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Chronic psychosocial stress alters NPY system: Different effects in rat and tree shrew

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ABSTRACT

The neuropeptide Y (NPY) system has been largely studied in relation to affective disorders, in particular for its role in the mechanisms regulating the pathophysiology of anxiety and depression and in the stress-related behaviours. Although NPY has been previously investigated in a variety of animal models of mood disorders, the receptor subtype mainly involved in the modulation of the stress response has not been identified. In the present study, the chronic psychosocial stress based on the resident–intruder protocol—an ethologically relevant paradigm known to induce behavioural and endocrine modifications which mimic depression-like symptoms—was used. Two different species were investigated: rat and tree shrew (*Tupaia belangeri*); the latter is regarded as an intermediate between insectivores and primates and it was chosen in this study for its pronounced territoriality. In these animals, the regulation of NPY and of Y_1, Y_2 and Y_5 receptors mRNA expression was evaluated after chronic stress and chronic antidepressant treatment by *in situ* hybridization in selected brain regions known to be involved in the pathophysiology of mood disorders. The animals were exposed to psychosocial stress for 35 days and concomitant daily fluoxetine treatment (10 mg/kg for rats and 15 mg/kg for tree shrews) after the first week of stress. The results confirmed a major role for hippocampal and hypothalamic NPY system in the pathophysiology of mood disorders. Although there were no evident differences between rat and tree shrew in the NPY system distribution, an opposite effect of chronic psychosocial stress was observed in the two species. Moreover, chronic antidepressant treatment was able to counteract the effects of stress and restored basal expression levels, suggesting the utility of these paradigms as preclinical models of stress-induced depression. Overall, although evident species differences were found in response to chronic psychosocial stress, the present study suggests a role for NPY receptors in the stress response and in the action of antidepressant drugs, providing further support for an involvement of this neuropeptidergic system in the pathophysiology of depression and anxiety.

1. Introduction

The study of the mechanisms involved in the etiology of affective disorders requires the investigation of animal models potentially reflecting the course and symptoms of human pathologies. In addition to genetic factors, which are known to predispose to psychopathologies (McGuffin and Katz, 1989), environmental stress plays an important role in the etiology of anxiety and depression, which are linked to maladaptive changes in the stress response (Kendler et al., 1995a,b). Since the majority of stressful stimuli leading to psychopathologies in humans are of social nature (Bjorkqvist, 2001; Kendler et al., 2003; Kessler, 1997; Kessler et al., 1985), the study of the consequences of social stress in experimental animal models is of great interest. Social defeat using the resident–intruder paradigm (Tornatzky and Miczek, 1994) represents a suitable and naturalistic experimental method to study the effects of stress (Fuchs et al., 1996). Subordinate animals exhibit physiological and behavioural changes similar to those of depressive-like state, such as increased adrenocorticotropin hormone (ACTH) and glucocorticoid activity (Buwalda et al., 1999, 2001), disturbances in sleep (Rüther, 1989), altered heart rate, blood pressure and core temperature (Meerlo et al., 1996; Sgoifo et al., 1999), impaired immunological function and reduced resistance to diseases (Engler et al., 2004; Stefanski and Engler, 1998), decreased locomotor and exploratory activities (Koolhaas et al., 1997; Meerlo et al., 1996; Rygula et al., 2005), reduced self-grooming (van Erp et al., 1994), impaired consummatory behaviour, with a consequent loss of body weight (Kramer et al., 1999; Rybkin et al., 1997), reduced aggression and sexual behaviour

Abbreviations: ACTH, adrenocorticotropin hormone; ANOVA, analysis of variance; CA1, CA1 region of hippocampus; CA2, CA2 region of hippocampus; CA3, CA3 region of hippocampus; CNS, central nervous system; DG, dentate gyrus; HPA, hypothalamic-pituitary-adrenocortical; NPY, Neuropeptide Y; PBS, phosphate buffered saline; SSC, saline sodium citrate; VMH, ventro-medial hypothalamus; VMHDM, ventro-medial hypothalamus, dorso-medial portion.

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(McGrady, 1984), increased submissive behaviour and anxiety (Ruis et al., 1999).

Neuropeptide Y (NPY) is a neuroactive peptide acting as a neurotransmitter and neuromodulator regulating many physiological functions (Colmers and Wahlestedt, 1993). Preclinical and clinical studies support the role of NPY in the regulation of emotions and stress-related behaviours, showing that increased NPY levels in specific regions of the central nervous system (CNS) induce antidepressant- and anxiolytic-like effects, whereas a down-regulation of this peptide induces opposite effects on the emotional responses (Heilig, 2004). Since the NPY system is altered by stressful challenges (Castagné et al., 1987), the study of its expression in animal models of depression could be useful in clarifying the role of NPY in emotional behaviours. Previous studies on genetic models (Bjørnebekk et al., 2006; Caberlotto et al., 1998, 1999; Husum et al., 2008, 2001; Jiménez-Vasquez et al., 2000a,b; Wortwein et al., 2006) or environmental models (Husum and Mathé, 2002; Husum et al., 2002; Jiménez-Vasquez et al., 2001) have demonstrated alterations of the NPY system in the CNS; however, the variety of regions affected by changes does not allow a simple interpretation, although the hippocampus seemed to be consistently involved.

Of the seven NPY receptor subtypes isolated to date, named Y_1–Y_7, a number of findings have indicated Y_1 and Y_2 receptors as the main modulators of NPY anti-stress activity (Heilig, 2004). The Y_1 receptor agonists exert an antidepressant- and anxiolytic-like action (Ishida et al., 2007), while the Y_1 antagonists induce anxiety and depressive-like behaviour in various animal models of anxiety (Primeaux et al., 2005; Redrobe et al., 2002; Sajdyk et al., 1999). Conversely, pharmacological or genetical blockade of Y_2 receptors has been shown to induce anxiolytic- and antidepressant-like profiles (Bacchi et al., 2006; Carvajal et al., 2006; Redrobe et al., 2003; Tschenett et al., 2003). Among the other NPY receptors, the Y_5 receptor subtype has been mainly studied in relation to food intake (Schaffhauser et al., 1997; Kask et al., 2001), however, based on its distribution in brain regions known for their role in emotional disorders (Dumont et al., 1998; Gerald et al., 1996), Y_5 has been suggested to be involved in the regulation of anxiety-like state and in the response to stressful stimuli (Sajdyk et al., 2002; Sørensen et al., 2004). The remaining receptor subtypes were not considered in this study; the Y_3 receptor subtype has not yet been cloned (Lee and Miller, 1998), while the Y_4 receptor was primarily found in peripheral tissues (Barrios et al., 1999). The Y_6 receptor gene was found to be completely absent in the rat (Burkhoff et al., 1998) and to be functional only in some species but not in human and other primates (Gregor et al., 1996; Matsumoto et al., 1996), whereas the Y_7 receptor has been cloned only in fish, amphibians and chicken (Fredriksson et al., 2004; Bromée et al., 2006).

Therefore, the present study was focused on the characterization of the three major NPY receptors—Y_1, Y_2 and Y_5—in order to understand their involvement in the regulation of the NPY role in the emotional states. This was investigated using a validated stress-induced animal model of depression, the chronic psychosocial stress, an ethological stress paradigm inducing depressive-like symptoms (Fuchs et al., 1996, 2001; Kramer et al., 1999) and evaluating the effects of the established antidepressant fluoxetine to counteract the alterations induced by stress. Two different animal species were investigated, a rodent (rat) and a pre-primate (tree shrew), the latter phylogenetically classified as an intermediate between insectivores and primates (Martin, 1990). In addition, since contrasting evidence about the receptor subtype mainly involved in the mediation of the role of NPY in the regulation of emotions still exists, the potential changes of the different receptor subtypes were also considered, in order to identify the receptor subtype primarily involved in the regulation of emotional processes.

2. Materials and methods

2.1. Animal care and treatments

Tissues from adult male rats and tree shrews were obtained from cohorts submitted to social conflict paradigm at the German Primate Center (Göttingen, Germany).

The experimental design of the study, the behavioural and hormonal characterisations of the rats used in the present study were previously described in detail (Rygula et al., 2006; Fig. 1). The rats used in the present study are the same as those included in the work of Rygula and colleagues. Three different experimental phases and four groups of animals ($n = 6$ rats per group) were generated: i) *Control*, ii) *Stress*, iii) *Control + fluoxetine*, iv) *Stress + fluoxetine*. The first experimental phase lasted for 7 days, during which all the animals were handled daily and body weight was recorded. The second phase also lasted for 7 days, during which the Wistar rats (intruders) of the *Stress* and *Stress + fluoxetine* groups were exposed daily to 1 h of psychosocial stress. The third experimental phase, lasting 28 days, consisted of the antidepressant treatment: stressed rats were maintained in the psychosocial conflict situation and were treated daily with fluoxetine or vehicle. Animals in the *Control + fluoxetine* and *Stress + fluoxetine* groups received fluoxetine (10 mg/kg body weight per day) orally directly before or after the defeat sessions. The drug (Fluoxetin Ratiopharm®, 4 mg/ml oral solution; Ratiopharm GmbH, Ulm, Germany) was administered using a bulb-headed cannula into the buccal cavity to minimize uncontrollable stress effects caused by daily injections. This dose has been demonstrated to be effective in reversing a series of endocrine and behavioural parameters modified by stress exposure (Rygula et al., 2006) and resulting with blood concentration of fluoxetine and its major active metabolite, norfluoxetine, similar to those reported in human patients treated with therapeutically active doses (Czéh et al., 2006). The animals of the *Control* and *Stress* groups were treated with vehicle only. On the last experimental day (day 42), all the animals were sacrificed, the brains were rapidly

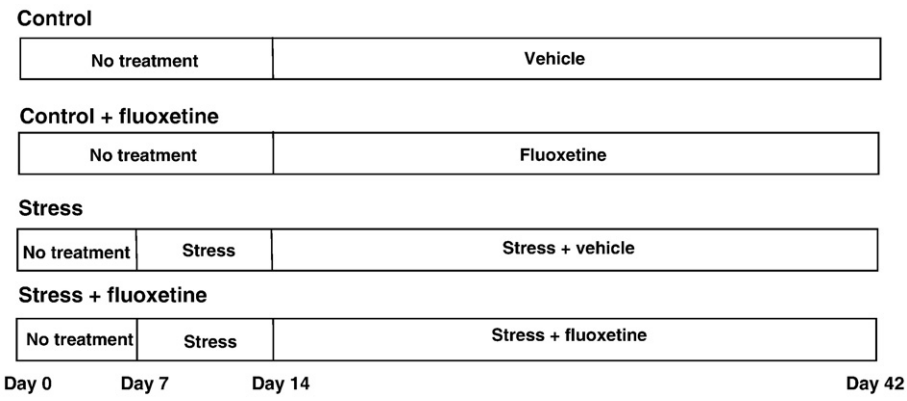

Fig. 1. Experimental groups and design of the study. For details, see the Materials and methods section and refer to Rygula et al. (2006).