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Research Report

Selective lesioning of nucleus incertus with corticotropin releasing factor-saporin conjugate

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ABSTRACT

The nucleus incertus (NI), a brainstem nucleus found in the pontine periventricular grey, is the primary source of the neuropeptide relaxin-3 in the mammalian brain. The NI neurons have also been previously reported to express several receptors and neurotransmitters, including corticotropin releasing hormone receptor 1 (CRF_1) and gamma-aminobutyric acid (GABA). The NI projects widely to putative neural correlates of stress, anxiety, depression, feeding behaviour, arousal and cognition leading to speculation that it might be involved in several neuropsychiatric conditions. On the premise that relaxin-3 expressing neurons in the NI predominantly co-express CRF_1 receptors, a novel method for selective ablation of the rat brain NI neurons using corticotropin releasing factor (CRF)–saporin conjugate is described. In addition to a behavioural deficit in the fear conditioning paradigm, reverse transcriptase polymerase chain reaction (RT-PCR), western blotting (WB) and immunofluorescence labelling (IF) techniques were used to confirm the NI lesion. We observed a selective and significant loss of CRF_1 expressing cells, together with a consistent decrease in relaxin-3 and GAD65 expression. The significant ablation of relaxin-3 positive neurons of the NI achieved by this lesioning approach is a promising model to explore the neuropsychopharmacological implications of NI/relaxin-3 in behavioural neuroscience.

1. Introduction

The nucleus incertus (NI), better known for its status as the principal source of neuronal relaxin-3, is a brainstem nucleus located ventral and medial to the posterodorsal tegmental nucleus (PDTg) in the pontine periventricular grey (Burazin et al., 2002; Goto et al., 2001; Olucha-Bordonau et al., 2003). The NI neurons were initially shown to express the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) (Ford et al., 1995) and have later been revealed to

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co-express the recently discovered neuropeptide, relaxin-3 (Ma et al., 2007). This small and distinct group of neurons has sparked off renewed interest due to its high expression of the corticotropin releasing hormone receptor type 1 (CRF_1) (Bittencourt and Sawchenko, 2000; Potter et al., 1994), which suggests a role of the NI in the stress response. Subsequent studies reported the presence of other receptors in the NI, e.g. 5-hydroxytryptamine (5-HT or serotonin) receptor subtype 1A ($5\text{-}HT_{1A}$) (Miyamoto et al., 2008), and relaxin/insulin-like family peptide receptor 3 (RXFP3) (Sutton et al., 2004). Other neuropeptides expressed by nucleus incertus include neuromedin B (Chronwall et al., 1985; Wada et al., 1990) and cholecystokinin (Kubota et al., 1983; Olucha-Bordonau et al., 2003). Of particular interest, expression of relaxin-3 is highly specific and most abundant in the NI and only a small number of neurons in other nearby regions such as the pontine raphe nucleus, periaqueductal grey and dorsal substantia nigra express relaxin-3 (Tanaka et al., 2005). As such, the NI – serves as a key point of regulation for functioning of the relaxin-3 neural circuitry.

The NI possibly exerts its actions through numerous projections to several parts of the brain, including the prefrontal cortex, medial septum (MS), hippocampus, amygdala, hypothalamus and the raphe nuclei (Goto et al., 2001) – areas that are implicated in various psychiatric conditions. Relaxin-3 binds to the RXFP3, which belongs to the family of relaxin peptide receptors that was recently elevated from orphan receptor status (van der Westhuizen et al., 2008). The precise functions of the NI and relaxin-3 remain largely undetermined but intriguing reports have been published in the past decade demonstrating the alleged role of the NI/relaxin-3/RXFP3 system in feeding behaviour (McGowan et al., 2006; McGowan et al., 2005), stress (Burazin et al., 2002; Smith et al., 2012), anxiety (Watanabe et al., 2011) and cognition (Cervera-Ferri et al., 2011; Farooq et al., 2013; Ma et al., 2009). Recent studies show c-Fos expression in the nucleus incertus occurs in response to acute antipsychotic treatments in rats (Rajkumar et al., 2013) and polymorphisms in human relaxin-3 and RXFP3 associated with metabolic disturbances in patients with schizophrenia treated with antipsychotic drugs (Munro et al., 2012). Thus the study of the NI and relaxin-3 is an exciting new frontier in behavioural neuroscience.

