Maternal Deprivation and Mood Stabilizing Drugs:

Effects on Rat Brain NPY

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Cover picture: Human/rat neuropeptide Y (NPY), which takes its name from the single letter code for tyrosine (Y; yellow) that makes up five of the thirty-six amino acids in the molecule. The tertiary structure of NPY is comprised by an N-terminal polyproline helix (blue), connected with a β-turn to an amphiphilic α-helix (red). These elements form the characteristic PP-fold, a trait of the NPY family of peptides, which brings the C- and N-termini of the molecule in close proximity and presents the combined moiety to the receptor. The COOH-terminal is amidated and projects away from the hairpin-loop (Schwartz et al., 1990). Reproduced with the kind assistance of Dr. Stefano Ricagno, Department of Medical Biochemistry and Biophysics, Karolinska Institutet.

SUMMARY

Experiences of early adverse life events are more frequent among adult depressed patients than healthy subjects. Studies with non-human primates and rats show that maternal deprivation leads to changes in hypothalamic-pituitary adrenal (HPA)-axis activity/reactivity and increased levels of anxiety and alcohol preference. Neuropeptide Y (NPY) is widely and abundantly distributed in mammalian brain, in particular in limbic areas. NPY modulates a number of behavioral and physiological functions including anxiety, food intake, cognition, hippocampal excitability and HPA-axis activity. Since accumulated clinical and experimental evidence suggests a role for NPY in the pathophysiology of mood disorders, the effects of maternal deprivation on rat brain NPY were investigated as a major aim of this thesis. Maternal deprivation for 180 min/day during postnatal day 2-14 or for 24 hrs on postnatal day 9 led to marked reductions in hippocampal levels of NPY-LI. These changes were associated with reduced saccharin preference and prepulse inhibition deficits, indicating that reductions in hippocampal NPY may be associated with anhedonia and cognitive disturbances. This notion is supported by the finding that central administration of NPY induced antidepressant-like activity in normal rats, in a manner comparable to that of imipramine.

A second aim of the present thesis was to investigate the effects of lithium and the antiepileptics topiramate and levetiracetam, which all have mood stabilizing effects in patients, on NPY-LI in maternally deprived, Flinders Sensitive Line (FSL) and amygdala-kindled rats, models that each display specific resemblances to hallmarks of depression. These treatments all lead to changes in hippocampal NPY mRNA, NPY-LI and NPY receptor binding, indicative of an increased NPYergic neurotransmission. Specifically, in maternally deprived animals, lithium reversed the separation-induced decrease of hippocampal NPY-LI levels to concentrations that were similar to those found in the control animals. Similarly, lowered hippocampal NPY-LI in the FSL rats was normalized following repeated, but not single, topiramate treatment, whereas topiramate had no effect on this measure in the FRL control animals. Levetiracetam delayed the development of kindling and also reduced the duration of afterdischarge and motor seizures. These behavioral manifestations coincided with the prevention of a kindling-induced upregulation of brain-derived neurotrophic factor and NPY mRNA in the dentate gyrus of the hippocampus and the prevention of a kindling-induced downregulation of Y1-, and Y5-like receptors in the dentate gyrus and Y2-like receptors in the CA3 area of the hippocampus. In contrast, none of these parameters were affected by levetiracetam in normal rats.

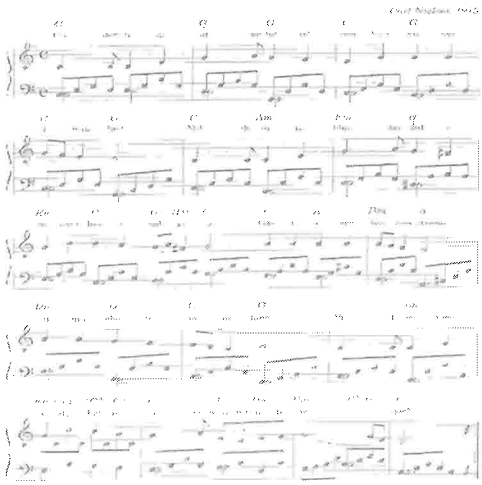
By extrapolation, the present findings therefore support the working hypothesis that a neurobiological correlate to an increased risk for mood disorders and comorbid anxiety may be a decreased concentration of NPY in the hippocampus, and that such a reduction in NPY may result from exposure to early life stress. In view of the observations that (1) NPY is a potent anticonvulsant and also exerts antidepressant properties, (2) NPY affects a variety of centrally mediated functions of possible significance to the symptomatic spectrum of mood disorders, (3) a dysfunctional NPY system has been reported in patients suffering from depression as well as epilepsy disorders, an expanded heuristic hypothesis can be proposed: a decreased concentration of NPY in the hippocampus may be associated with aspects of lowered mood, impaired cognitive function, alcohol-dependence, and increased stress sensitivity and seizure vulnerability. Lithium, the most commonly prescribed drug in mood-disorders, and antiepileptics, which all essentially possess mood stabilizing properties, may in part exert their therapeutic mechanisms of action by stimulating the synthesis of hippocampal NPY, thus being a common denominator for their mood stabilizing and anticonvulsant properties.

Keywords: maternal deprivation, stress, mood stabilizing drugs, NPY, mood disorders, depression, anhedonia, Flinders Sensitive Line rats, Amygdala-kindling, microdialysis, RIA, histochemistry.

»Den enkelte betyr intet, hans innsats betyr *noe.*
Den enkelte er bare en bit, bare en liten sten i en stor mosaikk.
Og denne mosaikken er fremtidens gulv.
Det vakreste mønsteret,
det herligste og sanneste i denne mosaikken
er vitenskapen«

From *"Salme ved reisens slutt",* Erik Fosnes Hansen,
J.W. Cappelens Forlag A/S, Oslo 1990.

Emil Nolde 1867-1956-»musikkapelle«



To my family

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ORIGINAL PAPERS

The present thesis is based on the following papers and manuscripts, which will be referred to in the text by their Roman numerals.

I. Husum H., Mikkelsen J.D., Hogg S., Mathé A.A., Mørk A. Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. Neuropharmacology 2000, 39: 1463-1473.

II. Husum H., Mathé A.A. Early life stress changes concentrations of neuropeptide Y and corticotropin-releasing hormone in adult rat brain. Lithium treatment modifies these changes. Neuropsychopharmacology 2002, 27: XX-XX *in press*.

III. Husum H., Termeer E., Mathé A.A., Bolwig T.G., Ellenbroek B.A. Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-gene related peptide in adult rats. Neuropharmacology 2002, 42: 798-806.

IV. Husum H., Gruber S.H.M., Bolwig T.G., Mathé A.A. Extracellular levels of NPY in the dorsal hippocampus of freely moving rats are markedly elevated following a single electroconvulsive stimulation, irrespective of anticonvulsive Y1 receptor blockade. Neuropeptides 2002, 36: XX-XX *in press*.

V. Husum H., van Kammen D., Termeer E., Bolwig T.G., Mathé A.A. Topiramate normalizes hippocampal levels of NPY in Flinders sensitive line 'depressed' rats and upregulates levels of NPY, galanin and CRH in hypothalamus: Implications for mood stabilizing and weight-loss inducing effects. Submitted.

VI. Husum H., Bolwig T.G., Sánchez C., Mathé A.A., Hansen S. Antiepileptogenic effect of levetiracetam against Amygdala-kindling in the rat is associated with a region-specific maintenance of normal hippocampal levels of BDNF and NPY mRNA, and Y1, Y2 and Y5 receptor subtypes. Manuscript.

ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
CAMP	cyclic 3',5'adenosine-monophosphate
CNS	Central nervous system
CSF	Cerebrospinal fluid
CRH	Corticotropin-releasing hormone
DSM IV	Diagnostic and statistical manual of mental disorders, 4th editiom
ECS	Electroconvulsive stimulations
ECT	Electroconvulsive therapy
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
GABA	γ-amino butyric acid
GAD	Glutamic acid decarboxylase
HPA-axis	Hypothalamic-pituitary-adrenal axis
i.c.v.	Intracerebroventricular
NPY	Neuropeptide Y
NPY-IR	NPY-immunoreactive
NPY-LI	NPY-like immunoreactivity
PND	Postnatal day
PP	Pancreatic polypeptide
PPI	Prepulse inhibition
PVN	Paraventricular nucleus
PYY	Peptide YY

FOREWORD

Mood disorders are among the most common psychiatric disorders in adults and comprise devastating behavioral conditions of unknown etiology that affect mood, cognition and perception. The World Health Organization has projected mood disorders to become the most disabling type of disorders by the year 2020.

Clinical and experimental studies suggest that the serotonin (5-HT) and noradrenaline neurotransmitter systems are involved in the pathophysiology and treatment of depression. These studies have led to the monoamine depletion and receptor sensitivity hypotheses of depression (Vetulani and Sulser, 1975;Schildkraut, 1965). Consistently with these hypotheses, currently available antidepressant drugs exert their pharmacological actions by increasing synaptic levels of 5-HT and noradrenaline. However, the time lag for increasing levels of monoamines is more rapid (hours/days) than the therapeutic onset of these treatments (weeks), indicating that these effects may not directly mediate the therapeutic action of antidepressant drugs (Riva and Creese, 1989). Moreover, monoamine depletion alone is not sufficient to cause depression in normal subjects (Salomon et al., 1997;Delgado et al., 1989). In addition, findings of lowered levels of monoamines and/or their metabolites in cerebrospinal fluid, plasma or urine from depressed patients are inconsistent (Harro and Oreland, 2001). Taken together, these findings indicate that the pathophysiology of depression can not simply be explained by a dysregulation of 5-HT and/or noradrenaline.

Advances in cellular and molecular neurobiology have provided new insight into the neurobiology of stress, generally considered to be a vulnerability factor for depression. This work, in combination with advances in our understanding of the long-term adaptations occurring in the brain in response to pharmacological treatment, is revealing potential molecular and anatomical sites that could contribute to the understanding of the pathophysiology and treatment of mood disorders. Specifically, studies have shown that antidepressant drug treatment and lithium regulate common intracellular signal transduction pathways and the expression of specific target genes, including neuropeptides.

One such target gene is neuropeptide Y (NPY). Clinical and experimental evidence suggest that NPY may be involved in the pathophysiology of mood disorders as well as the long-term adaptations that constitute one of the brain responses to treatment modalities that alleviate symptoms in mood disorders. In addition, NPY modulates a number of behaviors that are of significance to the symptomatology of depression. The present studies address the question of the role of NPY in the pathophysiology and alleviation of mood disorders by two experimental approaches: a developmental and a pharmacological approach. In both cases, the main focus is effects on rat brain NPY.

MOOD DISORDERS

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM IV, American Psychiatric Association, Washington D.C. 1994) mood disorders include, in addition to dysthymic and cyclothymic disorder:

- Major depression, characterized by recurrent (sometimes single) episodes of depression. The lifetime prevalence of major depression is between 4% and 25% and about twice as prevalent among women than men (Swendsen et al., 1998;Kessler et al., 1994).
- Bipolar disorder, previously referred to as manic-depressive illness, characterized by episodes of depression and mania (type I) or episodes of depression and hypomania (type II). The lifetime prevalence is around 1% in both women and men (Craddock and Jones, 1999).

These disorders constitute a major public health problem because of the great suffering they bring to afflicted individuals and their relatives, comorbidity and medical complications. In addition, patients diagnosed with either major depression or bipolar disorder have an estimated suicide risk that is 20 and 15 times higher, respectively, than in the general population (Harris and Barraclough, 1997).

Etiological factors in mood disorders

Genetic factors

The lifetime risk of major depression in a monozygotic co-twin of a depressed proband is in the range of 31-42%. For bipolar disorders the heritability is estimated to be in the range of 40-70% (Sullivan et al., 2000;Craddock and Jones, 1999). It is therefore evident that none of these disorders exhibit classic Mendelian recessive or dominant inheritance. More likely, their genetic predisposition involves the interaction of several susceptibility genes or more complex genetic mechanisms such as mutations and genomic imprinting. In addition, mood disorders are believed to result from an interaction between the effects of genetic predisposition and environmental factors.

Early life stress

One such environmental factor that has been recognized as significantly increasing the susceptibility to mood disorders is early life stress. For instance, experiences of childhood sexual abuse are over-represented among adult depressed patients compared to healthy subjects (Gladstone et al., 1999;McCauley et al., 1997;Boudewyn and Liem, 1995;Bifulco et al., 1991). Inadequate and deprivational parenting has also been identified as important antecedents to the development of depression (Parker et al., 1995;Parker, 1983). Furthermore, childhood separation experiences caused by parental illness, divorce or death are more prevalent among adult depressed and bipolar patients than healthy control subjects (Agid et al., 1999;Kendler et al., 1992;Tennant et al., 1982). Studies in non-human primates report that early maternal deprivation/inadequate attention during

infancy induces behavioral changes that resemble symptoms of human depression including disturbed sleep, decreased locomotion, anxiety, and affective disturbances (Suomi, 1997;Hinde et al., 1978).

Comorbidity with epilepsy

Epidemiological investigations have revealed a high degree of comorbidity between depression, diabetes, cardiovascular diseases and gastrointestinal dysfunction disorders as well as some CNS disorders (Mayer et al., 2001;Ballenger et al., 2001;Poewe and Luginger, 1999). For instance, the co-occurrence of depression with alcoholism and anxiety disorders has been extensively documented and has added to the hypothesis of shared etiologic factors contributing to the manifestation of these disorders (Lenze et al., 2001;Swendsen et al., 1998;Angst, 1996;Penick et al., 1994).

