the adult mammalian nervous system (Gage 2000), is presently not known. However, in view of the clinical evidence of reduced hippocampal volume in patients with repeated episodes of major depression and stress-induced degeneration of this region in rats, reversible by lithium, antidepressants, and ECS, all of which stimulate hippocampal NPY neurotransmission, this is a plausible assumption (Gould et al. 1992; Husum et al. 2000; Jacobs et al. 2000; Jiménez Vasquez et al. 2000; Mathé et al. 1997; Sheline et al. 1996; Stenfors et al. 1989; Widdowson and Halaris 1989; Zachrisson et al. 1995a,b). Indeed, an antistress action of hippocampal NPY has been postulated on the basis of experiments showing that rats overexpressing NPY in the hippocampus display a behavioral insensitivity to stress and fear (Thorsell et al. 2000). Lowering hippocampal NPY levels may therefore render maternally deprived rats more sensitive to stress and anxiety. In this context, it is of in-

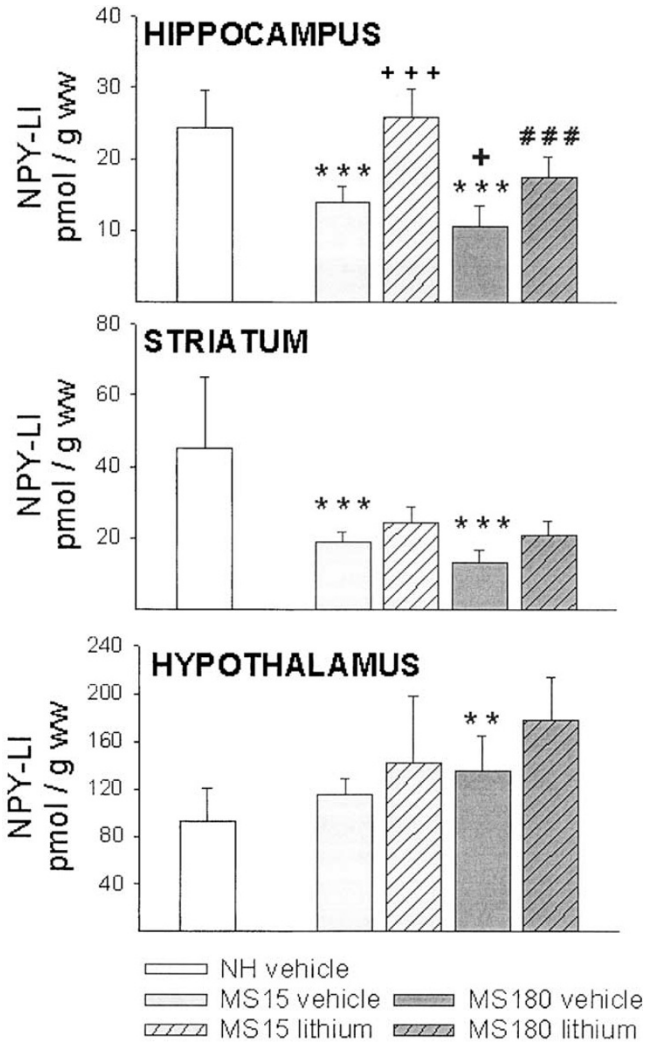

Figure 1. Effects of maternal deprivation (PND 2–14) and dietary lithium treatment (PND 50–83) on NPY-LI concentrations in rat hippocampus, striatum and hypothalamus. In the striatum and the hippocampus, NPY-LI levels were markedly decreased in both the MS15 and MS180 compared with the NH animals (*** $p < .001$). In the hippocampus, NPY-LI was reduced in vehicle-treated MS180 animals compared with vehicle treated MS15 animals ($^{+}$ $p < .05$). Lithium increased NPY-LI more in the MS15 ($^{+++}$ $p < .0001$) than in the MS180 group ($^{\#\#\#}$ $p < .001$) in this region. In the hypothalamus, NPY-LI levels were increased in the vehicle treated MS180 compared with NH animals (** $p < .01$). For details on overall group and treatment effects, see the Results section.

