Changed Concentrations of Tachykinins and Neuropeptide Y in Brain of a Rat Model of Depression: Lithium Treatment Normalizes Tachykinins

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Lithium's therapeutic mechanism of action is unknown. In lithium-treated normal rats, increased striatal concentrations of neurokinin A (NKA)-like immunoreactivity (LI), substance P (SP-LI) and neuropeptide Y (NPY-LI) have been reported. To investigate whether these effects might be of therapeutic relevance, Flinders Sensitive Line rats (FSL), an animal model of depression, and control Flinders Resistant Line (FRL) rats were during a 6-week period fed chow to which either lithium or vehicle was admixed. Following sacrifice, the peptides were extracted from dissected brain regions and measured by radioimmunoassay. NKA-LI and SP-LI were markedly decreased in striatum and increased in frontal cortex in FSL compared to control FRL animals. Lithium treatment abolished these differences. Basal concentrations of NPY-LI were decreased in hippocampus of FSL rats, but unaffected by lithium. The present study suggests that changed tachykinins and NPY may underlie the characterized depressive-like phenotype of the FSL rats. It is hypothesized that altering tachykinin peptidergic neurotransmission in striatum and frontal cortex constitutes a mechanism of action of lithium and that such a mechanism might be of therapeutic relevance.
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Lithium, reintroduced in psychiatry 50 years ago, is highly effective in the treatment of mania as well as long-term prophylaxis of bipolar disorder (Baastrup et al. 1970). Although the acute actions of lithium to reduce signaling through the phosphoinositol-protein kinase C and cyclic AMP protein kinase A second messenger systems are well documented (Mørk 1990; Lenox and Watson 1994; Manji and Lenox 1999; Manji et al. 1995, 1999), the mechanisms underlying the mood-stabilizing actions of lithium have not been fully elucidated. Neurokinin A (NKA) and substance P (SP) belong to the tachykinin family of peptides which are widely distributed in the central nervous system. NKA and SP are generated by post-translational processes from tachykinin precursor proteins. These proteins are encoded by three types of tissue-specific mRNAs denoted α, β,

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and γ preprotachykinin (ppt) mRNA and are different splicing alternatives of the ppt-A gene (Nawa et al. 1983; Kotani et al. 1986; Krause et al. 1987). Changes in tachykinin neurotransmission have been proposed as a possible mechanism of action of lithium. Thus, subchronic lithium treatment was found to increase ppt mRNA and NKA-like immunoreactivity (LI) and SP-LI in striatum and frontal cortex of Fischer rats (Hong et al. 1983; Sivam et al. 1989). Previous work from our laboratory confirmed the findings that concentrations of NKA-LI and SP-LI increase in striatum following long-term lithium treatment, and extended them by showing, that concentrations of neuropeptide Y (NPY), and somatostatin, both immunoreactivity and -mRNA, and neurotensin-LI were also increased in striatum and other brain regions of Sprague-Dawley rats (Zachrisson et al. 1995; Mathé et al. 1990a, 1994; Jousisto-Hanson et al. 1994). It thus appears that several neuropeptide systems in various brain regions are affected by long-term lithium treatment. The observed changes may be related to the therapeutic actions of lithium or could be effects of lithium *per se*. The present study addresses this question by investigating the effects of chronic dietary lithium in an animal model of depression. The Flinders Sensitive Line (FSL) was established by selective breeding of Sprague-Dawley rats for high sensitivity to diisopropylfluorophosphate (DFP), an anticholinesterase agent (Overstreet et al. 1979). The FSL rats exhibit several features which resemble the symptom pattern of depression. These include reduced bodyweight, reduced locomotor activity, increased rapid eye movement (REM) sleep and cognitive deficits (Overstreet 1993; Yadid et al. 2000). Furthermore, exposure of FSL rats to chronic mild stress results in a state of "anhedonia," measured as decrease in saccharin preference (Pucilowski et al. 1992). Lastly, chronic but not acute treatments with imipramine, desipramine or sertraline were reported to counteract the exaggerated immobility of FSL rats as tested in the forced swim test (Porsolt et al. 1978; Schiller et al. 1992; Overstreet 1993; Yadid et al. 2000). The FSL rat is therefore considered to be a model of depression with a good face and predictive validity. Consequently, in order to explore lithium actions that might be of possible therapeutical relevance, FSL and Flinders Resistant Line (FRL) control rats, were chronically given dietary lithium or vehicle and effects on concentrations of NKA-LI, SP-LI and NPY-LI measured in brain regions.