Depression is among the most common psychiatric co-morbidities in epileptic patients (Harden and Goldstein, 2002;Kanner and Balabanov, 2002;Jacoby et al., 1996). Conversely, a history of depression increases the risk for unprovoked seizures (Hesdorffer et al., 2000;Forsgren and Nystrom, 1990). This relationship between epilepsy and depression was first suggested around 400 BC when Hippocrates observed that "melancholics ordinarily become epileptics, and epileptics melancholics: what determines the preference is the direction the malady takes; if it bears upon the body, epilepsy, if upon the intelligence, melancholy" (Lewis, 1934). In general, epileptic patients have an estimated five-fold greater incidence of depression and of attempting suicide, with even higher incidences for temporal lobe epilepsy, than the general population (Piazzini and Canger, 2001;Lambert and Robertson, 1999;Harris and Barraclough, 1997;Forsgren and Nystrom, 1990). Depression is particularly observed in patients with partial seizure disorders of temporal and frontal lobe origin, i.e. seizures involving the limbic circuit, and are more frequent among patients with poorly controlled seizures (Piazzini and Canger, 2001;Jacoby et al., 1996;Robertson et al., 1987). In addition, risk factors for completed suicide among epilepsy patients have been found to include stressful life events (Roth et al., 1994).

Hallmarks of depression

Symptomatology

In the DSM IV system, an episode of depression is characterized by at least five symptoms, each occurring for a minimum of two weeks, of which either lowered emotional state and/or anhedonia should be present as core symptoms. Other symptoms include loss of weight (or increase in weight), sleep disturbances, psychomotor agitation, fatigue, feelings of worthlessness or inappropriate guilt, cognitive and concentration-difficulties or indecisiveness, and suicidal ideation.

Hypothalamic-pituitary-adrenal axis

Efficient activation and feedback inhibition of the hypothalamic-pituitary-adrenal (HPA) axis is of central importance to the individual in coping with stress and thus to long-term sense of well-being. The stress response of the HPA axis is organized by corticotropin-releasing hormone (CRH) neurons in the parvocellular component of the hypothalamic paraventricular nucleus (PVN), which in response to acute stress increase their release of CRH into portal blood via the median eminence. At the anterior pituitary, CRH stimulates the release of adrenocorticotrophic hormone (ACTH), which stimulates adrenocortical cell growth and the release of glucocorticoids, predominantly cortisol in man and corticosterone in the rat (see review by Holsboer, 2001;Holsboer, 2000). Negative feedback control of the HPA-axis is achieved at all levels through the actions of corticosteroid receptors in medial prefrontal cortical areas, the pituitary and the hippocampus (Brake et al., 2000;Sapolsky, 1994;Diorio et al., 1993;Herman et al., 1989;Sapolsky et al., 1984). However, in chronically stressed rats, an increased CRH activity is maintained in the PVN, and ACTH and corticosterone levels are elevated due to impaired negative feedback control (Fernandes et al., 2002;Pinnock and Herbert, 2001). These adaptive changes underlie the capacity to sustain an increased secretion of corticosteroids and to mount an additional corticosteroid response to superimposed stress (Holsboer, 2001).

Interestingly, major depression is associated with similar endocrinological changes. A post mortem study of brains from six depressed patients that committed suicide found a marked increase in the number of CRH-expressing cells in the PVN (Raadsheer et al., 1994). Cerebrospinal fluid (CSF) levels of CRH were shown to be elevated in depressed patients (Banki et al., 1987;Widerlöv et al., 1986;Nemeroff et al., 1984), while CRH-sensitive binding sites were reduced in prefrontal cortex of suicide victims (Nemeroff et al., 1988). In addition, a hypertrophy of the adrenal glands in depressed patients has repeatedly been demonstrated (Rubin et al., 1995;Nemeroff et al., 1992;Dorovinin-Zis and Zis, 1987), and an increased secretion of cortisol is seen in approximately 50% of patients with major depression (Westrin et al., 1999;Halbreich et al., 1985). Also, negative feedback control of the HPA axis by corticosteroids is impaired in depressed patients (Young et al., 1991;Carroll, 1982). Taken together, these clinical data suggest that the basal activity of the HPA axis is elevated in depressed subjects and that this change is associated with the pathology of major depression.

Hippocampal atrophy

It is a long-standing dogma that adult neurons are post mitotic and, as such, cannot be replaced once lost. However, in the past decade it has been shown that neurogenesis occurs also in the adult brain and that it is particularly pronounced in the dentate gyrus of the hippocampus in rodents, nonhuman primates and humans (Duman et al., 1997;Gould et al., 1992;Altman and Das, 1965). It has also been demonstrated that neurogenesis is inhibited by prolonged exposure to elevated levels of glucocorticoids (Sapolsky, 2000;Sapolsky, 1994). Volumetric magnetic resonance imaging scans have shown that patients in remission with a history of severe depression have reduced hippocampal

volumes (Sapolsky, 2000;Sheline et al., 1999;Sheline et al., 1996). These findings are believed to be the result of an increased cumulative exposure to glucocorticoids as a consequence of HPA-axis hyperactivity.

Animal models of depression

During the past decades several animal models of depression have been developed by means of genetic selection for specific behaviors. Such models include for instance Fawn-Hooded (Rezvani et al., 2002) and Wistar Kyoto rats (Pare and Tejani-Butt, 1996). Other models mimic environmental stress by subjecting animals to pre or post-natal stress or repeated stress in adulthood. These include for instance the chronic mild stress paradigm (Willner et al., 1987) and the congenital learned helplessness model in which rats are selectively bred for susceptibility and resistance to behavioral deficits induced by uncontrollable shock (Henn et al., 1985;Overmier and Seligman, 1967). These genetic and behavioral paradigms induce a state of anhedonia in the animals that can be alleviated by antidepressant treatment.

The present thesis is focused on the use of three rat models, all displaying resemblances to hallmarks of depression, and each focusing on one of the etiological factors outlined above.

Flinders Sensitive Line rats

The Flinders sensitive line (FSL) and Flinders resistant line (FRL) rats were established at Flinders University in Adelaide, Australia, by selective breeding of Sprague-Dawley albino rats for high (FSL) and low (FRL) sensitivity to the irreversible anticholinesterase agent diisopropylfluorophosphate (DFP) (Russell et al., 1982;Overstreet et al., 1979). Effects on drinking behavior, body weight, and core body temperature were used as indices for sensitivity to DFP but are also apparent following treatment with physostigmine and muscarinic agonists such as oxotremorine and arecoline (Overstreet, 1986;Russell et al., 1982;Overstreet et al., 1979). The FSL rat is a genetic animal model of depression since genetic factors are believed to underlie the hypersensitivity to DFP, a phenomenon that has been observed among depressed patients (Risch et al., 1981;Janowsky et al., 1980;Risch et al., 191). A number of other symptoms of human depression are mimicked by the FSL rats including: anhedonia, i.e. reduced saccharin preference, and submissive behavior (Overstreet, 1993;Pucilowski et al., 1993;Pucilowski et al., 1992;Pucilowski et al., 1991); reduced bodyweight (Husum et al., 2001;Friedman et al., 1996;Overstreet, 1993;Russell et al., 1982); reduction in REM sleep onset and increased REM sleep episodes (Shiromani et al., 1991;Shiromani et al., 1988); cognitive deficits and learning disturbances (Overstreet, 1993); reduced locomotor activity (Overstreet, 1993;Overstreet, 1986); and increased immobility in the forced swim test which is reversible by chronic but not acute treatment with antidepressant drugs (Schiller et al., 1992;Overstreet, 1986). The FSL rat thus presents a model of depression with good face and predictive validity.

Regarding HPA axis abnormalities, corticosterone and ACTH levels are similar in FSL and FRL rats (Friedman et al., 1996;Overstreet, 1993;Owens et al., 1991), while levels of CRH-LI in the median eminence and hypothalamus were in one study found to be lower in FSL than FRL rats (Owens et al., 1991).

The maternal deprivation model of early life stress

The implication of early adverse life events as a vulnerability factor for the development of adult life psychopathology has encouraged numerous investigations into the biological consequences of early maternal deprivation in rats. The separation paradigms employed differ with regard to duration, frequency and time of the day of the separation period, strain of rats used, age of pups undergoing separation, and control-handling procedures. Regardless of the particular procedure used, maternal deprivation has been observed to increase levels of CRH mRNA in the hypothalamus and median eminence, induce adrenal hypertrophy, and a prolonged stress-induced ACTH and corticosterone response (Penke et al., 2001;Ladd et al., 1996;Rots et al., 1996;Plotsky and Meaney, 1993;Pihoker et al., 1993). There is also some evidence for lowered glucocorticoid and mineralocorticoid receptor binding in the hippocampus of maternally deprived rats (Penke et al., 2001;Rots et al., 1996;Sutanto et al., 1996). These reports suggest that maternal deprivation leads to HPA axis hyperactivity in rats, which may be a consequence of CRH hypersecretion from the median eminence, possibly due to impaired negative feedback mechanisms. In contrast, brief handling during the pre-weaning period, resulting in more mother-pup interactions, leads to a permanent upregulation of the hippocampal type II glucocorticoid receptors, lower CRH mRNA levels in the PVN, and a blunted ACTH and corticosterone-response to restraint stress (Liu et al., 1997;Viau et al., 1993;Meaney et al., 1989a;Meaney et al., 1989b;Meaney et al., 1988). Taken together, these studies imply that early life handling/separation leads to permanent changes in the reactivity of the HPA axis.

Studies suggest that hippocampal degeneration may be more pronounced in individuals with an enhanced HPA axis reactivity, which entails an increased cumulative exposure to glucocorticoids (Sapolsky, 2000;Sapolsky, 1994;Gould et al., 1992;Sapolsky et al., 1984;Landfield et al., 1981). Interestingly, rearing-induced hyperactivity of the HPA-axis is associated with an exacerbated age-related loss of glucocorticoid receptor-expressing neurons in the CA1 and CA3 areas of the hippocampus and pronounced spatial memory deficits in the Morris swim maze (Meaney et al., 1988). Recently, similar findings were obtained in adult rats to which CRH was centrally administered on postnatal day (PND) 10 (Brunson et al., 2001), indicating that CRH hypersecretion may underlie the effects of early-life stress on hippocampal integrity and function. Interestingly, pups from rat dams that spent more time licking/grooming their offspring had higher NMDA receptor and brain-derived neurotrophic factor (BDNF) mRNA levels as adults as well as increased synaptogenesis in the hippocampus and an improved performance in the Morris Water Maze, compared to offspring subjected to less mother-pup interactions (Liu et al., 2000). Conversely, pups subjected to maternal deprivation had lower NMDA receptor and BDNF mRNA levels in the

hippocampus as adults (Roceri et al., 2002). This suggests that these systems are highly sensitive to brief manipulations in early life, with long-lasting consequences on learning and some forms of memory.

Apart from impairments in hippocampal-mediated learning and memory, maternally deprived rats have also been reported to display an increased anxiety level (Penke et al., 2001;Plotsky et al., 1995), sexual dysfunction (Rhees et al., 2001) and distinct alcohol preference compared to control-reared animals (Plotsky et al., 1995).

Amygdala-kindling

Although interictal depression is the most commonly recognized presentation of depression among epileptic patients, reports of periictal and ictal depression also exist (see Harden and Goldstein, 2002;Kanner and Balabanov, 2002;Lambert and Robertson, 1999). Further, in temporal lobe epilepsy patients, sudden feelings of sadness, hopelessness and despair and suicidal attempts can occur as part of the seizure itself (Kanner and Balabanov, 2002;Lambert and Robertson, 1999;Mendez and Doss, 1992). Interestingly, prospective studies have shown that feelings of depressed mood and irritability occurring hours to days before a seizure are often relieved by the convulsion (Hughes et al., 1993;Devinsky and Bear, 1991;Blanchet and Frommer, 1986). These observations imply that epilepsy and depression may share a common antecedent at the biochemical level and that depression symptomatology may be associated with a lowered seizure threshold.

The Amygdala-kindling reflects aspects of epilepsy as well as mood disorders. In this model, the rat becomes sensitized to an initially sub-convulsive electrical stimulus of the amygdaloid complex. That is, successive stimulations will elicit seizures and ultimately induce 'status epilepticus' in which seizures occur spontaneously (Post and Weiss, 1996;Erickson et al., 1996a). This kindling phenomenon shows some resemblance to the increased vulnerability to relapse with increased number of disease episodes and increased illness severity in mood disorders (Kessing et al., 1998a). Furthermore, the kindling epileptogenesis is attenuated or prevented by antiepileptic drugs as well as electroconvulsive treatment, that also display mood stabilizing properties in humans (Reissmuller et al., 2000;McElroy et al., 2000;Calabrese et al., 1999;Post et al., 1996;Post et al., 1991;Silver et al., 1991;Post and Weiss, 1989;Post et al., 1984;Okuma et al., 1979;Baastrup et al., 1970).

Kindling of the amygdaloid complex results in elevated anxiety, greater response to fear-potentiated startle, and reduced exploratory behavior in rats (Haimovici et al., 2001;Anisman et al., 2000;Hori et al., 1997;Helfer et al., 1996;Adamec, 1990). Evidence implies that changes in HPA-axis activity may play a role in the kindling epileptogenesis. For instance, intracerebroventricular (i.c.v.) administration of CRH was shown to decrease the afterhyperpolarization period of dentate granule neurons, to enhance the progression of Amygdala-kindling and to induce electroencephalographic (EEG) and motor seizure activity in the rat (Smith et al., 1997;Weiss et al., 1986;Ehlers et al., 1983). Further, levels of CRH mRNA in the dentate gyrus and plasma ACTH and corticosterone levels were increased in rats following Amygdala-kindling (Smith et al., 1997;Weiss et al.,

1993;Szafarczyk et al., 1986). These findings suggest that CRH hyperactivity could facilitate epileptogenesis.

In humans, volumetric and histochemical analyses have shown that temporal lobe epilepsy is associated with patterns of hippocampal cell loss, in particular in the subgranular polymorphic region of the dentate gyrus and in the pyramidal layer in Ammon's horn, most notably in the CA1 area (Sundstrom et al., 2001;Furtinger et al., 2001;Mathérn et al., 1995;de Lanerolle et al., 1989;Babb et al., 1984;Dam, 1980;Margerison and Corsellis, 1966). In the rat, Amygdala-kindling has been observed to induce apoptosis in the dentate gyrus, CA1 and CA3 areas of the hippocampus (Haas et al., 2001;Umeoka et al., 2000;Frantseva et al., 2000)

In view of the comorbidity between epilepsy and depression, hippocampal atrophy in both conditions, attenuation of progression of kindling mood stabilizing agents, and kindling-induced changes in behavior and HPA axis activity, the amygdala-kindling model appears also to be an appropriate model for the study of mood disorders.

Mood stabilizing agents

In addition to using animal models, the study of the biology of pharmacological mood stabilization may provide insight into the pathophysiology of mood disorders.

Lithium and antiepileptics are the two major groups of drugs used in the prophylactic treatment of mood disorders. Antiepileptic drugs are thought to act mainly by reducing electrical excitability of cell membranes through use dependent block of sodium channels and enhancing GABA-mediated synaptic transmission. However, a common therapeutic mechanism describing the action of mood stabilizing agents has not yet been identified.

Much effort has been invested in elucidating the mechanisms of action of mood stabilizing drugs with a focus on signal transduction pathways. Specifically, lithium and valproate have been shown to regulate protein kinase C (PKC) signaling with implications for immediate and long-term effects on the regulation of synaptic function and neuronal excitability. Some important targets of PKC signaling include myristoylated alanine-rich C kinase substrate (MARCKS), a key phosphoprotein substrate for PKC, and glycogen synthase kinase (GSK-3), a transcription factor activator, and mitogen activated protein (MAP)-kinase. The interactions of these kinase cascades regulate the activity of immediate early genes (IEG) including the fos and jun gene-families (for reviews see Manji and Lenox, 1999;Lenox and Manji, 1998;Manji et al., 1995;Mørk, 1990;Berridge et al., 1989;Berridge and Irvine, 1989). The activator protein-1 (AP-1) is a collection of homo- and heterodimeric complexes composed of phosphorylated products from those two families (Chiu et al., 1988). AP-1 regulatory elements (5'-TGAC/GTCA3') exist in the promoter region of several genes, including genes for various neuropeptides, and regulate the transcriptional frequency of such genes (Hughes and Dragunow, 1995;Chiu et al., 1988). Interestingly, therapeutic concentrations of lithium and valproate as well as repeated electroconvulsive stimulations have been shown to

markedly enhance AP-1 DNA binding activity in the frontal cortex and the hippocampus (Manji and Lenox, 1999;Chen et al., 1999;Ozaki and Chuang, 1997;Hope et al., 1994). Mood stabilizing drugs may thus, via effects on PKC and other kinase activities, regulate the expression of multiple genes in critical neuronal circuits, effects that are likely to be of significance for their therapeutic mechanism of action.

Lithium and neuropeptide Y

Cloning of the promoter sequence of the human and rat neuropeptide Y (NPY) gene has revealed several putative AP-1 like binding sites (Minth and Dixon, 1990;Larhammar et al., 1987). Interestingly, lithium has been shown to stimulate NPY gene transcription in vitro (Kalasapudi et al., 1990). Further, when administered to rats, lithium increases NPY mRNA levels in the hippocampus and NPY mRNA and NPY-like immunoreactivity (LI) in the striatum (Husum et al., 2001;Zachrisson et al., 1995a;Mathé et al., 1994;Weiner et al., 1992;Mathé et al., 1990). To the best of our knowledge, no other publications have reported on the effects of other mood stabilizing drugs on the activity of the NPY system. Interestingly, a connection between NPY and mood disorders has been suggested by clinical and experimental findings.

NEUROPEPTIDE Y

NPY and mood disorders: clinical findings

With the exception of a single contradictory report (Irwin et al., 1991), depressed patients have repeatedly been shown to have lower plasma levels of NPY-LI as compared to age-matched healthy controls (Westrin et al., 1999;Nilsson et al., 1996;Hashimoto et al., 1996). Noticeably, one study found patients who had repeatedly attempted suicide to have the lowest levels of NPY (Westrin et al., 1999). Furthermore, the pharmacological alleviation of depression symptoms associated with cerebral infarction was associated with marked increases in plasma NPY-LI levels (Koide et al., 1995).

Reduced levels of NPY-LI concentrations in CSF from depressed patients have also been reported (Gjerris et al., 1992;Widerlöv et al., 1988a;Widerlöv et al., 1988b;Widerlöv et al., 1986). Yet, contradictory findings exist as some studies failed to detect any difference in CSF NPY-LI between depressed patients and suicide attempters, and healthy controls (Roy, 1993;Träskman-Bendz et al., 1992;Sunderland et al., 1991;Berrettini et al., 1987). It has been suggested that depressed patients may have abnormal processing or metabolism of NPY, which may explain the findings of reduced NPY-LI as well as discrepancies between studies (Ekman et al., 1996;Arranz et al., 1996;Heilig and Widerlöv, 1995;Maeda et al., 1994). Indeed, no association between NPY gene polymorphism and mood disorders was found, suggesting that secondary events account for the lowered NPY levels observed in the majority of studies of depressed patients (Detera-Wadleigh et al., 1987).

Postmortem studies have reported lower NPY-LI in frontal cortex and caudate putamen in suicide victims with a previous history of depression (Widdowson et al., 1992). However, subsequent studies from the same and other laboratories have failed to replicate these findings (Arranz et al., 1996;Ordway et al., 1995). Levels of Y2 receptor mRNA expression were found to be higher in the prefrontal cortex of psychiatric patients with suicide as cause of death (Caberlotto and Hurd, 2001). In another study, no association between NPY mRNA in the frontal cortex and suicide as cause of death was found, but NPY mRNA was markedly reduced in the prefrontal cortex in patients diagnosed with bipolar disorder (Caberlotto and Hurd, 1999).

Neuropeptide Y: genealogy and structure

Two decades have passed, since the discovery of NPY by Tatemoto, Carlquist & Mutt (Tatemoto et al., 1982). This thirty-six amino-acid neuropeptide belongs to a family of structurally related peptides in which pancreatic polypeptide (PP) was the first to be isolated from chicken pancreas (Kimmel et al., 1969). Later, the gut hormone peptide YY (PYY) and the non-mammalian fish pancreatic peptide Y (PY) were added to this family of peptides commonly referred to as the NPY family of peptides. All peptide members are rich in tyrosine residues, are amidated at the C-terminal, and, with the exception of PP, share a high degree of sequence homology across vertebrate species (Larhammar et al., 1993).

The NPY gene is located on chromosome 7 and 4 in humans and rats, respectively, and the corresponding mRNA encodes a prehormone sequence consisting of 97 and 98 amino acids, respectively, in these two species (Minth and Dixon, 1990;Higuchi et al., 1988a;Larhammar et al., 1987;Takeuchi et al., 1986;Minth et al., 1984). The prehormone consists of four elements i.e. an N-terminal hydrophobic signal peptide, NPY, an amidation motif and the C-flanking peptide of NPY (CPON) (Higuchi et al., 1988a). Following the processes of post-translational modifications in the endoplasmic reticulum, NPY and CPON are co-stored in dense core vesicles that are present in dendrites, cell bodies, and axons in the central and peripheral nervous system (Burke et al., 1997;Pickel et al., 1995). In response to action potential activity, the two peptides are presumably co-released, but no biological function has so far been assigned to CPON (Wahlestedt and Reis, 1993;Higuchi et al., 1988a).

The tertiary structure of NPY is shown on the cover of this thesis. Human and rat NPY are identical and sequence analysis of NPY across vertebrate species has revealed a remarkable phylogenetic conservation of this peptide that is only surpassed by somatostatin (Larhammar et al., 1993;Blomqvist et al., 1992). As will be described in the following, NPY is abundantly and widely distributed in mammalian brain and affects a variety of centrally mediated functions of possible relevance to the symptomatology of mood disorders.

Distribution of NPY cell bodies in the central nervous system

Early studies have shown that NPY-IR cell bodies and fibers are found in most parts of the brain and spinal cord and that NPY is one of the most abundant neuropeptides in the CNS. NPY-positive neurons comprise primarily local circuit short-axon interneurons, although long projection neurons in the corpus callosum, stria terminalis, diagonal band of Broca, and various brainstem nuclei suggest that NPY-IR cells also project in long pathways throughout the brain (DeQuidt and Emson, 1986;Allen et al., 1983).

In the CNS, the distribution of NPY-IR neurons in rat and human brain seems to follow a common fundamental pattern with respect to cell locations, cell morphology and axonal innervation (Kohler et al., 1986;Adrian et al., 1983;Allen et al., 1983). The difference, however, is the greater complexity and abundance of the NPY-IR axonal plexuses in the human brain. One prominent feature of the NPY distribution in the brain is the rich occurrence of NPY in forebrain areas. Moderate to large numbers of NPY-IR cell bodies have been detected in the following regions: olfactory bulb, striatum, nucleus accumbens, neocortex: particularly in deep layers IV-VI; the corpus callosum, septum, ventral pallidum, amygdaloid complex, hippocampal formation, thalamus, bed nucleus of the stria terminalis, medial preoptic area, hypothalamus, supramammillary nucleus, inferior coliculus, interpeduncular nucleus, periaqueductal central grey, locus coeruleus, dorsal tegmental area, superior olive, reticular nucleus, medial longitudinal fasciculus, nucleus of the solitary tract, dorsal raphe, spinal nucleus of the trigeminal nerve, all levels of substantia gelatinosa and dorsal grey commissure lumbosacrally (Wahlestedt et al., 1989;DeQuidt and Emson, 1986;Chan-Palay et al., 1985;Everitt et al., 1984;Adrian et al., 1983;Allen et al., 1983). In view of the results presented in this thesis, the distribution of NPY-positive neurons in selected brain regions as well as NPY receptors in the hippocampus will be described in greater detail.

Hippocampal formation

The hippocampal formation consists of the archicortical structures Ammon's horn, dentate gyrus, and the subicular complex. The largest density of NPY-IR is found in the sub-granular zone of the hilus and polymorphous layer of the dentate gyrus, within dentate pyramidal basket cells, polymorphic cells, and spheroid cells (DeQuidt and Emson, 1986;Kohler et al., 1986). No NPY-IR cells are found in the granule cell layer of the dentate gyrus, while few medium-sized cells are found throughout the dentate gyrus molecular layer. The Ammon's horn contains a moderate to low number of medium-sized NPY-positive cells. In the CA1 and CA3 areas, the highest concentration of NPY-IR cells is seen within or adjacent to the pyramidal cell layer, e.g., in strata oriens and radiatum and rarely in the stratum lacunosum-moleculare. The subicular complex contains medium- to large-sized NPY-IR cells in dorsal and ventral parts, though less than the rest of the hippocampal formation (DeQuidt and Emson, 1986;Kohler et al., 1986;Chan-Palay et al., 1986). Double-labeling experiments have shown that NPY-positive neurons in the hippocampal formation frequently co-express GAD (glutamic acid decarboxylase, i.e. GABA) and/or somatostatin (Kohler et al., 1987).

Hypothalamus

The highest concentrations of NPY in the brain are found within hypothalamic nuclei, particularly the PVN, arcuate nucleus, suprachiasmatic nucleus, median eminence, and dorsomedial nucleus (DeQuidt and Emson, 1986;Allen et al., 1983). Except for the suprachiasmatic nucleus, which is innervated by NPY fibers primarily from the intergeniculate leaflet of the thalamus, these nuclei are innervated by NPY fibers originating in the arcuate nucleus and in neurons co-expressing catecholamines in the brain stem (DeQuidt and Emson, 1986;Everitt et al., 1984). Arcuate NPY-positive neurons also project to the PVN, which has a dense plexus of varicose NPY-IR fibers, with the highest density in the parvocellular subdivisions (Li et al., 1998;DeQuidt and Emson, 1986;Bai et al., 1985). Other fibers in the PVN originate in adrenergic (group A1) and noradrenergic nuclei in the brainstem (C1, C2 and C3) (Sawchenko et al., 1985;Everitt et al., 1984). An excessive density of NPY-IR fibers is also seen in the sub-ependymal layers of the median eminence and the infundibular stalk (DeQuidt and Emson, 1986;Allen et al., 1983). Many NPY-positive cells in the hypothalamus express glucocorticoid receptors (Dean and White, 1990;Härfstrand et al., 1989;Rivet et al., 1989).

