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BRAIN RESEARCH

Research Report

Altered neuropeptide Y and neurokinin messenger RNA expression and receptor binding in stress-sensitised rats

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ABSTRACT

A single session of footshocks in rats causes long-lasting sensitisation of behavioural, hormonal and autonomic responses to subsequent novel stressful challenges as well as altered pain sensitivity. These changes mimic aspects of post-traumatic stress disorder in humans. Our aim was to identify neuropeptide substrates in the central nervous system involved in stress sensitisation. Male Wistar rats were exposed to ten footshocks in 15 min (preshocked) or placed in the same cage without shocks (control). Two weeks later, rats were placed in a novel cage, subjected to 5 min of 85 dB noise, and returned to their home cage. Rats were killed either before or 1 h after noise and their brains processed for in situ hybridization for neuropeptide Y (NPY) and beta-preprotachykinin-I (PPT) mRNA. Additional groups of rats were killed under basal conditions and brains processed for NPY and neurokinin receptor binding with radiolabelled ligands. Two weeks after footshock treatment NPY mRNA expression was increased in the basolateral amygdala and showed preshock × noise interaction in the locus coeruleus (down after noise in controls, lower basal and unchanged after noise in preshocked). PPT expression in the lateral parabrachial nucleus also showed preshock × noise interaction (up after noise in controls, higher basal and down after noise in preshocked), and was increased after noise in the periaquaeductal grey. NK1 receptor binding in the agranular insular cortex and arcuate nucleus of the hypothalamus and NK2 receptor binding in the amygdala was lower in preshocked rats than in controls. Altered expression of NPY in the basolateral amygdala and locus coeruleus could contribute to or compensate for behavioural and autonomic sensitisation in preshocked rats. Altered PPT expression in the parabrachial nucleus may be involved in the altered pain processing seen in this model. Lower NK1 and NK2 receptor numbers in cortex, hypothalamus and amygdala may reflect secondary adaptations to altered neuropeptide release. These long-term changes in brain neuropeptide systems could offer novel leads for pharmacological modulation of long-term stress-induced sensitisation.

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Abbreviations: Arc, arcuate hypothalamic nucleus; AI, agranular insular cortex; BLA, basolateral amygdaloid nucleus; Ce, central amygdaloid nucleus; Cg, cingulate cortex; CON, control; CNS, central nervous system; DR, dorsal raphe nucleus; IL, infralimbic cortex; LC, locus coeruleus; LPBC and LPBE, lateral parabrachial nucleus central and external division; Me, medial amygdaloid nucleus; NK, neurokinin; NPY, neuropeptide Y; PaAP, paraventricular hypothalamic nucleus anterior parvicellular part; PAG, periaquaeductal gray; PoDG, polymorph layer of the dentate gyrus; PPT, beta-preprotachykinin-I; PrL, prelimbic cortex; PRS, preshocked; PV, paraventricular thalamic nucleus; PVP, paraventricular thalamic nucleus, posterior part; SolC, nucleus of the solitary tract, commissural part; SolM, nucleus of the solitary tract, medial part; SP, substance P; SSC, standard sodium citrate

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1. Introduction

Exposure to a brief but intense stressful experience, a single session of footshocks, causes long-lasting sensitisation of behavioural, hormonal and autonomic responses to subsequent novel stressful challenges in rats (summarised in Stam, 2007). Compared to controls, rats previously exposed to a single session of footshocks ('preshocked') show increased latency to emerge from a dark compartment to a brightly lit space, increased immobility in the open field and noise test, but increased escape behaviour in the shuttlebox test, for weeks to months after the shock experience. The hypothalamus–pituitary–adrenal axis response to novel stressors (open field, noise) is increased in preshocked rats 2 weeks after the shocks, and preshocked rats also display sensitised autonomic responses after exposure to an electrified rod inserted into the home cage. These changes mimic aspects of the anxiety disorder post-traumatic stress disorder in humans, and the altered behavioural responsivity found in preshocked rats is reversed by various anxiolytics (Stam, 2007). Our goal is to identify molecular substrates in the central nervous system that are lastingly changed after stress, in order to understand the mechanisms involved and identify novel treatment targets.