MATERIALS AND METHODS

Animals

Male FSL and FRL rats were selected from the breeding colonies maintained at the University of North Carolina Medical School. At 50 days of age, the rats were shipped to the Karolinska Institutet where the studies were initiated after a 2-week adaptation period. The animals were housed 5 per cage at a constant room temperature of $22 \pm 1°C$ in a 12-h light/dark cycle (lights on at 06:00) with free access to chow and tap water. The experiments were approved by the 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.

Test Procedure

FSL and FRL rats were divided into two subgroups. During a 6-week period, treatment groups were fed a standard rat chow (R36, Lactamin, Stockholm, Sweden) which had been admixed with 2.19 g Li_2SO_4/kg chow. The vehicle groups were fed a standard rat chow with admixed vehicle. In addition, 0.45% NaCl was available to the rats receiving lithium-supplemented chow in order to prevent lithium toxicity (Ellis and Lenox 1990; Lenox et al. 1992). The experimental design took into account previous experiments demonstrating that under such conditions lithium serum concentrations will be approximately 0.5–0.9 mM after 4 weeks. In one study, serum lithium concentrations in Sprague-Dawley rats were measured to be 0.53 ± 0.15 mM (Mathé et al. 1994) which is within the therapeutic range of concentration of lithium (Lenox and Manji 1998). Following 6 weeks of treatment the rats were sacrificed by focused high-energy microwave irradiation since this markedly increases the measurable concentrations of peptides in brain tissue compared to decapitation (Theodorsson et al. 1990a; Mathé et al. 1990b). The brains were rapidly removed and dissected on ice into frontal cortex, striatum, hippocampus, occipital cortex and hypothalamus. Individual brain regions were weighed and then stored at $-80°C$ until further use.

Extraction of Brain Tissue Samples

The frozen samples were homogenized and boiled in 1 mol/L acetic acid. After centrifugation, the supernatants were lyophilized and stored at $-20°C$. The procedure is described in detail elsewhere (Mathé et al. 1990b).

Radioimmunoassay

The tissue concentrations of NKA-LI, SP-LI and NPY-LI were analyzed by competitive radioimmunoassays as previously reported (Theodorsson et al. 1990b; Heilig and Ekman 1995; Mathé et al. 1998). NKA-LI was analyzed using antiserum K12, kindly provided by Dr. E. Theodorsson. The antibody reacts with NKA$_{3-10}$ (48%), NKA$_{4-10}$ (45%), neurokinin B (26%) and neuropeptide K (61%) but not with SP. SP-LI was analyzed using anti-

serum RIN 7451 (Peninsula Laboratories) which is directed toward the N-terminal portion of SP and shows <0.01% cross-reactivity to NKA, neurokinin B or neuropeptide K. NPY-LI was analyzed using NPY antiserum, kindly provided by Drs. M. Heilig and R. Ekman. This antibody does not cross-react with pancreatic polypeptide or peptide YY. It cross-reacts 100% with NPY_{2-36}, 5% with NPY_{5-36} and 0.5% or less with shorter C-terminal NPY fragments (Heilig and Ekman 1995). The IC_{50} values of the NKA-LI, SP-LI and NPY-LI assays were 22.3, 26.6 and 22.4 pmol/L, respectively, with the lower sensitivity limit of all assays being 1.28 pmol/L. The intra assay coefficients of variation for NKA-LI, SP-LI and NPY-LI were 5–7%. All samples from a given brain region for each peptide were assessed in the same RIA run.

Data Analysis

Two-factor ANOVA with post hoc Tukey test was employed for all statistical analyses, with the exception of hippocampal NPY-LI data. Since these data failed the normality test, Mann-Whitney Rank Sum Test was employed. Results were considered significant for $p < .05$ and are presented as mean ± SD. Statistical analysis was carried out using the Sigmastat® statistical package.