Neocortex

All areas of neocortex (frontal, somatosensory, occipital and temporal cortex) exhibit a moderate number and a layered distribution of NPY-positive cells with the densest staining seen in layers II-III and IV-VI (DeQuidt and Emson, 1986;Kohler et al., 1986;Chan-Palay et al., 1985;Adrian et al., 1983). To a large extent these cortical, non-pyramidal interneurons co-localize somatostatin and/or GAD (Aoki and Pickel, 1990;Aoki and Pickel, 1989;Vincent et al., 1982), while a sub-population of NPY/somatostatin-positive neurons co-express the tachykinins neurokinin A and substance P (Jones et al., 1988).

Striatum

In the striatum, the highest density of NPY-IR cell bodies and fibers is located in the nucleus accumbens and in the bed nucleus of the stria terminalis area adjacent to the striatum. The majority of these cells are medium-sized, aspiny neurons, which to a large extent co-localize somatostatin, or GABA (Aoki and Pickel, 1990;Aoki and Pickel, 1989;Vincent et al., 1983). Dopaminergic axons, projecting from the substantia nigra, form synapses onto NPY-positive neurons in the striatum (Vuillet et al., 1989). Evidence suggests that the NPY expression in these neurons is under inhibitory dopaminergic control (Gruber and Mathé, 2000;Lindefors et al., 1990).

NPY receptors

The actions of NPY are mediated by receptors belonging to the seven-transmembrane guanosine-nucleotide binding protein (G protein) coupled receptor superfamily (Michel et al., 1998). Five NPY receptors, the Y1, Y2, Y4, Y5 and Y6 (Gregor et al., 1996) receptors have been identified and cloned and are all, except Y6, functional in human and rat brain (Michel et al., 1998;Blomqvist and

Herzog, 1997;Gehlert, 1994). In addition, a putative Y3 receptor has been pharmacologically identified, but not cloned (Higuchi et al., 1988b). Upon binding of ligand, all known NPY receptors couple to pertussis-toxin sensitive G_i/G_o-proteins and inhibit the formation of cAMP. For this reason NPY is considered to be an inhibitory neuromodulator (Michel et al., 1998;Karelson et al., 1995). However, NPY receptors may also elevate intracellular calcium, depending on the specific repertoire of G proteins and effector systems present in any cell type (Herzog et al., 1992).

NPY receptor ligand profiles

The different NPY receptor subtypes show a low degree of primary amino acid sequence identity (Blomqvist and Herzog, 1997). Yet, there are extensive similarities in their binding affinities for the endogenous ligands NPY and the closely related PYY, see table I. All NPY receptors require the amidated peptide to become activated. However, the Y1 receptor binds only the intact 36-amino acid peptides with high affinity, whereas other NPY receptors (Y2, Y3, Y5) bind also C-terminal fragments of NPY and PYY with high affinity. Thus, attempts to assess the physiological significance of NPY receptors or to discriminate between different NPY receptor subtypes are hampered by the lack of specific peptide ligands.

Table I. Summary of characterized NPY receptors in the rat and peptide ligands with high, intermediate and low affinity for the receptors.

Receptor	High-affinity agonists ($IC_{50} < 10^{-9}$ M)	Intermediate-affinity ($IC_{50} < 10^{-7}$ M)	Agonists with no or low affinity ($IC_{50} > 10^{-6}$M)
Y1	NPY, PYY, [Pro^{34}]PYY/NPY, [Leu^{31}Pro^{34}]NPY/PYY	NPY_{2-36}, BIBP3226	hPP, rPP, NPY_{13-36}
Y2	NPY, PYY NPY_{2-36}, NPY_{13-36}		hPP, rPP, [Leu^{31}Pro^{34}]NPY/PYY [Pro^{34}]PYY, BIBP3226
Y3	NPY, NPY_{13-36}	[Pro^{34}]NPY	PYY, PP, [Leu^{31}Pro^{34}]NPY/PYY
Y4	HPP, rPP	PYY, [Pro^{34}]PYY [Leu^{31}Pro^{34}]NPY/PYY	NPY, BIBP3226, C-terminal NPY/PYY fragments
Y5	NPY,PYY, hPP, NPY_{2-36}, NPY_{3-36}, , [Pro^{34}]PYY, [Leu^{31}Pro^{34}]NPY/PYY	NPY_{13-36}	rPP, BIBP3226

hPP = human PP, rPP = rat PP. (Redrobe et al., 1999;Michel et al., 1998;Dumont et al., 1998a;Blomqvist and Herzog, 1997;Grundermar, 1997;Gerald et al., 1996).

Y1

Early studies showed that N-terminal truncation of NPY markedly reduced the biological activity at the denoted Y1 receptor (Wahlestedt et al., 1986). In 1990, a G-protein coupled neuropeptide receptor was cloned from the rat (Eva et al., 1990). After *in vitro* transfection into cell lines, this

receptor was demonstrated to bind NPY, but not shorter C-terminal fragments of NPY, and subsequently identified as the Y1 receptor subtype (Krause et al., 1992). High levels of Y1 mRNA hybridization have been found in the neocortex, the olfactory tubercle, the hippocampal dentate gyrus, several thalamic nuclei and the medial preoptic and arcuate nucleus of the hypothalamus. The amygdala and basal ganglia present the lowest Y1 mRNA levels (Parker and Herzog, 1999;Caberlotto et al., 1998;Larsen et al., 1995;Larsen et al., 1993). Autoradiographic studies using [^{125}I][Leu^{31}Pro^{34}]-PYY and [^{3}H]BIBP3226, a selective non-peptide Y1 receptor antagonist (Rudolf et al., 1994), essentially reveal a similar distribution of Y1-like binding and –mRNA in the rat brain, except at the level of the caudate putamen and the arcuate nucleus where [^{125}I][Leu^{31}Pro^{34}]-PYY binding is present and absent, respectively (Dumont et al., 1996a;Dumont et al., 1996b;Larsen et al., 1995;Larsen et al., 1993;Dumont et al., 1993). The Y1 receptor distribution is species specific and in humans, in contrast to rat, abundant levels of the Y1 receptor are found only in the dentate gyrus of the hippocampus (Dumont et al., 1998b;Jacques et al., 1997).

Y2

The Y2 receptor is the predominant NPY receptor subtype in the human brain (Jacques et al., 1997). In contrast to the Y1 receptor, Y2-like receptors do not require the intact 36-amino acid peptide for binding, but also bind C-terminal fragments of NPY/PYY such as NPY_{13-36} and NPY_{3-36} (Grandt et al., 1996;Colmers et al., 1991;Wahlestedt et al., 1986). Evidence suggests that such fragments are generated endogenously in several species (Batterham et al., 2002;Grandt et al., 1996;Eberlein et al., 1989). In the rat, levels of Y2 receptor mRNA are prevalent in the olfactory tubercle, piriform cortex, the hippocampal formation, the supraoptic and arcuate nucleus of the hypothalamus, while lower levels are generally found in the neocortex, the amygdala, and the thalamus (Parker and Herzog, 1999;Parker and Herzog, 1998). Autoradiographic studies using iodinated NPY_{3-36} and NPY_{13-36} have revealed a similar distribution of Y2-like binding (Dumont et al., 1998b;Dumont et al., 1996b;Dumont et al., 1993). In the CNS, the function of the Y2 receptor has been linked to suppression of transmitter release, for instance at the mossy fiber projecting pathway where the release of glutamate is regulated by this receptor (see page 30). In the human brain, a subpopulation of Y2 receptor mRNA expressing neurons was found to co-express NPY mRNA in several brain regions, indicating that this receptor may act as a prejunctional inhibitory autoreceptor (Caberlotto et al., 2000). These studies indicate that the Y2 receptor, like in the peripheral nervous system at the sympathetic neuroeffector junction, is generally located prejunctionally and mediates inhibition of neurotransmitter release (Wahlestedt et al., 1986).

Y3

The ‘putative Y3 receptor’ is the newest of the established NPY receptor subtypes (Michel et al., 1998). It has not been cloned but merely pharmacologically defined by its characteristically low affinity for PYY, while NPY is a fully efficacious agonist on this receptor in various rat tissue preparations (Michel et al., 1998;Higuchi et al., 1988b). Putative Y3-like binding has been detected

in the rat brainstem, specifically in the nucleus tractus solitarius, where it has been proposed to reduce blood pressure and heart rate (Grundemar et al., 1991a;Grundemar et al., 1991b). The presence of a putative Y3 receptor has also been demonstrated in the hippocampus by means of electrophysiology (Monnet et al., 1990) but not replicated by others (McQuiston and Colmers, 1992).

Y4

The Y4 receptor, has been cloned and classified as the PP-preferring receptor as it has higher affinity for rat and human PP than for NPY (Lundell et al., 1996;Bard et al., 1995). The Y4 receptor is the least abundant NPY receptor in the CNS. Moderate levels of Y4 mRNA are found in specific brain stem nuclei, including the inferior olive and lateral reticular nucleus, while Y4 mRNA is only scarcely expressed in the hippocampal formation, neocortex, thalamic and hypothalamic nuclei and the amygdala (Parker and Herzog, 1999;Parker and Herzog, 1998). In the rat, iodinated PP binding was observed in the medial preoptic area and interpeduncular area, nucleus tractus solitarius, area postrema, and dorsal vagal nucleus while cortical areas and the hippocampus contained negligible levels of labeling (Schober et al., 2000;Trinh et al., 1996).

Y5

The cDNA for the rat and human Y5 receptor subtype was cloned most recently (Gerald et al., 1996;Hu et al., 1996). Intense staining for Y5 mRNA has been found in the olfactory tubercle, piriform cortex, the hippocampal formation and several thalamic nuclei (Parker and Herzog, 1999). Moderate levels of Y5 mRNA are found in the PVN and the supraoptic hypothalamic nuclei, while Y5 mRNA levels are generally low in the neocortex, amygdala and brain-stem areas (Durkin et al., 2000;Parker and Herzog, 1999;Parker and Herzog, 1998). Immunocytochemistry as well as autoradiographic studies using iodinated [$Leu^{31}Pro^{34}$] in the presence of BIBP3226 have described a similar distribution of Y5-like binding sites in rat brain (Bregola et al., 2000;Grove et al., 2000;Dumont et al., 1998a). However, some discrepancy was observed in the hypothalamus and hippocampus, where Y5-like binding sites were less prominent than expected from the distribution of mRNA (Bregola et al., 2000;Grove et al., 2000;Dumont et al., 1998a). In the neocortex, Y5 receptors were localized to somatic and proximal dendritic processes of neurons in layers 2, 3 and 4 (Grove et al., 2000). Interestingly, double-label immunofluorescence experiments have shown that all CRH-expressing neurons and a few GABAergic neurons in these areas co-express Y5-like receptor immunoreactivity (Grove et al., 2000).

Y6

The Y6 gene is expressed in a number of species including mouse, rabbit, dog, cow and chicken, but is completely absent in the rat (Burkhoff et al., 1998). Despite a functional inactivation of the human Y6 receptor due to a frame shift mutation, Y6 receptor transcripts have been detected in

various peripheral organs in humans, but not in the brain (Burkhoff et al., 1998;Matsumoto et al., 1996).

NPY receptors in the hippocampus

In the rat hippocampus, levels of different NPY receptor mRNAs have been quantitatively compared by means of in situ hybridization histochemistry. In the granular cells of the dentate gyrus, Y5 mRNA staining is far more abundant than Y2 mRNA and particularly Y1 mRNA, while Y4 mRNA has the lowest signal (Parker and Herzog, 1999;Parker and Herzog, 1998). In the pyramidal cells throughout the CA1-CA3 areas the same order of prevalence is observed, although levels of Y5, Y2 and Y1 mRNA are more similar (Parker and Herzog, 1999;Parker and Herzog, 1998). Yet these findings may not reflect the levels of functional expressed receptors in the rat. Emulsion receptor autoradiography and receptor immunocytochemistry indicates that the Y1 receptor accounts for 70-80% of the NPY receptor population in the molecular layer of the dentate gyrus and that Y1 and Y2 receptors are equally distributed in the granular layer and the hilus of the dentate gyrus. The Y2 receptor is the most prevalent in the CA3 area (76-82%) and Y5-like binding is expressed mainly in the CA3 area of the ventral hippocampus (St-Pierre et al., 1998;Dumont et al., 1998a;Widdowson et al., 1997;Gobbi et al., 1996;Dumont et al., 1996b;Dumont et al., 1993).

Several studies have investigated the identity of hippocampal NPY receptor presenting cells. Thus, a proportion (20%) of cultured hippocampal astrocytes were shown to express NPY receptors, preferentially of the Y1 receptor subtype (St-Pierre et al., 2000;Hösli and Hösli, 1993a). Astrocytes are known to promote the differentiation and survival of neurons and regulate synaptogenesis during early development (Hösli and Hösli, 1993b). These findings may therefore indicate a neuroproliferative action of NPY acting at Y1 receptors in the hippocampus, in addition to those reported to occur in rodent olfactory epithelium (Hansel et al., 2001). Y1 receptors have also been localized on glutamate as well as NPY-positive neurons in the hippocampus, indicating that this receptor may regulate the release of glutamate and NPY as a hetero and auto-receptor, respectively (St Pierre et al., 2000). However, the Y2 but not the Y1 receptor subtype regulated the release of NPY from NGF-differentiated PC-12 cells (Chen et al., 1997). Furthermore, postmortem studies of human hippocampus suggest that Y2 but not Y1 receptor mRNA is co-expressed in NPY-expressing cells in the hippocampus (Caberlotto et al., 2000).