The expression of Fos, a protein product of the proto-oncogene *c-fos*, has been used as a non-specific but stimulus intensity-dependent marker of neuronal activation (Morgan and Curran, 1995). When an electrified rod is introduced into the home cage and touched 2 weeks after the footshocks, increased numbers of Fos-positive cells are found in preshocked compared to control rats in brain areas involved in fear and anxiety and in neuroendocrine and autonomic control (Bruijnzeel et al., 1999). Painful balloon distension of the large and small intestine also induces differential Fos expression in selected brain areas of preshocked and control rats (de Lange et al., 2005; Stam et al., 2002). Little is known yet about the molecular substrates that underlie sensitised neuronal activation and the associated behavioural, autonomic and pain sensitisation. So far, antagonists of corticotropin-releasing factor have not been found to antagonise behavioural sensitisation, and it is increased synthesis and storage of vasopressin, not corticotropin-releasing factor, in the hypothalamic neurons projecting to the median eminence that appears to drive sensitised responsivity of the hypothalamus–pituitary–adrenal axis (for an overview see Stam, 2007). Based on cumulative evidence for their involvement in behavioural and autonomic responses to stress, pain processing, and sensitisation, two alternative neuropeptide systems may contribute to functional changes following a single session of footshocks. Neuropeptide Y (NPY) generally reduces stress responses and anxiety (for reviews see Griebel, 1999; Kask et al., 2002). In contrast, the tachykinin substance P (Ebner et al., 2004; Ebner and Singewald, 2006; Griebel, 1999) in the brain is mostly anxiogenic. Stress-induced colonic hypermotility is reduced by intra-cerebroventricular (i.c.v.) application of NPY (Gué et al., 1992). Central nervous system NPY has antinociceptive properties (Broqua et al., 1996; Wang et al., 2001). Tachykinins have complex effects on pain sensitivity depending on the site of administration and condition of the animal (Altier and Stewart, 1999; Rosen et al., 2004; Santos and Calixto, 1997). There is preliminary evidence that NPY and tachykinin systems may be involved in the trauma-induced physiological and behavioural changes in humans (Geracioti et al., 2006; Morgan et al., 2003).

We have studied mRNA expression of NPY and preprotachykinin-I (PPT, also referred to as PPT-1, PPT-A, or TAC1) in our footshock sensitisation model in pre-selected brain areas that were previously demonstrated to show sensitised Fos-induction in preshocked animals, are involved in anxiety, autonomic responses, and pain processing, and are known to contain NPY or PPT mRNA. Because stress-induced changes in neuropeptide systems may also lead to altered neuropeptide receptor numbers, we also studied NPY and neurokinin (NK) receptor binding in two additional groups of preshocked rats and controls, again in pre-selected brain areas previously demonstrated to show sensitised Fos-induction in preshocked animals, or (arcuate nucleus) recently demonstrated to show acute changes in peptidergic expression following footshocks (Kas et al., 2005). We chose to measure mRNA expression and receptor binding 2 weeks after footshocks, since behavioural, physiological and pain sensitisation are fully expressed at this time point (Stam, 2007).

2. Results

2.1. *Experiment 1*

2.1.1. *Behaviour*

During the first 5 min of the noise test preshocked animals displayed sensitised behavioural responses to the noise test compared to controls (Fig. 1): decreased locomotion ($t=4.7$, $P=0.001$) and rearing behaviour ($t=3.7$, $P=0.005$), and increased immobility (Fig. 1, $t=4.9$, $P=0.001$). No significant differences were found during the noise-off period (results not shown).

2.1.2. *NPY mRNA expression*

Messenger RNA expression levels were determined in control and preshocked animals, either under basal conditions or after a novel challenge, the noise test. NPY mRNA expression was significantly increased in the basolateral amygdala (Fig. 2A,B)

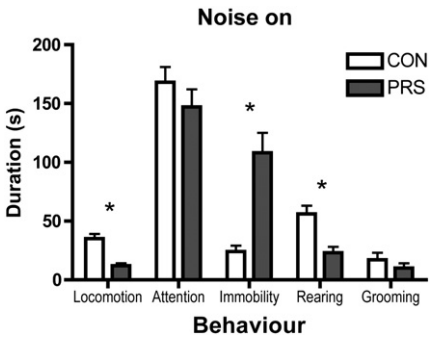

Fig. 1 – Duration of the five mutually exclusive behavioural components during the 'noise on' phase of the noise test, 2 weeks after a 15-minute session of footshocks (PRS, $n=6$) or no shocks (CON, $n=6$). Preshocked animals displayed significantly less locomotion and rearing and significantly more immobility. Data are means with SEM. * = significant difference between control and preshocked groups. For detailed statistics see the Results section.