RESULTS

The lithium-treated rats did not display overt symptoms of toxicity; normal grooming and sleeping behavior were observed. At the end of the experiment, bodyweights of lithium or vehicle-treated FSL rats were not different (mean ± SD: 228 ± 20 g and 223 ± 12 g, respectively), while in the FRLs, the lithium-treated rats weighed less than the vehicle-treated rats (248 ± 12 g vs. 291 ± 20 g).

Peptide-LI Concentrations in Vehicle and Lithium-Treated FSL and FRL Rats

Neurokinin A and Substance P.   As shown in Figure 1, in the striatum, concentrations of NKA-LI and SP-LI were significantly lower in FSL compared to FRL rats ($p < .05$). Post hoc analysis revealed that this was due to the markedly lower basal NKA-LI and SP-LI concentrations in FSL rats which were 64% and 63%, respectively, of the basal concentrations in controls ($p < .01$). Lithium increased striatal NKA-LI and SP-LI in FSL rats to concentrations which were no longer significantly different from those in the FRL rats. The effect of lithium was observed only in the FSL strain.

As shown in Figure 2, in the frontal cortex, in contrast to the striatum, basal NKA-LI and SP-LI concentrations were 29% and 37% higher, respectively, in the
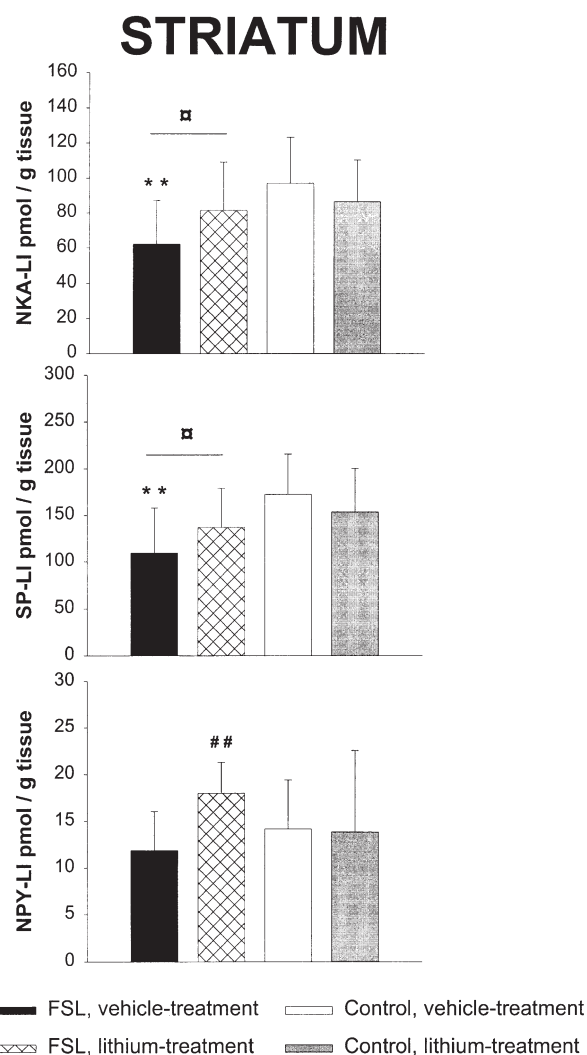

Figure 1. Concentrations of peptide-LI in striatum. See footnote for Table 1. NKA-LI and SP-LI concentrations were lower in FSL compared to FRL rats (XXX = $p < .05$). In the vehicle treatment groups, NKA-LI and SP-LI concentrations were lower in FSL compared to FRL rats (** = $p < .01$). Lithium treatment increased NKA-LI and SP-LI concentrations in FSL rats so that they were no longer different from those in FRL rats. Lithium had no effect in FRL rats. Basal concentrations of NPY-LI were not different between FSL rats and FRL rats. Lithium increased NPY-LI in FSL (# # $p < .01$) but not in the FRL rats. For statistical analysis, two-way ANOVA with post hoc Tukey test was used.