In the hilus of the dentate gyrus of the rat, the majority of Y5-like receptor binding was localized to GABAergic neurons (Grove et al., 2000). In contrast to Y1 and Y2 receptors, Y5 receptors were not expressed on NPY-positive hippocampal neurons but in close apposition to NPY-IR fibers (Grove et al., 2000). Taken together, these findings suggest that NPY may regulate the hippocampal release of GABA and glutamate via Y5 and Y1 receptors in the hilus of the dentate gyrus, while Y2 receptors may be autoregulatory prejunctional receptors that regulate the release of NPY in the dentate gyrus.

Functional roles of NPY in the central nervous system

Cardiovascular effects

NPY is a potent modulator of autonomic reflexes that are governed by various brain stem nuclei. Central administration of NPY leads to hypotension, bradycardia and decrease in total peripheral resistance in rats (Chen et al., 1990;Härfstrand, 1986). The central effects of NPY are thus in opposition to its peripheral actions where it is co-released with noradrenaline and mediates vasoconstriction (see Zukowska-Grojec, 1995). The hemodynamic actions of central NPY are believed to originate in the nucleus of the solitary tract as they were elicited by microinjection of NPY into this area and abolished in the presence of an NPY antiserum (Tseng et al., 1988). NPY_{13-36} was observed to counteract the vasodepressor effects of NPY and [$Leu^{31}Pro^{34}$]NPY in this area, indicating complementary roles for Y2 and Y1-like receptor subtypes in mediating these actions of NPY (Yang et al., 1993;Aguirre et al., 1990).

A few more words about peripheral NPY are appropriate at this point. Since the activation of the sympathetic system is one of the hallmarks of stress, it is not surprising that adrenal levels of tyrosine hydroxylase and NPY-LI as well as plasma levels of NPY, NA, and cortisol, are elevated in humans and animals following exposure to stress or intense exercise (Morgan et al., 2001;Serova et al., 1998;Zukowska-Grojec, 1995). Interestingly, NPY has been reported to penetrate the blood-brain-barrier (BBB) in a non-satiable manner, indicating that peripheral NPY may enter the brain via simple diffusion across the BBB (Kastin and Akerstrom, 1999). This may underlie the observation of a negative association between plasma levels of NPY and psychological distress in humans exposed to stress, indicating an association between anxiolytic effect and peripheral release of NPY (Morgan et al., 2002).

Anxiety

In several models of fear or anxiety, such as the Geller-Seifter punished responding test (Heilig et al., 1993;Heilig et al., 1992), fear-potentiated startle (Broqua et al., 1995), Vogel's drinking conflict test (Britton et al., 1997;Heilig et al., 1989), elevated plus-maze test (Britton et al., 2000;Nakajima et al., 1998;Broqua et al., 1995), and social interaction test (Sajdyk et al., 1999), the actions of centrally administered NPY are similar to those observed with typical anxiolytic drugs. Correspondingly, NPY-deficient mice show a clear preference for non-central areas in the open field test and for the closed arms in the elevated plus-maze, indicative of an increased level of anxiety in these animals compared to controls (Bannon et al., 2000;Palmiter et al., 1998). Moreover, NPY transgenic animals that overexpress NPY mRNA in the CA1 area, display an insensitivity to stress-induced anxiety as assessed in the elevated plus maze and the punished drinking test (Thorsell et al., 2000). Since infusion of Y1 receptor antisense oligonucleotides into amygdala induces anxiety while Y1 receptor agonists reduces anxiety in rats, it has been suggested that Y1 receptors in the amygdala are involved in the regulation of fear and anxiety (Sajdyk et al., 1999;Britton et al., 1997;Broqua et al., 1995;Wahlestedt et al., 1993;Heilig et al., 1993). In contrast,

Y2 receptors in the amygdala have been implicated in mediating both anxiolytic and anxiogenic effects (Nakajima et al., 1998;Kask et al., 1998)

Alcohol consumption

Genomic screen for genes influencing voluntary alcohol consumption in selectively bred alcohol-preferring rats has outlined a quantitative trait locus on chromosome 4, which includes the NPY gene (Bice et al., 1998). Further studies showed that alcohol-preferring rats had lower levels of NPY in the amygdala, frontal cortex and the hippocampus (Ehlers et al., 1998). Indeed, an inverse relationship between brain NPY levels and ethanol intake appears to exist as NPY-deficient mice have an increased intake of alcohol while NPY overexpressing mice have a lowered ethanol intake, compared to controls (Thiele et al., 1998). However, these findings were not corroborated in another study (Thorsell et al., 2000). The Y1 receptor has been proposed to be involved in this action of NPY (Thiele et al., 2002;Kelley et al., 2001).

Energy balance

Intrahypothalamic administration of NPY stimulates voracious feeding and suppresses energy expenditure in rats, while antagonizing hypothalamic NPY signaling by infusing antibodies against NPY, delays the onset of feeding (Shibasaki et al., 1993;Stanley et al., 1986;Stanley et al., 1985;Levine and Morley, 1984;Clark et al., 1984). Conversely, food-deprivation increases the synthesis of NPY in the PVN of the hypothalamus (Kalra et al., 1991;Brady et al., 1990;Sahu et al., 1988). NPY neurons projecting from the arcuate nucleus to the PVN, the primary integration site for the maintenance of energy balance, express leptin receptors (Håkansson et al., 1996;Gold et al., 1977). The positive effects of NPY on energy balance have been shown to be inversely linked to the activity of leptin, a hormone that is released by adipocytes and suppresses feeding and stimulates metabolism (Thorsell et al., 2002;Håkansson et al., 1996). Most data suggest that Y1 and Y5 receptors are mediating these effects of NPY on energy balance although also the Y2 receptor has recently been implicated (Batterham et al., 2002;Kaga et al., 2001;Campbell et al., 2001;Bannon et al., 2000;Naveilhan et al., 1999;Criscione et al., 1998). However, other mechanisms appear also to be involved since food intake, body weight and adiposity are unaltered in NPY deficient mice, although fasting-induced food intake is significantly reduced (Bannon et al., 2000;Erickson et al., 1996b).

Learning and Memory

Spatial learning depends on the integrity of the hippocampus and the formation of long-term potentiation (LTP) in this region (Eichenbaum and Otto, 1993). Injections of NPY into the hippocampus enhanced memory retention in rats, while intrahippocampal injection of NPY antibodies induced amnesia in rats (Flood et al., 1989). The Y2 receptor has been implicated in mediating these actions of NPY as NPY_{20-36} reversed the amnesia induced by the cholinergic antagonist scopolamine (Flood and Morley, 1989;Flood et al., 1987). On the other hand, NPY was

recently shown to attenuate the formation of long-term potentiation in the rat dentate gyrus (Whittaker et al., 1999). Furthermore, NPY-deficient mice exhibit normal spatial learning in the Morris water maze and in the inhibitory avoidance paradigm, a model designed to measure memory retention (Bannon et al., 2000;Palmiter et al., 1998). NPY transgenic mice also display normal memory retention in the Morris water maze (Thorsell et al., 2000). Thus, the impact of NPY on cognitive behavior likely involves complex mechanisms that remain to be established.

Sexual behavior and reproduction

Central administration of NPY has been shown to inhibit sexual behavior in male and female rats (Clark et al., 1997;Poggioli et al., 1990). This effect has been shown to be caused by a change in motivational behavior rather than sexual function and is therefore believed to reside in the medial preoptic area, which is innervated by NPY fibers projecting from the arcuate nucleus (Clark, 1995;Bai et al., 1985). NPY has been shown to regulate the activity of the hypothalamic-pituitary-gonadal axis and suppress reproduction in virgin female rats (Toufexis et al., 2002). For instance, NPY infusion suppresses leutenizing hormone secretion and disrupts the estrous cycle in female rats (Catzeflis et al., 1993). This effect of NPY appears to be mediated by the Y5 receptor (Toufexis et al., 2002). However, NPY-deficient mice are fertile and appear to reproduce at a normal rate (Erickson et al., 1996b).

Effect on the HPA-axis

The abundance of NPY-positive nerve terminals and NPY receptors in anatomic sites involved in HPA-axis regulations supports a physiological role of endogenous NPY in endocrine function. Indeed, i.c.v. administration of NPY increases plasma levels of ACTH, corticosterone, and aldosterone in rats (Albers et al., 1990;Leibowitz et al., 1988;Wahlestedt et al., 1987;Haas and George, 1987;Härfstrand et al., 1987). These actions of NPY are mainly exerted in the PVN, where NPY fibers originating in the arcuate nucleus make synaptic contacts with CRHergic neurons, and increase secretion of CRH into the hypophysial portal secretion (Liposits et al., 1988;Wahlestedt et al., 1987). The Y5 receptor may be involved in this effect as double label immunofluorescence studies have detected Y5 receptor-IR on a subpopulation of CRHergic neurons in the parvocellular region of the PVN (Campbell et al., 2001). Reports also indicate that NPY may release CRH from the median eminence (Tsagarakis et al., 1989;Haas and George, 1987). The median eminence is moderately innervated by NPY-positive fibers and NPY has been shown to potentiate the effect of CRH on ACTH release from the pituitary (Koenig, 1990;McDonald et al., 1988). Thus, NPY modulates the activity of the HPA-axis both within the hypothalamus by altering CRH neuronal activity and release, and also at the levels of the pituitary corticotrope by enhancing the action of CRH. Many NPY-positive neurons in the hypothalamus express glucocorticoid receptors (Härfstrand et al., 1989), and dexamethasone and adrenalectomy stimulates and reduces NPY synthesis in this area, respectively (Dean and White, 1990;Rivet et al., 1989). These studies indicate that the activity of NPY neurons is under positive control of glucocorticoids.

Excitatory transmission

Application of NPY to hippocampal slices reduces the excitatory spike populations evoked in CA1 pyramidal regions by electrical stimulation and excitatory postsynaptic potentials at the mossy fiber-CA3 and CA3-CA3 synapses within the hippocampus (Klapstein and Colmers, 1993;Bleakman et al., 1992;Colmers et al., 1987). Presynaptic Y2 receptors located on terminals of mossy fibers and Schaffer collaterals are thought to mediate this action of NPY on excitatory transmission by reducing the release of glutamate (Woldbye et al., 1997;Bleakman et al., 1992;Colmers et al., 1991). Furthermore, the absence of this inhibitory signal is believed to underlie the hyperexcitability of NPY-deficient mice. In contrast, during standard recording conditions, no clear electrophysiological deficits are observed in hippocampal slices from NPY-deficient mice (Baraban et al., 1997). Furthermore, application of NPY produces similar levels of inhibition in NPY-deficient mice and littermate controls (Baraban et al., 1997).

Seizure activity

Accumulating evidence suggests a critical antiepileptic role of NPY in the brain. For instance, NPY-deficient mice develop spontaneous seizures, are hyperexcitable, and display aggravated seizures when challenged with convulsive agents such as penetylenetetrazole (PTZ) (Erickson et al., 1996b). In addition, kainic acid induces fatal seizures in the NPY-deficient mice (Palmiter et al., 1998). However, when NPY is administered i.c.v. prior to kainic acid, lethality is prevented (Palmiter et al., 1998).

Furthermore, inbred seizure-prone animals have increased levels of NPY in the hippocampus and entorhinal cortex (Jinde et al., 1999;Takahashi et al., 1997;Sadamatsu et al., 1995) while NPY knockdown mice display an increased vulnerability to seizures (DePrato Primeaux et al., 2000;Baraban et al., 1997;Erickson et al., 1996b). Lastly, transgenic animals that overexpress NPY mRNA in CA1 pyramidal neurons, are more resistant to the development of kainic acid-induced seizures and display an impaired kindling-induced epileptogenesis (Vezzani et al., 2002).

In normal rats, administration of NPY reduces epileptiform afterdischarges and time spent in experimentally elicited seizures, and also retards kindling acquisition (Reibel et al., 2000a;Woldbye, 1998;Woldbye et al., 1997;Woldbye et al., 1996a). Several NPY receptor subtypes may convey this anticonvulsive role of NPY since blocking Y1 receptors or stimulating Y2 and possibly Y5 receptors, inhibits seizure activity (Vezzani et al., 2000a;Vezzani et al., 2000b;Gariboldi et al., 1998;Woldbye et al., 1997).

NPY and mood disorders: experimental findings

Flinders Sensitive Line rats

A number of studies have investigated the integrity of the NPY system in the FSL and FRL rats. In one study, levels of NPY mRNA were lower in the hippocampus proper and nucleus accumbens,

but higher in the arcuate nucleus of FSL compared to FRL rats (Caberlotto et al., 1998). Further, hippocampal levels of NPY-LI are reduced in FSL compared to FRL (Husum et al., 2001;Jiménez Vasquez et al., 2000a;Jiménez Vasquez et al., 2000b). Y1 receptor mRNA levels were lower in the dentate gyrus, occipital and anterior cingulate cortex, and retrosplenial cortex, while levels of Y1-like binding in the hippocampus appeared to be higher in FSL animals (Caberlotto et al., 1999;Caberlotto et al., 1998). In contrast, no strain differences in Y2 receptor mRNA or Y2-like binding levels have been observed among FSL and FRL animals (Caberlotto et al., 1999;Caberlotto et al., 1998). It thus appears that abnormalities in the biosynthesis of NPY and Y1 receptors may underlie aspects of their characterized 'depressed' phenotype.

Early life stress

In a single study, maternal deprivation for six hours daily on postnatal days 2-6 and 9-13 was shown to reduce NPY-LI in the hippocampus and increase NPY-LI in the hypothalamus of male and female rats (Jiménez Vasquez et al., 2001). Stress-induced depression-like symptoms resulting from uncontrollable shocks alters basal NPY mRNA levels in the hippocampus (Lachman et al., 1992). In addition, subjecting adult animals to acute or chronic stress has been shown to increase levels of NPY mRNA in the arcuate nucleus of the hypothalamus and modulate NPY mRNA levels in the amygdala (Conrad and McEwen, 2000;Makino et al., 2000a;Krukoff et al., 1999;Thorsell et al., 1999;Thorsell et al., 1998). These studies imply that discrete changes in brain NPY occur in response to the recruitment of distinct stress mechanisms, which depend on the onset, duration, severity and type of the applied stress.

Kindling

Kindling induces morphological and biochemical changes in hippocampal neurons. In the hilus of the dentate gyrus these changes are particularly apparent and associated with cell loss (Schwarzer et al., 1996;Vezzani et al., 1996;Schwarzer et al., 1995;Rizzi et al., 1993). The progression of kindling epileptogenesis has been observed to increase levels of NPY mRNA and -IR in the polymorphic cell layer of the dentate gyrus and in sprouting mossy fibers (Vezzani et al., 1996;Schwarzer et al., 1995). In animals subjected to repeated perforant path stimulation, another experimental model of epilepsy, a selective loss of NPY neurons in the dentate hilus was also observed (Sloviter, 1987). Together, these results resemble findings on tissue resected from temporal lobe epilepsy patients, which have demonstrated that especially NPY-positive interneurons located in the subgranular polymorphic layer of the hilus are lost (Sundstrom et al., 2001;Mathérn et al., 1995;de Lanerolle et al., 1989). Further, remaining hilar neurons increase their NPY synthesis, which positively correlates with BDNF levels, and undergo extensive sprouting thus forming aberrant circuits onto dentate granule cells and their dendrites in the molecular layer (Furtinger et al., 2001;Takahashi et al., 1999;Mathérn et al., 1995;de Lanerolle et al., 1989). These changes in NPY are thought to increase the excitability of the hippocampal circuit as NPY has been shown to reduce the glutamatergic perforant path-evoked synaptic response in humans and animals (Patrylo et al.,

1999;Greber et al., 1994;Klapstein and Colmers, 1993;Colmers et al., 1991;Sloviter, 1991;Sloviter, 1987). In view of the observations of lowered hippocampal NPY in animal models of depression and reduced CSF levels of NPY in depressed patients, this change may also be of significance for the frequently occurring symptoms of depression in epilepsy patients.

Electroconvulsive therapy and NPY

Electroconvulsive therapy (ECT) is an effective treatment primarily for major depression and both phases of bipolar disorders, and may also be used as maintenance therapy to prevent relapse (Rabheru and Persad, 1997;Small et al., 1991). ECT increases levels of NPY-LI in cerebrospinal fluid in depressed patients and in patients with Parkinson's disease (Mathé et al., 1996;Fall et al., 1995). Repeated electroconvulsive stimulations (ECS), an experimental model of ECT, appear to increase the synthesis of NPY in the brain. Thus, levels of NPY mRNA and the number of NPY-expressing neurons are increased in the dentate gyrus of the hippocampus (Woldbye et al., 1996b;Zachrisson et al., 1995b;Kragh et al., 1994;Mikkelsen et al., 1994). Further, levels of NPY-LI in hippocampal homogenate are increased in normal and 'depressed' rats following repeated ECS (Jiménez Vasquez et al., 2000a;Mathé et al., 1998;Mathé et al., 1997;Stenfors et al., 1995;Stenfors et al., 1994;Stenfors et al., 1992;Wahlestedt et al., 1990;Stenfors et al., 1989;Detera-Wadleigh et al., 1987). The functional significance of these changes in terms of altered release is unknown. However, reduced hippocampal levels of NPY-sensitive binding have been suggested to reflect a compensatory downregulation of NPY receptors in response to an increased release of NPY from hippocampal terminals following repeated ECS (Madsen et al., 2000;Greisen et al., 1997).

Antidepressant drugs and NPY

Antidepressant drugs, i.e. tricyclic antidepressants, selective serotonin and noradrenaline re-uptake inhibitors and monoamine oxidase inhibitors, alleviate depression symptoms. These drugs have also been shown to affect NPY systems in rat brain, although the results are not consistent. Citalopram and desmethylimipramine did not affect brain NPY-LI in normal rats following 6 weeks of treatment (Heilig and Ekman, 1995). However, fluoxetine and desipramine were observed to further increase elevated levels of NPY-LI and NPY mRNA in the arcuate nucleus of FSL and chronically stressed rats, respectively (Makino et al., 2000b;Caberlotto et al., 1999). Studies have shown that chronic desipramine and imipramine increased NPY-LI concentration in the hippocampus, and striatum and reduced ^{3}H-NPY binding in the frontal cortex, hippocampus and striatum (Widdowson and Halaris, 1991;Widdowson and Halaris, 1989). However, a previous report failed to detect any change in NPY-sensitive binding in the cerebral cortex of imipramine-treated rats (Cummins et al., 1987). Clorgyline and pargyline have also been shown to increase NPY-LI in the hippocampus and nucleus accumbens in rats (Widdowson and Halaris, 1989).

AIMS

The central aims of this thesis were to investigate

- The effects of early maternal deprivation, a model of early life stress, on NPY-LI in adult rat brain.
- The effects of mood stabilizing agents on NPY synthesis, release and NPY receptor-like binding in three rat models reflecting aspects of mood disorders: early life stress (maternal deprivation), genetic predisposition (FSL), and relapse/sensitization (amygdala-kindling).

MATERIALS AND METHODS

Animals

The maintenance and use of animals met the guidelines of the Danish Committee on Care and Use of Laboratory animals (Papers I and VI) and Stockholm's Ethical Committee for Protection of Animals (Papers II-V) and were conducted in accordance with the Karolinska Institutet's guidelines for the Care and Use of Laboratory animals.

Sprague-Dawley (Papers I, II, and IV) or male Wistar rats (Papers III and VI) were supplied from Møllegård, Denmark or B & K Universal, Sollentuna, Sweden. Unless stated otherwise, the animals were housed 4-6 animals per cage and kept at room temperatures of 21-23°C in a 12h light/dark cycle (lights on at 06:00) with free access to chow and tap water *ad libitum.* Purchased animals were allowed 4-7 days of adaptation in the animal facilities prior to the initiation of experiments.

Flinders sensitive line rat

Paper V was generated by the use of male FSL and FRL rats selected from the rat colonies maintained at the animal facility at the Karolinska Institutet.

Maternal deprivation

The effects of repeated episodes of maternal deprivation were investigated in paper I, and on saccharin preference (described on page **Fel! Bokmärket är inte definierat.**). For those experiments, timed-pregnant Sprague-Dawley rats (B & K Universal, Sollentuna, Sweden) arrived at the animal facility on gestational day 12. The dams were checked for delivery once daily (08:00). Day of delivery was designated 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. Groups of non-handled pups (NH) were left undisturbed until PND 23 when all pups were weaned. From this day onwards, the rats were kept 5 per cage.

The effects of a single episode of maternal deprivation were investigated in paper III. For this experiment, male and nulliparous female Wistar rats of 3 months of age were caged together in pairs at the animal facility at University of Nijmegen. Two weeks later the males were removed and the females were checked for delivery twice daily (08:00 and 17:00). On PND 1, the litters were weighed and culled to 6 males and 2 females. On PND 9 half of the litters were subjected to maternal deprivation for 24 h while the other half were briefly removed from their home cages on both PND 9 and 10 (Ellenbroek et al., 2000). The pups were weaned on PND 21. At this point, the rats were housed 3 per cage in standard Macrolon cages with either sawdust or with a grid floor, a characterized mild stress paradigm (Weiss et al., 1999).

During both separation procedures the rat pups were placed in a neonatal incubator to prevent metabolic changes caused by change in body temperature.

Amygdala-kindling

Rats were anaesthetized with hypnorm:dormicum:water (1:1:2, 2 ml/kg s.c.) and mounted in a stereotaxic frame with the incisor bar set at –2 mm (Paper VI). A tripolar stainless steel electrode (Bilaney Consultants, Germany) was implanted into the basolateral amygdaloid nucleus, aimed at the following coordinates relative to Bregma: AP: -2.8 mm; ML: -4.8 mm; DV: -8.4 mm (Paxinos and Watson, 1986). Following 10 days of recovery, the afterdischarge thresholds (ADT) were individually determined by increasing the stimulus current from 75 µA, by 25 µA at 1 min intervals until an afterdischarge had been elicited. Animals with an ADT above 500 µA were discarded from the study. On the following day levetiracetam (20 mg/kg, i.p. per day) or vehicle treatments (0.9% NaCl, 5 ml/kg) were initiated. The rats were subsequently stimulated with the ADT stimulus 1 h after drug administration three times a week (Monday, Wednesday, Friday). The seizure severity was evaluated according to the Racine classification scale (Racine, 1972). In addition, AD duration and duration of tonic-clonic seizures were assessed. One hour following the 10^{th} kindling-stimulation (=partially kindled), one random half of the vehicle and levetiracetam treated animals were decapitated. The remaining vehicle- and levetiracetam-treated animals were decapitated one hour following the 25^{th} kindling-stimulation (=fully kindled). At each time-point, trunk-blood was collected in heparin-coated tubes and the brains were rapidly removed into isopentane (-40ºC) for 30 sec, and then transferred to the -80ºC freezer until analyses.

Treatments

ECS was used as an experimental model of ECT. Under brief halothane-anesthesia, Sprague-Dawley rats (Møllegård, Denmark) were given trans-auricular electrostimulations of unidirectional, square wave pulses of 50 mA, 0.5 s, 50 Hz once daily for 10 days. On the day following the last ECS, histochemical and microdialysis experiments were initiated (Paper I). In paper IV, a single ECS was delivered via ear clips by a Grass stimulator (150 V, 50 Hz, 20 m sec stim 1 sec) to Sprague-Dawley rats (B & K, Sollentuna, Sweden) undergoing microdialysis, in order to assess its effect on the release of NPY-LI.

Citalopram, a selective serotonin re-uptake inhibitor (SSRI), is indicated for the treatment of depression, anxiety and panic disorders. In paper I, citalopram (10 mg/kg i.p.) or vehicle (0.9% NaCl, 5 ml/kg i.p.) was administered to adult male Sprague-Dawley rats (Møllegård, Denmark) once daily for 28 days. Histochemical brain analyses and microdialysis experiments were commenced in the morning following the last day of treatment.

Lithium was administered via the diet to adult Sprague-Dawley rats (Paper I: Møllegård, Lille-Skensved, Denmark; Paper II: derived from pregnant Sprague-Dawley dams from B & K,

Sollentuna, Sweden). Concentrations of lithium in the diet were around 25-35 mEq for the first week and 50-60 mEq for the following 3 weeks. Throughout the treatment period, the animals had free access to NaCl in the form of either salt-stone or 0.9% NaCl. Serum levels of lithium were between 0.50 and 1.15 mM, which is within the therapeutic concentration range (Manji et al., 1995). In respective studies, microdialysis experiments, RIA, and histochemical experiments were commenced without discontinuing the lithium treatment.

Topiramate is an antiepileptic drug and is a sulfamate-substituted fructose derivative that is currently approved for the treatment of epileptic disorders (Shank et al., 2000). In paper V, topiramate (40 mg/kg i.p.) or vehicle (0.9% NaCl with added 3 mM Na_2HPO_4/NaH_2PO_4, pH 7.0, 6.7 ml/kg) was administered once daily to adult male FSL and FRL rats. This treatment regimen yields plasma concentrations of topiramate of 44.05 ± 9.57 μM that are within the anticonvulsant range of concentration (Reissmuller et al., 2000). Due to practical limitations in breeding capacity, two experimental protocols were run separately: (1) a single injection, (2) a series of ten injections. Two hours following the last injection, animals were decapitated, brains were quickly removed and on ice dissected into frontal cortex, occipital cortex, hippocampus, striatum, and hypothalamus and stored at -80°C until RIA analysis.

Levetiracetam is an anticonvulsant drug with antiepileptogenic properties that is currently indicated for the treatment of epilepsy. In paper VI, levetiracetam (20 mg/kg i.p.) or vehicle (0.9% NaCl, 5 ml/kg) were administered once daily for 28 days in naïve Wistar rats (Møllegård, Lille Skensved Denmark) or 1 hr prior to an electrical stimulation in Wistar rats undergoing Amygdala-kindling. Naïve rats, partially kindled rats (10 stimulations) and fully kindled rats (25 stimulations) were decapitated two hours following the last injection of drug. The brains were rapidly removed and transferred to –80°C until histochemical analysis. Plasma concentrations of levetiracetam were 42.49 ± 5.5 μg/ml, which is within the anticonvulsant range of concentration (Loscher et al., 1996).

BIBP3226 (N-2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl-D-arginamide]) is a selective non-peptide Y1 receptor antagonist with very low affinity for the Y5 receptor (Rudolf et al., 1994). In paper IV, BIBP3226 was locally perfused via a microdialysis probe into the dorsal hippocampus of adult Sprague-Dawley rats (B & K, Sollentuna, Sweden). The concentration of 10 μM BIBP3226 in the perfusion medium was chosen based on a presumed 10% passage of drug through the microdialysis probe. The effects of BIBP3226 on basal and ECS-induced outflow of NPY were assessed.