A strategy to achieve potent and selective lesioning of target brain structures has been to utilise cell-surface protein binding peptides or antibodies conjugated with saporin, a monomeric ribosomal inactivating protein (Heckers et al., 1994; Li et al., 2008; Thorpe et al., 1985; Waite et al., 1994). Selectivity is achieved because, as a ribosomal toxin, the saporin is only toxic when internalised by the corresponding receptor. The corticotropin releasing factor (CRF)–saporin conjugate toxin, used in the present study, is expected to selectively ablate CRF_1 expressing cells (Hummel et al., 2010; Maciejewski-Lenoir et al., 2000). On the premise that relaxin-3 expressing neurons in the NI predominantly co-express CRF_1 receptors (Tanaka et al., 2005), the present investigation attempted to establish a method for selective ablation of the NI using the CRF–saporin conjugate.

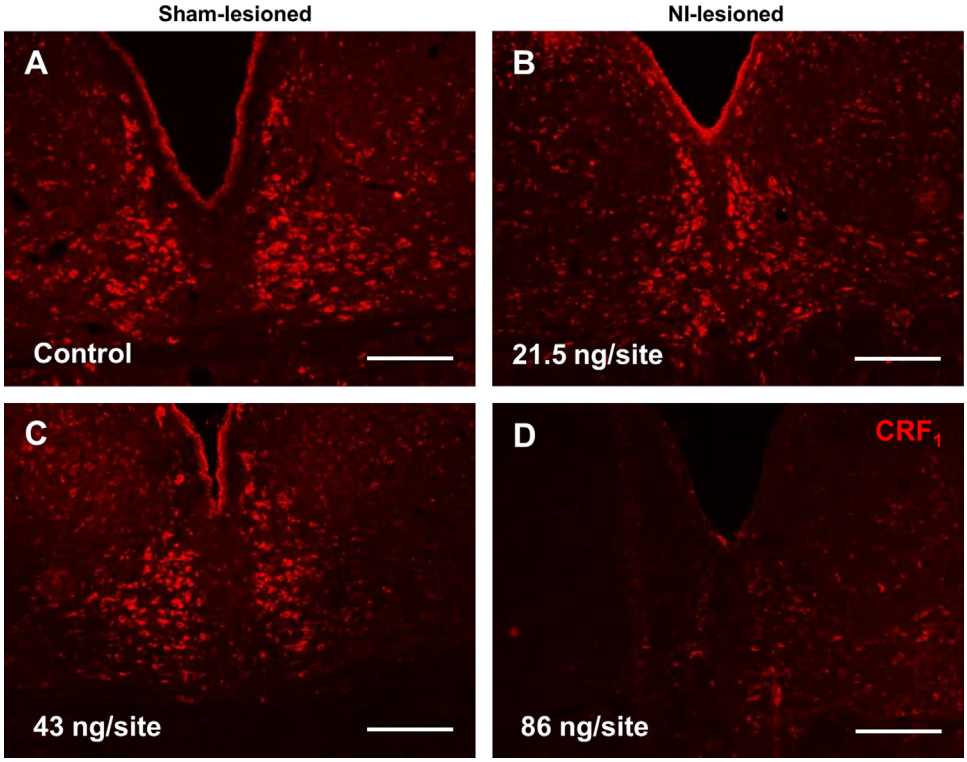

Fig. 1 – Selection of CRF–saporin dose for ablation of NI neurons. Images show representative examples of the CRF_1 positive cells in the NI of (A) naïve control ($n=3$), (B) 21.5 ng/site CRF–saporin injected ($n=3$), (C) 43 ng/site CRF–SAP injected ($n=3$) and (D) 86 ng/site CRF–SAP injected ($n=3$) rats. The 86 ng/site (172 ng in total) dose of CRF–SAP produced the greatest reduction in CRF_1 expressing cells in the NI. Scale bars are 100 μm.