FSL compared to the FRL rats ($p < .01$). Lithium treatment reduced concentrations of NKA-LI and SP-LI in the FSL rats ($p < .001$) but had no effect in the FRL rats. Thus following lithium treatment, there was no longer any difference in tachykinin levels in the frontal cortex among FSL and FRL rats.

Table 1 demonstrates that NKA-LI and SP-LI in the occipital cortex, hippocampus and hypothalamus did not differ between the FSL and the FRL rats. Lithium
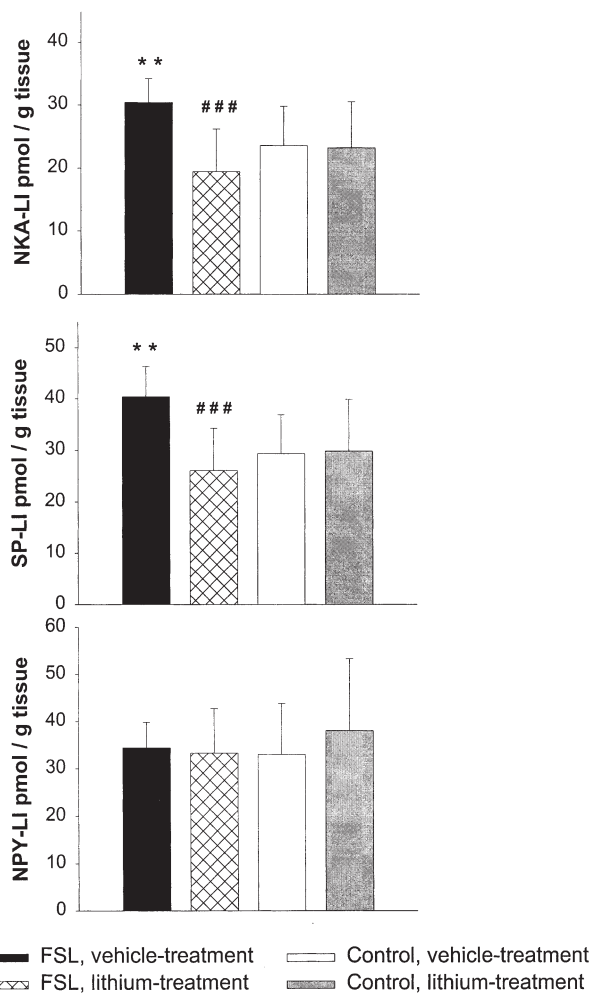
FRONTAL CORTEX

Figure 2. Concentrations of peptide-LI in frontal cortex. See footnote for Table 1. Basal concentrations of NKA-LI and SP-LI were higher in the frontal cortex of FSL in comparison to FRL rats (** = $p < .01$). Lithium treatment reduced concentrations of NKA-LI and SP-LI in FSL rats (### = $p < .001$); that is, they were no longer different from those in FRL rats. Lithium had no effect in the FRL rats. Basal concentrations of NPY-LI in the frontal cortex of FSL and FRL rats were not different. Lithium did not affect NPY-LI in frontal cortex of FSL or FRL rats. For statistical analysis, two-way ANOVA with post hoc Tukey test was used.

also had no effect on NKA-LI or SP-LI in any of these regions.

Concentrations of NKA-LI and SP-LI were highly correlated in all regions (R > 0.8, $p < .001$).

Neuropeptide Y.   As shown in Table 1, in the hippocampus, NPY-LI concentrations were significantly lower in FSL compared to FRL rats ($p < .05$). Basal concentrations of NPY-LI in FSL rats did not significantly differ from FRL in other brain regions (Figures 1 and 2, Table 1). Lithium treatment had no significant effects

Table 1. Neuropeptide-LI in Hippocampus, Occipital Cortex and Hypothalamus

	FSL		FRL	
	Vehicle	**Lithium**	**Vehicle**	**Lithium**
Hippocampus				
NKA-LI	11.5 ± 1.6	16.1 ± 2.1	13.9 ± 2.7	12.7 ± 2.3
SP-LI	16.2 ± 3.0	21.9 ± 3.3	21.4 ± 5.8	17.9 ± 3.8
NPY-LI	8.09 ± 1.5*	8.69 ± 1.6*	10.0 ± 1.1	10.4 ± 1.5
Occipital cortex				
NKA-LI	7.18 ± 4.8	6.78 ± 2.7	8.25 ± 4.2	8.19 ± 3.7
SP-LI	8.86 ± 4.6	8.15 ± 2.7	9.50 ± 4.7	9.97 ± 3.8
NPY-LI	30.6 ± 7.1	29.1 ± 6.5	30.2 ± 5.5	32.5 ± 7.3
Hypothalamus				
NKA-LI	146 ± 33	147 ± 31	147 ± 36	139 ± 44
SP-LI	171 ± 60	162 ± 34	164 ± 55	152 ± 43
NPY-LI	22.1 ± 11	20.5 ± 6.5	15.1 ± 7.9	22.9 ± 7.7

Following a 6-week period of dietary lithium or vehicle, FSL "depressed" and FRL control rats were sacrificed by focused microwave irradiation. The brains were dissected and hippocampus, occipital cortex and hypothalamus analyzed for neuropeptide-like immunoreactivity ($n = 11$ in all groups). Concentrations of NKA-LI, SP-LI and NPY -LI are expressed as pmol peptide-LI/g wet weight tissue ± SD. Non-parametrical analysis of variance showed that concentrations of NPY-LI were significantly lower in FSL compared to FRL rats (* = $p < .05$). Lithium had no effect on NPY-LI concentrations in either strain.

on NPY-LI concentrations in FSL or FRL rats, except in the striatum of FSL rats (Figure 1) where a 51% increase was observed ($p < .01$).

DISCUSSION

The main findings of this study are that the FSL rats displayed marked differences in basal concentrations of NKA-LI and SP-LI in striatum and frontal cortex in comparison to the FRL control strain. In the FSL rats, concentrations of both peptides were decreased in the striatum and elevated in the frontal cortex. Following lithium treatment, NKA-LI and SP-LI concentrations in these two regions were normalized, that is the FSL peptide-LI concentrations became similar to those measured in the FRL rats. No baseline differences and no effects of lithium were found in other brain regions. As far as NPY, in the hippocampus, NPY-LI concentrations were lower in FSL compared to FRL rats. Lithium treatment increased NPY-LI concentrations in striatum of FSL rats but had no effect in FRL rats.

The FSL rats have been selectively bred to display high sensitivity to DFP, an anticholinesterase agent (Overstreet et al. 1979; Russell et al. 1982). The FSL rats are hypersensitive to muscarinic agonists, a characteristic also found in depressed patients who are more sensitive to the behavioral and physiological effects of such agents than healthy controls (Janowsky et al. 1980; Risch et al. 1981). As reviewed in the introduction, a number of other symptoms of human depression are mimicked

by the FSL rats (Overstreet 1993). In the forced swim test, chronic treatment with imipramine, desipramine or sertraline, but not lithium, counteracted the exaggerated immobility of FSL rats, while no effect was observed in the FRL rats (Shiromani et al. 1990; Schiller et al. 1992; Overstreet 1993; Yadid et al. 2000; Zangen et al. 1997). The observation that lithium was without effect in this test does not obviate the validity of either the FSL rats as a model of depression or the validity of the swim test, as lithium treatment is essentially prophylactic and not antidepressant. Furthermore, increased levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) in limbic regions of FSL compared to the FRL strain are decreased by chronic treatment with antidepressants in FSL but not changed in FRL rats (Zangen et al. 1997) which, conceivably, parallels the observed behavior in the swim test. Another characteristic of the FSL rats is that their bodyweight is reduced in comparison to FRL rats. Indeed, in the present study the vehicle-treated FSL rats weighed about 20% less than the vehicle-treated FRL rats at the end of the experiment.

Increases in NKA-LI and SP-LI in striatum and frontal cortex of Fischer rats following various regimens of lithium treatment have previously been reported (Hong et al. 1983; Sivam et al. 1989). Using an experimental design similar to the one used in the present study, we have previously reported similar NKA-LI and SP-LI changes in striatum of Sprague-Dawley rats, while frontal cortex was not affected (Mathé et al. 1990a, 1994; Jousisto-Hanson et al. 1994). In addition, NPY-LI was also increased in striatum following lithium treatment (Mathé et al. 1990a, 1994). In the present study, lithium treatment did not affect NKA-LI, SP-LI or NPY-LI in any of the analyzed brain regions of FRL rats.

The FSL rats were originally established by selective breeding from randomly bred Sprague-Dawley rats obtained from Hawthorn Park Research Animals, Sydney, Australia (Overstreet et al. 1979). The criterion of sensitivity to DFP, as assessed by changes in drinking behavior, bodyweight and core body temperature, was used to distinguish the FSL animals from the "resistant" FRL animals (Overstreet et al. 1979). Thus the "resistant" line was resistant to DFP only when compared to the FSL rats, but not when compared to a random stock group of the original Sprague-Dawley rat population. The fact that the effects of lithium on NKA-LI, SP-LI and NPY-LI in Sprague-Dawley rats (B&K-universal, Sollentuna, Sweden) that were previously observed in our laboratory, were not found in the FRL rats in the present study may be explained by the fact that two different strains of Sprague-Dawley rats (from Australia and Sweden) were used in these experiments and that the FRL is an outbred strain selected for resistance to DFP.

Carbamazepine, a compound that has similar effects to those of lithium in affective disorders (Okuma et al.

1979; Post et al. 1984, 1998) also increases SP-LI in rat striatum (Mitsushio et al. 1988; Kuang et al. 1991). Striatum and frontal cortex are heavily innervated by dopaminergic neurons and haloperidol, a dopamine receptor blocker, has been reported to attenuate or block the lithium and carbamazepine induced NKA-LI and SP-LI and ppt mRNA increases in rat striatum (Hong et al. 1983; Mitsushio et al. 1988; Sivam et al. 1989). Furthermore, haloperidol treatment decreased ppt mRNA and SP-LI in striatum (Angulo et al. 1990; Lindefors 1992) and repeated injections of apomorphine, a dopamine agonist, led to increases in NKA-LI and SP-LI and ppt mRNA in striatum, effects which were blocked by haloperidol (Li et al. 1987).

Similarly, Zachrisson et al. (1997) reported that a series of electroconvulsive stimuli (ECS) altered levels of preprotachykinin and NK_1 receptor mRNA, possibly as a consequence of its effects on the dopaminergic system. Taken together these findings suggest a positive regulatory action of dopamine on the tachykinin system. Interestingly, Zangen et al. (1999) recently reported that the dopamine turnover is reduced in the striatum while increased in the frontal cortex in FSL rats compared to FRL control rats. This finding supports the notion that the altered tachykinin levels in striatum and frontal cortex of the FSL rats may be secondary to a changed dopamine transmission. Moreover, it is also consistent with clinical reports which, based on findings for D_2-receptor occupancy among depressed patients and controls, suggest that striatal dopamine-release is reduced in depressed patients (Ebert et al. 1996; D'Haenen and Bossuyt 1994). Further, PET studies have localized decreased activity in the subgenual prefrontal cortex in familial cases of unipolar and bipolar depression (Drevets et al. 1997) and histopathological studies have demonstrated a significant reduction in neuronal and glial density in the same area (Öngur et al. 1998; Rajkowska et al. 1999) which further implicates this area in the pathophysiology of depression.

In the present study levels of NKA-LI and SP-LI were found to be higher in FSL rats than FRL rats in the frontal cortex, a difference which was abolished after lithium treatment. Interestingly, NK_1 receptor blockade had an anxiolytic effect in rats (File 1997) while substance P was observed to have an anxiogenic profile in rats (Aguiar and Brandao 1996). In this respect it is of interest that MK 869, an NK_1 antagonist, displayed antidepressant and anxiolytic activity in an animal model and a randomized control trial of depressed patients (Kramer et al. 1998). Although the efficacy of MK 869 has not been definitely confirmed (Enserink 1999), these data as a whole suggest that NK_1 receptors may play a role in the therapy and perhaps the pathophysiology of mood disorders. In one study, the ratio of NK_1 receptor densities in the superficial laminae of the cingulate cor-

tex compared to the deep laminae was found to be decreased in subjects with unipolar depression. The authors suggested that this alteration was likely caused by a decrease in NK1 receptors in the superficial laminae (Burnet and Harrison 2000). Such a change could be a compensatory regulation in response to an increased SP and/or NKA release in this area, which the present study strongly supports.

Several mechanisms could account for the altered SP-LI and NKA-LI tissue concentrations in the FSL rats. Because concentrations of NKA-LI and SP-LI were highly correlated in all brain regions it seems plausible to assume that the lithium-induced changes may be a consequence of an altered transcription of the ppt-A gene (Sivam et al. 1989) and not of an altered post-translational processing (Hong et al. 1983; Sivam et al. 1989). This is in contrast to the effects of repeated ECS, where consistent increases only in NKA-LI and an unaltered SP-LI are found in rat hippocampus, but not in striatum (Stenfors et al. 1989, 1992, 1994; Mathé 1999). These findings support the notion that selective peptide and region effects of lithium as well as ECS on neuropeptides may indeed constitute one of their therapeutic mechanisms of action. The reduced basal concentrations of NKA-LI and SP-LI in striatum and increased basal concentrations in frontal cortex of FSL rats may suggest that a hypo- and hyper-function, respectively, of NKA and SP peptidergic transmission in these areas may be associated with the depressive-like phenotype of FSL rats. In view of the fact that lithium treatment had no effect on tachykinin concentrations in the FRL rats, it is conceivable that normalizing NKA-LI and SP-LI concentrations in striatum and frontal cortex in the FSL rats may constitute one therapeutic mechanism of lithium's action.

On the basis of measurements of NPY-LI in cerebrospinal fluid and plasma (Widerlöv et al. 1986, 1988; Nilsson et al. 1996; Hashimoto et al. 1996) and reduced concentrations of NPY-LI and NPY mRNA in post-mortem brain of bipolar patients (Widdowson et al. 1992; Caberlotto and Hurd 1998) it has been speculated, that an NPYergic hypofunction may underlie the pathophysiology of affective disorders. However, contradictory results have also been reported (Träskman-Bendz et al. 1992; Roy 1993; Ordway et al. 1995). The present study in which basal concentrations of NPY-LI in the hippocampus were significantly lower in FSL than FRL rats supports results from a parallel study of FSL rats (Jiménez Vasquez et al. 2000) and is consistent with the hypothesis that NPY plays a role in the neural processes underlying the depressive-like phenotype of the FSL rats. Hippocampal NPY-LI concentrations in Fawn Hooded rats (also considered to be an animal model of depression) were also found to be lower compared to Sprague-Dawley and Wistar rats (Mathé et al. 1998). Furthermore, it has been repeatedly shown that a se-

ries of ECS increases NKA-LI and NPY-LI but not SP-LI in hippocampus of Sprague-Dawley, Wistar, Fawn Hooded and FSL rats (Stenfors et al. 1992, 1995a, 1995b; Mathé et al. 1997, 1998; Jiménez Vasquez et al. 2000). Moreover, antidepressant drugs seem to also affect NPY-ergic neurotransmission in limbic structures of rats (Widdowson and Halaris 1991; Caberlotto et al. 1998; Husum et al. 2000). Consequently, one heuristic hypothesis is that limbic effects on NPY and NKA may be one common denominator of antidepressant treatments, while the effects in other brain regions (i.e., frontal cortex and striatum) are associated with the prophylactic effects of lithium and antipsychotic effects of ECT (Mathé 1999).

In conclusion, on the basis of the presented results it is suggested that changed concentrations of NKA-LI and SP-LI in striatum and frontal cortex may be associated with the depressive-like phenotype of the FSL rats and, by inference, play a role in the pathophysiology of affective disorders. Lastly, regulating NKA-LI and SP-LI concentrations in those two brain regions may be of relevance to the prophylactic effects of lithium.

ACKNOWLEDGMENTS

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