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Research Report

Partial reduction in neural cell adhesion molecule (NCAM) in heterozygous mice induces depression-related behaviour without cognitive impairment

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ABSTRACT

The neural cell adhesion molecule (NCAM) plays an important role in brain plasticity. Using mice deficient in all isoforms of NCAM we have previously demonstrated that constitutive deficiency in the NCAM gene (NCAM–/–) resulted in cognitive impairment, anhedonic behaviour and a reduced ability to cope with stress. This was accompanied by reduced basal phosphorylation of the fibroblast growth factor receptor 1 (FGFR1) and reduced phosphorylation of calcium-calmodulin kinase (CaMK) II and IV and cAMP response element binding protein (CREB). The present study was aimed to investigate how partial deficiency in NCAM in mice (NCAM+/–) affected phenotype. We found that NCAM+/– mice showed a longer period of immobility in the tail suspension test, increased latency to feed in the novelty-suppressed feeding test and reduced preference for sucrose in sucrose preference test. Both NCAM+/– and NCAM–/– mice showed reduced extinction of contextual fear. In contrast to NCAM–/– mice, NCAM+/– mice did not demonstrate memory impairment in either object recognition or contextual fear conditioning tests. Levels of phosphorylated FGFR1 in the hippocampus and prefrontal/frontal cortex of NCAM+/– mice were partially reduced and no changes in the phosphorylation of CaMKII, CaMKIV or CREB in the hippocampus were found. We conclude that a constitutive partial reduction in NCAM proteins results in a behavioural phenotype related to depression without impairment in cognitive functions, also affecting the level of FGFR1 phosphorylation without major alterations in CaMKII and CaMKIV intracellular signalling. Partial reduction in FGFR1 phosphorylation might explain the observed behavioural phenotype in NCAM+/– mice.

1. Introduction

One of the molecules involved in the regulation of brain plasticity is the neural cell adhesion molecule (NCAM). The neural cell adhesion molecule is a membrane-bound glycoprotein that belongs to the immunoglobulin superfamily of cell adhesion molecules. It is predominantly expressed on the surface of neuronal and glial cells at pre- and postsynaptic zones. The neural cell adhesion molecule has been implicated in cell–cell adhesion, neurite outgrowth, synaptic plasticity, neuronal development and neurogenesis (Persohn and Schachner, 1987; Rønn et al., 2000; Amoureux et al., 2001; Kiss and Muller, 2001; Schuster et al., 2001).

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Neural cell adhesion molecule is present in three isoforms, which result from alternative splicing of a single gene. The three isoforms are called NCAM-180 and NCAM-140, which are transmembrane isoforms, and NCAM-120, which is a glycosylphosphatidylinositol-linked isoform. All three isoforms share the same extracellular domain comprising five Ig-like modules and two membrane-proximal fibronectin type-3 modules. The adhesive properties of NCAM can be regulated by the addition of long, linear homopolymers of alpha-2,8-linked sialic acid residues (polysialic acid, PSA), which attenuate NCAM-mediated cell interactions and thereby promote structural plasticity and affect cell functions (Bruses and Rutishauser, 2001; Rutishauser, 2008).

Altered levels of PSA and neural cell adhesion molecule have been associated with several neuropsychiatric disorders (reviewed in Brennaman and Maness, 2010). Accumulating evidence suggests that NCAM is involved in depression (Sequeira et al., 2007; Aonurm-Helm et al., 2008a; Tochigi et al., 2008). Adult mice that lack all three major isoforms of the NCAM (NCAM–/–) exhibit anhedonia, impaired cognitive functions and a reduced ability to cope with stress (Cremer et al., 1994; Bukalo et al., 2004; Aonurm-Helm et al., 2008a; Jürgenson et al., 2010).

It has been shown that NCAM and PSA-NCAM regulate plasticity by interacting with several interaction partners (Walmod et al., 2004). A major interaction partner of NCAM is the fibroblast growth factor receptor 1 (FGFR1, Doherty and Walsh, 1996, Cavallaro et al., 2001; Kiselyov et al., 2003; reviewed in Kiselyov et al., 2005; Francavilla et al., 2007).