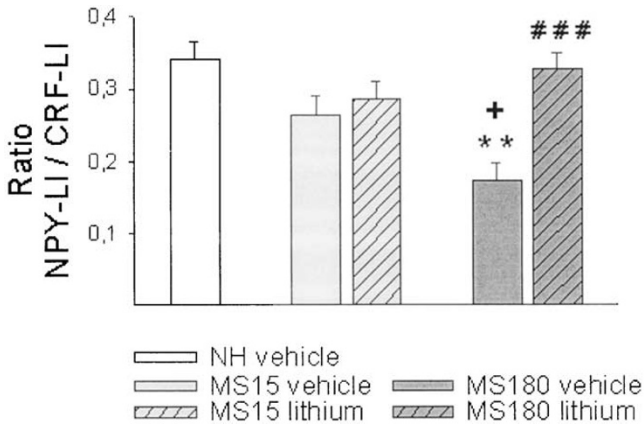

Figure 2. Effects of maternal deprivation (PND 2–14) and dietary lithium treatment (PND 50–83) on the NPY-LI/CRH-LI ratio in the hippocampus. The ratio was lower in the MS180 animals compared with both the NH (** $p < .01$) and the MS15 group ($^{+}$ $p < .05$). Lithium normalised this ratio in the MS180 group ($^{\#\#\#}$ $p < .001$) but had no effect in the MS15 group. For details on overall group and treatment effects, see the Results section.

terest that reduced hippocampal NPY levels were associated with increased ethanol consumption in rats and mice and the authors suggested that the enhanced ethanol consumption might constitute a compensatory anti-anxiety effect (Ehlers et al. 1998; Thiele et al. 1998). Indeed, maternally deprived rats display a larger stress-induced increase in corticosterone and ACTH release and exaggerated anxiety behavior and alcohol preference compared with controls (Ladd et al. 1996; Plotsky et al. 1995; Plotsky and Meaney 1993).

In previous experiments, in FSL and Fawn Hooded rats, genetic models of depression that have a reduced dopamine turnover in the striatum, no changes in NPY-LI were observed in the striatum (Husum et al. 2001; Jiménez Vasquez et al. 2000; Mathé et al. 1998; Zangen et al. 1999). However, in maternally deprived rats, both increased levels of dopamine in the striatum and increased levels of tyrosine hydroxylase mRNA levels in the substantia nigra have been reported, indicating that dopaminergic neurotransmission may be enhanced in this region (Matthews et al. 2001; Rots et al. 1996). In the present study, maternal deprivation reduced striatal concentrations of NPY. Since marked interactions between dopamine agonists/antagonists and NPY have been observed in striatum, it is conceivable that the decrease in striatal NPY in maternally deprived animals may lead to or, alternatively, is a consequence of an enhanced dopaminergic activity in this region (Gruber and Mathé 2000; Lindefors et al. 1990; Maeda et al. 1993; Tatsuoka et al. 1987).

We also investigated the effect of lithium on brain NPY-LI levels. The lithium treatment used yielded serum lithium levels that were at the lower therapeutic range of lithium concentrations (Lenox and Manji 1998). Lithium increased levels of NPY-LI in the hippocampus and striatum of both MS15 and MS180 rats to levels that were similar to those in the NH group. Lithium, an effective agent in the prophylaxis of affective disorders (Baastrup et al. 1970), was previously

found to increase levels of preproNPY mRNA and NPY-LI in rat hippocampus, striatum and cortical areas indicating that stimulation of NPY gene transcription may indeed be one therapeutical mechanism of action of lithium (Husum et al. 2000, 2001; Mathé et al. 1994; Zachrisson et al. 1995b).

CRH and NPY systems interact in the brain and when CRH or NPY are administered centrally to the rat, opposing actions on feeding, anxiety and sleep have been reported (Ehlers et al. 1997; Heilig et al. 1994; Heinrichs et al. 1993). Thus, an imbalance in these neuropeptide systems may be consistent with a dysregulation of affect and sleep, which are classical symptoms of major depression. This notion is supported by findings of elevated CRH and reduced levels of NPY in CSF from depressed patients (Mathé et al. 1996; Westrin et al. 1999; Widerlöv et al. 1986). In line with such findings, in the present study the ratio of NPY/CRH in the hippocampus was markedly reduced in the MS180 animals. Moreover, following lithium, this measure was normalized in the MS180 group, while unchanged in the MS15 group indicating that lithium restores the normal ratio of these two peptides in this region.

CRH and NPY are present in high concentrations in the hypothalamus where they interact and mediate neuroendocrine and autonomic functions. Adult maternally deprived rats display an increased neuroendocrine stress response, as shown previously (Liu et al. 1997; Meaney et al. 1989; Plotsky and Meaney 1993). Consequently, we measured hypothalamic CRH-LI and NPY-LI. CRH-LI was significantly increased in the MS180 animals, in agreement with previous findings of increased CRH mRNA in the PVN, increased CRH-LI in the median eminence and plasma from the hypothalamic-hypophysial portal vessel, and reduced CRH binding sites in the pituitary gland of maternally deprived rats, all indicative of hypothalamic CRH hypersecretion (Arborelius et al. 1999; Ladd et al. 1996; Plotsky and Meaney 1993). The CRH increase was paralleled by an NPY-LI increase in the hypothalamus of MS180 animals. Electron microscopy has revealed synapses between NPY-containing neurons and dendrites and cell bodies of CRH neurons in the PVN and several studies have shown that NPY stimulates the synthesis and release of CRH in the hypothalamus (Haas and George 1987; Liposits et al. 1988; Suda et al. 1993; Tsagarakis et al. 1989; Wahlestedt et al. 1987). Thus, the elevated levels of CRH-LI in the MS180 animals may be secondary to an increased hypothalamic level of NPY-LI.

No difference in baseline serum corticosterone levels was observed between NH, MS15, and MS180 rats, in agreement with previous findings (Plotsky and Meaney 1993). Interestingly, serum corticosterone was significantly elevated by lithium. Although this finding may seem to be counterintuitive, it is in agreement with previous studies in rodents (Ghosh et al. 1990; Meador-Woodruff and Greden 1988). Following lithium treatment, CRH-LI tended to decrease in the hypothalamus of the MS180 group while the effect of maternal deprivation on NPY-LI was potentiated, that is NPY-LI was further increased. These findings parallel those of another study where NPY mRNA levels in the arcuate nucleus of the hypothalamus were increased by stress and potentiated by desipramine treatment, which had no effect in control rats (Makino et al. 2000). The mechanism underlying these changes is a matter of speculation. However, since CRH exerts an inhibitory control on NPY neurons in the PVN and there is a negative feedback glucocorticoid-CRH loop (Plotsky and Sawchenko 1987), a possible explanation is that the lithium-induced corticosterone increase will inhibit CRH, in turn leading to decreased inhibition of the NPY system. In this context it is noteworthy that dexamethasone, a selective glucocorticoid type II receptor agonist, increases preproNPY mRNA and NPY-LI in specific hypothalamic regions, including the arcuate nucleus and the PVN (Corder et al. 1988; Larsen et al. 1994; McKibbin et al. 1992). Consequently, the stimulatory effect of lithium on hypothalamic NPY levels may also at least in part be mediated by corticosterone.

In conclusion, the present study demonstrates that early life stress leads to long-term changes in NPY-LI in the hippocampus, striatum, and hypothalamus, areas of major importance for affect, psychosis, and neuroendocrinology. Lithium treatment counteracted these effects in the hippocampus and striatum by increasing NPY-LI to levels similar to those found in the control animals. The present results are consistent with our previous findings of altered NPY-LI in brains from rat genetic models of depression and that lithium and antidepressant treatments, including ECS, selectively increase brain NPY-LI in a distinct time course and regional fashion. Considering the potential significance of the present findings we have performed baseline studies with two collaborator groups in two different countries. Although using different sources and strains of rats as well as different maternal separation procedures, similar effects on brain NPY-LI were found (Husum et al. 2001b; Jiménez Vasquez et al. 2001). Cumulatively, these findings suggest that early life stress has long-term effects on brain NPY and that this effect may be involved in the development of depression and vulnerability to stress, and that one of lithium's therapeutic mechanisms of action is to increase brain NPY.

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