Histochemical analysis

The animals were sacrificed by decapitation. The brains were rapidly removed and stored at -80°C until further processing. Later, coronal sections were cut through the hippocampus from –3.30 – -3.50 mm relative to Bregma (Paxinos and Watson, 1986). Four consecutive sections were thaw-mounted on each glass-slide, which were dried on a hotplate and then stored at –80 °C.

In situ hybridization histochemistry:

Slides were defrosted, fixed in 4% formaldehyde in PBS (0.13 M NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4), dehydrated in graded series of ethanol and air-dried. Oligonucleotides (DNA technology ApS, Aarhus, Denmark) complimentary to parts of the exonic sequences of CPON and BDNF were labeled at the 3'end with [α^{35}S]dATP (>3000 Ci/mmol, Amersham, Denmark) using terminal deoxynucleotidyl transferase (Boehringer Mannheim, Denmark). The labeled probes were added to give a specific activity of 3.0 x 10^5 cpm/100µl to the hybridization buffer. 100 µL hybridization mixture was added onto each slide. The slides were cover-slipped and placed in a humidified chamber at 42°C over-night. Finally, the slides were washed in SSC, dehydrated in a graded series of ethanol and air-dried. The slides were exposed to Hyperfilm β-max with a ^{14}C-microscale (both Amersham) for 3 weeks and then developed in Kodak D19 film developer. Levels of preproNPY and BDNF mRNA in the CA1, CA3 and dentate gyrus, represented by optical densities, were measured using a computer image analysis system (Image-Pro® Plus, Media Cybernetics, Silver Spring, Maryland).

NPY receptor autoradiography:

Slides were defrosted and pre-incubated for 20 min in HEPES-buffer, pH 7.4. The slides were incubated for 60 min in HEPES-buffer + 0.1 nM [^{125}I][Tyr^{36}] mono-iodo-PYY (4000 Ci/mmol, Amersham, Denmark) (total binding) *or* buffer A + 0.1 nM [^{125}I][Tyr^{36}] mono-iodo-PYY *and* 1 µM NPY (non-specific binding, Bachem, Bubendorf, Switzerland) *or* 1 µM NPY_{13-36} (Bachem) *or* 1 µM BIBP3226 (Amersham) *or* 1 µM ^{31}Leu^{34}Pro-NPY (Bachem) *or* 1 µM NPY_{13-36} (Bachem). Subsequently, the slides were washed in HEPES-buffer 2 x 30 min and dried under a stream of cool air. The slides were exposed to Hyperfilm β-max for 4 days with ^{125}I microscales (both Amersham) and then developed in Kodak D19 developer. ^{125}I-PYY binding, represented by optical densities, was measured in the dentate gyrus, CA1, and CA3 areas of the hippocampus, using the computer analysis system listed above.

Microdialysis

The rats were anesthetized with pentobarbital (60 mg/kg i.p.) and mounted in a stereotaxic frame with the incisor bar set at –2mm. A microdialysis probe (Paper I: CMA/12, CMA, Sweden, Paper II: MAB-2, Metalant, Sweden; both 2 mm membrane) was implanted into the dorsal hippocampus aimed at the following coordinates: AP: -4.3 mm, ML: -2.6 mm relative to bregma and DV: -4.2 mm relative to the dura (Paxinos and Watson, 1986). The position of the probe was secured with micro scull screws and dental cement. After surgery the animals were singly housed and allowed a two-day recovery. On the day of the experiment, the rat was placed in a bowl-shaped cage and the implanted microdialysis probe was connected to a microinfusion pump (CMA, Stockholm, Sweden). In paper I the microdialysis probe was perfused at a flow rate of 0.85 µl/min with an artificial cerebrospinal fluid (aCSF: 149 mM NaCl, 2.8 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$,

0.25 mM ascorbic acid, 5.4 mM glucose, 1% bovine serum albumine, pH 6). 2 h fractions of extracellular NPY-LI levels were collected. In paper IV, the microdialysis probe was perfused at a flow rate of 3 µL/min with aCSF (1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 3 mM KCl, 147 mM NaCl, 1.13 mM NaH_2PO_4, 0.2% egg-albumin and 0.03% bacitracin, pH 7.2) to which various substances were added depending on the type of experiment: calcium-free aCSF (as aCSF with 1 mM EGTA added and $CaCl_2$ omitted), high-potassium aCSF (as aCSF with 60 mM KCl, KCl substituted isosmotically for NaCl, with or without 1.3 mM $CaCl_2$), perfusion with 10 µM BIBP3226 in aCSF. All microdialysis fractions were immediately stored at –20ºC and later transferred to -80ºC until analysis. At the end of the experiment, the animals were given a lethal injection of pentobarbital and the brains were taken out for later probe placement verification by cryostat sectioning. Only animals with a correctly placed probe were included in the experiment.

Peptide extraction

The frozen brain tissue samples were homogenized using a metal rod tissue homogenizer for 30 sec in 2-3 ml 1 M acetic acid, ultrasonicated (10 min) and boiled for 10 min in a heating block. After centrifugation (1600 x *g*, 30 min, 4ºC, the supernatants were isolated and pellets reconstituted in deiodinated H_2O. The samples were then subjected to identical sonication, boiling and centrifugation. Supernatants were again isolated and pooled with supernatants from the first extraction. Following lyophilization (over-night) the samples were reconstituted in phosphate buffer (0.05M, pH 7.4) and stored at –20ºC.

Radioimmunoassay

RIA was performed using a sensitive NPY-antibody, a generous gift from Drs. M. Heilig & R. Ekman. This antibody does not cross-react with pancreatic polypeptide or peptide YY. It cross-reacts 100% with NPY, NPY_{2-36}, 5% with NPY_{5-36} and 0.5% or less with shorter C-terminal NPY fragments (Heilig and Ekman, 1995). All microdialysate fractions within an experiment were analyzed as singletons in the same RIA run. Brain microdialysates and standard samples were pre-incubated with antibody for 48 h at 4°C. After addition of Bolton-Hunter labeled ^{125}I-NPY (~6000 cpm pr assay tube, Amersham, Bucks, U.K.), all samples were incubated for additional 24 h at 4°C. Free radioligand was separated from antibody-bound radioligand by addition of a sheep anti-rabbit antibody-coated Sepharose suspension (Pharmacia & Upjohn, Uppsala, Sweden). After 30 min of incubation at room temperature and centrifugation for 20 min at 1.600 x *g*, 4°C, the supernatant was aspirated and discarded. The radioactivity in the pellets containing the bound fraction was measured in a γ-counter. The lower detection limit of the assay was 0.45 pmol/L and the intra assay coefficient of variation was 5%.

The concentrations of NPY-LI were analyzed using Bolton-Hunter labeled ^{125}I-NPY (4000 Ci/mmol, Amersham, Sweden) and NPY antiserum (a generous gift from Drs. R. Ekman and M.

Heilig). This antibody shows no cross-reactivity 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). Samples were analyzed for peptide-LI in triplicates or singletons (microdialysates) and all samples from one brain region were assayed in the same RIA run. The lower detection limit for the RIA was 0.45 pmol/L, and the intra-assay coefficient of variation was 5-7%.

Behavioral tests

Forced swim

The forced swim test is selective for antidepressant action of drugs (Porsolt et al., 1978). Animals were anesthetized and intracerebrally cannulated with patent guide cannulae aimed at the dorsal part of the right lateral ventricle. For two weeks the animals were habituated on a daily basis in order to minimize stress on the day of i.c.v. injections. On the first day of the test, all animals were placed for 15 min in a plexiglass cylinder (Height: 45 cm; Diameter: 19 cm) containing water (25°C) at a depth of 20 cm. One hour after the pre-swim the animals were injected i.c.v. with NPY (1.5, 3.0 or 6.0 µg) or vehicle (aCSF: 5µl). On the following day, the animals were again placed in the cylinders following treatments, which had been applied 5 h or 15 min before the swim. The 5 min swim was video recorded and an observer blind to the treatment of the animals later scored the immobility of the animals.

Prepulse inhibition

In all mammals, the presentation of a loud stimulus usually elicits a startle response including a high amplitude blink. Prepulse inhibition (PPI) is defined as the decrease in the magnitude of the startle response produced by the prior presentation of a weak stimulus. The PPI has been used as an operational model to assess the suppression of a motor response that is normally elicited by a sensory stimulus. Disruption of PPI is considered to model attention impairments, cognitive deficits such as distractibility, and thought disorders, all with relevance to various psychiatric disorders (Karper et al., 1996;Braff and Geyer, 1990).

In paper III, the adult rats were individually placed in the startle chamber consisting of a plexiglas tube (diameter 8.2 cm, length 25 cm) mounted on a plastic frame and installed in a sound attenuated chamber. The rats were allowed to habituate for a period of five min during which a background white noise was present. Following this period the rats received 10 startle trials, 10 no-stimulus trials, and 30 prepulse inhibition trials. The movements of the rat in response to the stimuli were recorded by means of a piezoelectric accelerometer, which recorded and transduced the motion of the tube into a computer. The movement of the rat during the no-stimulus trial represents a control trial for detecting differences in overall activity.

Saccharin preference

Reduced consumption of and preference for saccharin solution in rats is interpreted in terms of a state of anhedonia that is reversible by antidepressant treatment (Willner et al., 1987).

Male pups from Sprague-Dawley dams (B & K Universal, Sollentuna, Sweden) were subjected to repeated maternal separation during PND 2-14 (MS180), control handling (MS15) or left undisturbed (NH) in complete following of the procedures described on page 34. For this experiment, however, male animals were kept two per cage following weaning. The number of cages in the study were: NH: n=15; MS15: n=16; MS180: n=16. Saccharin preference experiments were commenced on PND 55 and conducted on three occasions with one-week intervals. Regular drinking bottles were filled with 200 ml of freshly prepared 0.02% saccharin in tap water or regular tap water, capped, and then weighed. In the afternoon (15:00), saccharin and a tap water bottle replaced the normal drinking bottle in each cage. The locations of the bottles were randomly varied. The next day the bottles were removed and weighed and regular drinking bottles were returned to the cages. The saccharin preference for each cage was computed as weight of drunk saccharin solution/weight of drunk tap water * 100. The results are presented in the results section on page 41.

Statistical analyses

Individual sets of data were first analyzed for normality. In case of a non-Gaussian distribution of data, non-parametric statistical analysis was employed (Paper I). Otherwise, multi-factor analysis of variance was used (Papers II-VI). For the evaluation of behavioral changes in prepulse inhibition (Paper II), kindling model (Paper VI), and saccharin preference and changes in extracellular levels of NPY-LI in the second microdialysis experiment (Paper IV), repeated measure analysis of variance was employed. Details regarding use of specific statistical tests and multiple comparisons are described in the individual papers.

RESULTS AND DISCUSSION

Effects of maternal deprivation on rat brain NPY

Experiences of early adverse life events are over-represented among adult depressed patients compared to healthy subjects (Gladstone et al., 1999;McCauley et al., 1997;Boudewyn and Liem, 1995;Bifulco et al., 1991). Such experiences include incidents of childhood separation caused by parental divorce, illness, or death (Agid et al., 1999;Kendler et al., 1992;Tennant et al., 1982). Studies with non-human primates show that maternal deprivation or inadequate attention from a care-giver during infancy leads to changes in HPA-axis activity/reactivity and behavioral characteristics, which resemble symptoms of human depression (Suomi, 1997;Hinde et al., 1978). In rodents, separation-induced hypersecretion of CRH from the median eminence, possibly due to impaired negative feed-back mechanisms (Penke et al., 2001;Ladd et al., 1996;Rots et al., 1996;Sutanto et al., 1996;Plotsky and Meaney, 1993;Pihoker et al., 1993) is associated with an increased level of anxiety and alcohol preference, and sexual dysfunction compared to control-reared animals (Penke et al., 2001;Plotsky et al., 1995).

Since clinical and experimental evidence suggesting a role for NPY in the pathophysiology of mood disorders is accumulating, the effects of maternal deprivation on rat NPY were investigated as one of two central aims of this thesis.

In paper II, brain levels of NPY-LI were assessed in adult male rats that had been separated from the dams for 180 min per day (MS180) or control-handled during PND 2-14. Marked reductions in levels of NPY-LI were observed in the hippocampus and the striatum while hypothalamic levels of NPY-LI were increased. In addition, a single period of maternal deprivation for 24 h on PND 9 also reduced NPY-LI levels and was associated with disruption of pre-pulse inhibition (Paper III). Further, in contrast to the effect of 24 h maternal deprivation, the chronic stress associated with rearing the rats on a grid floor from weaning had no effect on hippocampal NPY-LI in either maternally deprived or control-handled rats, but did increase NPY-LI in the frontal cortex. These findings suggest that discrete changes in brain NPY reflect the recruitment of distinct stress mechanisms depending on the character of the applied stress. One other study has recently found similar changes in the hippocampus and hypothalamus of male and female rats that were maternally separated for 6 h daily during PND 2-6 and 9-13 (Jiménez Vasquez et al., 2001). These results imply that particularly hippocampal NPY-LI is affected by early life stress/maternal deprivation. In view of previous results with genetic and behavioral animal models of depression, this finding contributes to our hypothesis that a reduction in hippocampal NPY is a common trait for animal models of depression (Paper V;Husum et al., 2001;Jiménez Vasquez et al., 2000a;Mathé et al., 1998;Caberlotto et al., 1998;Criscione et al., 1998)

The functional consequences of the observed changes in brain NPY were both directly and indirectly addressed in the present thesis. In paper I, i.c.v. administration of NPY was shown to

reduce immobility in normal rats, in a manner comparable to that of imipramine. This study indicates that NPY may possess an antidepressant potential and has since been replicated by other groups (Redrobe et al., 2002;Stogner and Holmes, 2000). In an additional study, using identical experimental procedures as those employed in paper II, maternal deprivation reduced the preference for saccharin (Fig 1), indicating that a reduction in hippocampal and striatal NPY may be associated with a state of 'anhedonia'. In addition, lowering of hippocampal NPY was associated with a reduction in basal startle amplitude and disruption in pre-pulse inhibition (Paper III). Disruption of PPI is considered to model attention impairments, cognitive deficits such as distractibility, and thought disorders, all with relevance to various psychiatric disorders (Karper et al., 1996;Braff and Geyer, 1990). It has been shown that 'early' neonatal lesions of the ventral hippocampus lead to a delayed emergence of deficient PPI in adult animals (Lipska et al., 1995). This finding is in line with neurodevelopmental theories purporting that early life insult can lead to adult psychopathology. Studies suggest that hippocampal degeneration may be more pronounced in individuals with an enhanced HPA axis reactivity, entailing an increased cumulative exposure to glucocorticoids (Sapolsky, 2000;Sapolsky, 1994;Gould et al., 1992;Sapolsky et al., 1984;Landfield et al., 1981). In rats, rearing-induced hyperactivity of the HPA-axis and CRH administration early in life was associated with reduced NMDA receptor and BDNF levels and an exaggerated age-related loss of glucocorticoid receptor-expressing neurons in the CA1 and CA3 areas in the hippocampus (Roceri et al., 2002;Brunson et al., 2001;Liu et al., 2000;Meaney et al., 1988). It is not known if hippocampal NPY neurons, like hypothalamic NPY neurons, express glucocorticoid receptors and, as such, could be vulnerable to excessive glucocorticoid exposure (Dean and White, 1990;Härfstrand et al., 1989;Rivet et al., 1989). However, BDNF has been shown to regulate the synthesis of NPY in vitro and in vivo (Croll et al., 1994;Reibel et al., 2000b). The observed reductions in hippocampal NPY-LI following maternal deprivation could therefore be secondary to a lowering of BDNF following glucocorticoid/HPA-axis hyperactivity. An inverse relationship has been proposed to exist between hippocampal NPY, and anxiety, and alcohol intake (Thorsell et al., 2000;Thiele et al., 1998;Ehlers et al., 1998). Consequently, in addition to anhedonia and disrupted PPI, the observed reductions in hippocampal NPY-LI levels may ultimately be of relevance to increased anxiety behavior and alcohol preference that has been observed following maternal deprivation (Penke et al., 2001;Plotsky et al., 1995).

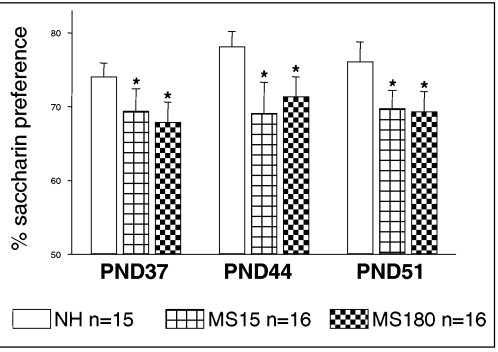

Fig 1. Rats were subjected to maternal deprivation for 180 min/day (MS180), 15 min/day (MS15) or no handling (NH) during postnatal days 2-14. Following weaning, the rats were kept two per cage. In early adolescence, the baseline preference for 0.02% saccharin over regular tap water was assessed on three occasions with a one-week interval. Both MS15 and MS180 groups had a lowered saccharin preference compared to NH-controls (* $P < 0.05$, two-way repeated measure ANOVA followed by Student-Newman-Keul's for multiple comparisons). n-values refer to number of cages.

Effects of mood stabilizing agents on rat brain NPY

If a dysfunctional NPY system in the brain is associated with the pathophysiology of depression aspects of mood disorders, it might be expected that agents that prevent the occurrence of depression symptoms may stimulate NPY neurotransmission in brain. Mood stabilizing agents, i.e. lithium and a number of antiepileptic drugs, are effective in the prophylactic treatment of mood disorders. Previous reports have shown that NPY gene transcription and NPY-LI tissue concentrations are increased following chronic lithium treatment or repeated ECS in normal rats (Husum et al., 2001;Jiménez Vasquez et al., 2000a;Mathé et al., 1998;Mathé et al., 1997;Stenfors et al., 1995;Zachrisson et al., 1995a;Stenfors et al., 1994;Mathé et al., 1994;Weiner et al., 1992;Stenfors et al., 1992;Wahlestedt et al., 1990;Mathé et al., 1990;Stenfors et al., 1989). In paper I these findings were replicated. In addition, it was demonstrated that both chronic dietary lithium and repeated ECS markedly increase the release of NPY in the dorsal hippocampus of freely moving 'normal' Sprague-Dawley rats. In paper IV it was shown that also a single ECS increases release of NPY in the dorsal hippocampus, and that it is possible by means of microdialysis to study the calcium-dependent release of NPY-LI from functional neurons. Furthermore, NPY-sensitive binding sites were lowered following chronic lithium treatment and repeated ECS, probably reflecting a compensatory downregulation of NPY receptors in response to an increased release of NPY. In contrast, citalopram increased NPY-sensitive binding in the hippocampus but had no effect on NPY gene-transcription or extracellular levels of NPY-LI, implying an increased stimulation of NPY-sensitive receptors. Overall, these results indicate that lithium, ECS, and citalopram by different modes of action increase NPY neurotransmission in the hippocampus of 'normal' Sprague-Dawley rats.

In subsequent papers (Papers II and V) the effect of lithium and topiramate on brain NPY-LI were investigated in maternally deprived animals and 'depressed' FSL rats, respectively. In MS180 animals, lithium reversed the separation-induced decrease of hippocampal and striatal NPY-LI levels to concentrations that were similar to those found in the MS15 control animals. Although lithium also increased hippocampal NPY-LI in the MS15 group, the effect was of smaller magnitude. Similarly, lowered hippocampal NPY-LI levels in the FSL rats were normalized following repeated, but not single, topiramate treatment, whereas topiramate had no effect on hippocampal NPY-LI in the FRL control animals. Previous studies have shown that also repeated ECS increased hippocampal NPY-LI levels in the FSL rats (Jiménez Vasquez et al., 2000a;Jiménez Vasquez et al., 2000b). Taken together, these studies suggest that ECS, lithium, and topiramate all normalize lowered hippocampal NPY-LI levels in 'depressed' rats and that one common denominator of these treatments is enhancement of NPY neurotransmission in the hippocampus. A functional explanation for this finding will be discussed against the background of the findings in paper VI.

In paper VI, 'normal' Wistar rats were subjected to amygdala-kindling with concomitant pretreatment with either vehicle or the antiepileptic drug levetiracetam. Significant differences in

the progression of kindling were observed between the two treatment groups: Levetiracetam markedly delayed the development of kindling and also reduced the duration of afterdischarge and motor seizures. These behavioral manifestations coincided with the prevention of a kindling-induced upregulation of BDNF and NPY mRNA in the dentate gyrus of the hippocampus. Similarly, levetiracetam pretreatment prevented a kindling-induced downregulation of Y1- and Y5-like receptors in the dentate gyrus and Y2-like receptors in the CA3 area of the hippocampus. In contrast, none of these parameters were affected by levetiracetam in normal rats. These findings suggest that levetiracetam maintains a normal NPYergic neurotransmission on Y1, Y2, and Y5 receptors in the epileptic hippocampus.

The amygdala-kindling model has features which model some aspects of mood disorders: (1) the process of sensitization may resemble the increased vulnerability to relapse with increased number of disease episodes and increased illness severity (Kessing et al., 1998a;Kessing et al., 1998b); (2) treatments which delay the epileptogenesis or dampen motor seizures have mood stabilizing properties in man (Reissmuller et al., 2000;McElroy et al., 2000;Calabrese et al., 1999;Post et al., 1996;Post et al., 1991;Silver et al., 1991;Post and Weiss, 1989;Okuma et al., 1979;Baastrup et al., 1970); (3) epileptic disorders and depression share a high degree of comorbidity and may thus in part have a shared etiology (Harden and Goldstein, 2002;Kanner and Balabanov, 2002;Piazzini and Canger, 2001;Lambert and Robertson, 1999;Harris and Barraclough, 1997;Jacoby et al., 1996;Forsgren and Nystrom, 1990;Robertson et al., 1987). Consequently, one heuristic hypothesis may be that a reduction in NPYergic transmission in the hippocampus underlies a progressively increasing vulnerability to seizures and, by extrapolation, aspects of depression. Indeed, studies with NPY-deficient mice and pharmacological assessments of NPY in animal seizure models suggest that NPY is a potent anticonvulsant (Woldbye, 1998;Palmiter et al., 1998;Woldbye et al., 1997;Woldbye et al., 1996a;Erickson et al., 1996b). In addition, findings in human subjects suggest that a dysfunctional NPY system may underlie aspects of both epilepsy and mood disorder pathology (Caberlotto and Hurd, 2001;Furtinger et al., 2001;Caberlotto and Hurd, 1999). Furthermore, treatment with topiramate, ECS, or levetiracetam, all displaying both anticonvulsant and mood stabilizing properties, lead to changes in NPY parameters suggestive of an increased NPY neurotransmission in the hippocampus of ‘depressed’ rats. Thus, increased hippocampal excitability and symptoms of depression may converge on an impaired hippocampal NPY function, which can be ameliorated by mood stabilizing drugs.

CONCLUSIONS

- Clinical investigations show that experiences of early life separation and inadequate attention from a care-giver are significantly more frequent in depressed patients. Further, evidence implies that NPY may play a role in the pathophysiology of mood disorders. Consequently, the first aim of this thesis was to investigate the effect of early maternal deprivation on brain levels of NPY in rats. It was found that maternal deprivation for 3 hrs per day during PND 2-14 (MS180) or for 24 hrs on PND 9 consistently reduced hippocampal NPY-LI levels in adult rats, thus confirming a recent finding from our group (Jiménez Vasquez et al., 2001). These manipulations were associated with a lowered saccharin-preference and disruption of pre-pulse inhibition, respectively, indicative of an increased level of anhedonia and impaired sensory motor gating in maternally deprived animals. Other studies have shown that maternal deprivation leads to increases in anxiety, alcohol consumption and stress-hyperresponsivity (Rhees et al., 2001;Penke et al., 2001;Plotsky et al., 1995). In contrast, NPY possesses antidepressant and anxiolytic activity in rodents and may convey a behavioral insensitivity to stress (Paper I;Halford, 2001;Britton et al., 2000;Stogner and Holmes, 2000;Thorsell et al., 2000;Sajdyk et al., 1999;Nakajima et al., 1998;Britton et al., 1997;Broqua et al., 1995;Heilig et al., 1993;Heilig et al., 1992;Heilig et al., 1989). By extrapolation, the present findings therefore support the working hypothesis that a neurobiological correlate to an increased risk for mood disorders and comorbid anxiety may be a decreased concentration of NPY in the hippocampus (Jiménez Vasquez et al., 2000b;Mathé, 1999;Mathé et al., 1998), and that such a reduction may result from exposure to early life stress.
- The second aim of this thesis was to investigate the effects of mood stabilizing drugs on rat brain NPY. It was found that lithium, topiramate and ECS treatment consistently led to increases in NPY gene transcription, NPY-LI tissue concentrations and release of NPY-LI in vivo as well as reductions in NPY-sensitive binding sites in the hippocampus, thus confirming and extending the results obtained by our groups and also by others. In kindled animals levetiracetam prevented the loss of Y1- and Y5-like receptors in the dentate gyrus and Y2-like receptors in the CA3 area of the hippocampus. These effects were associated with a significant delay in kindling sensitization, indicative of a reduced vulnerability to 'relapse'. Consequently, these changes may be of therapeutic relevance to the purported mood stabilizing effects of this drug. In view of the observations that (1) NPY is a potent anticonvulsant and also exerts antidepressant properties, (2) NPY affects a variety of centrally mediated functions of possible significance to the symptomatic spectrum of mood disorders, (3) a dysfunctional NPY system has been reported in patients suffering from depression as well as epilepsy disorders, and based on our hypothesis that in the CNS a dysregulation of the NPY system may constitute a biological underpinning of mood disorders, while enhancement of NPYergic neurotransmission may be one common denominator in antidepressant treatment modalities (Jiménez Vasquez et

al., 2000b;Mathé, 1999;Mathé et al., 1998) an expanded heuristic hypothesis can now be proposed: a decreased concentration of NPY in the hippocampus may be associated with aspects of lowered mood, reduced neurogenesis, impaired cognitive function, alcohol-dependence, and increased stress sensitivity and seizure vulnerability. Lithium, the most commonly prescribed drug in mood-disorders and antiepileptics, which all essentially possess mood stabilizing properties, may in part exert their therapeutic mechanisms of action by stimulating the synthesis of hippocampal NPY, thus being a common denominator for their mood stabilizing and anticonvulsant properties.

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