Homophilic and heterophilic interactions of NCAM initiate signal transduction pathways, such as the mitogen-activated protein kinase (MAPK) cascade and calcium/calmodulin dependent kinase II (CaMKII) and calcium/calmodulin dependent kinase IV (CaMKIV) pathways, and activates the transcription factor cAMP-response element binding protein (CREB) at Ser133 (Schmid et al., 1999; Kolkova et al., 2000; Jessen et al., 2001; Povlsen et al., 2003; Griffith, 2004; Ditlevsen et al., 2008). Our recent studies showed that NCAM–/– mice demonstrated reduced ability to cope with stress as evidenced by an increased immobility time in the tail suspension test and anhedonia as evidenced by reduced preference for sucrose solution in the sucrose preference test (Aonurm-Helm et al., 2008a). These behaviours, but not cognitive impairment, were ameliorated by the antidepressants amitriptyline and citalopram and the NCAM mimetic peptide FGL (Aonurm-Helm et al., 2008a). Other studies have also demonstrated some morphological alterations in the brain of NCAM–/– mice, such as reduced olfactory bulbs size and reduced mossy fibre density in the hippocampus (Cremer et al., 1997; Stork et al., 1997). Furthermore, NCAM–/– mice showed reduced phosphorylation of the major NCAM interaction partner fibroblast growth factor receptor 1 (FGFR1) and impaired activity of several NCAM-mediated intracellular signalling pathways, demonstrated by the reduced levels of phosphorylated CREB and phosphorylated CaMKII and CaMKIV in the hippocampus (Aonurm-Helm et al., 2008b, 2010). However, the impaired intracellular pathways responsible for the cognitive dysfunction and those implicated in the formation of the depression-like phenotype remain unclear. It has been shown previously that mice with a partial reduction in NCAM expression (NCAM+/–) display increased anxiety and inter-male aggression with a post-aggression test increase in corticosterone plasma concentration, similarly to NCAM–/– mice (Stork et al., 1997, 1999). These data suggests that even a partial reduction in NCAM proteins may cause alterations in behavioural phenotype.

We therefore studied whether a partial reduction in NCAM was capable of inducing similar alterations in behaviour to those observed in NCAM–/– mice. We also studied the intracellular signalling pathways that were previously shown to be impaired in NCAM knockout mice.

For this purpose, we studied depression-related behaviours and memory function in heterozygous NCAM+/– mice and measured the phosphorylation levels of FGFR1, CREB, CaMKII and CaMKIV in hippocampal tissues, all of which have been previously shown to be dysregulated in NCAM–/– mice.

2. Results

2.1. *NCAM and PSA-NCAM protein levels*

Western blots were performed to measure the amount of NCAM isoforms and PSA-NCAM in hippocampal and prefrontal/frontal cortical lysates from wild-type, NCAM+/– and NCAM–/– mice. There was a 50% reduction in the immunoreactivity of all NCAM isoforms and PSA-NCAM in the hippocampus of NCAM+/– mice compared with wild-type mice. The tissue from NCAM–/– mice did not demonstrate any immunoreactivity related to NCAM or PSA-NCAM (Fig. 1). Similar reductions in NCAM and PSA-NCAM levels were also observed in prefrontal/frontal cortex (Supplementary data, Fig. 1).

2.2. *Tail suspension, novelty-suppressed feeding and sucrose preference tests in NCAM+/– and NCAM–/– mice*

To evaluate the ability to cope with stress and determine anhedonic behaviour in NCAM+/– animals, we employed the tail suspension test (TST), novelty-suppressed feeding test and the sucrose preference test. The TST was first introduced as a test with predictive validity for detecting drugs with antidepressant-like activity (Porsolt et al., 1987; Bai et al., 2001; reviewed in Cryan and Mombereau, 2004 and Cryan et al., 2005). More recent studies, however, demonstrated relatively good face validity of the TST for describing depression-like phenotypes in genetic-, stress- or toxin-induced models of depression in mice (Dantzer et al., 2008; Ito et al., 2011; Monje et al., 2011; Popova and Tibeikina, 2010). As shown in Fig. 2A, the NCAM+/– mice spent a significantly longer time immobile than their wild-type littermates. For comparison, we also tested NCAM–/– knockout mice and observed increased immobility time similar to our earlier publication (Aonurm-Helm et al., 2008a). A one-way ANOVA demonstrated a highly significant genotype effect: $p<0.0001$, $F=10.57$, d.f.=27 (n=8–10). Post-hoc analyses showed a significant increase in immobility time in both NCAM+/– ($p<0.01$) and NCAM–/– ($p<0.001$) mice (Fig. 2A). To rule out the possibility that the longer period of immobility in the TST was due to an impairment in locomotion, we measured general locomotor activity. A one-way ANOVA revealed an effect of genotype: