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The angiotensin converting enzyme inhibitor, captopril, prevents the hyperactivity and impulsivity of neurokinin-1 receptor gene 'knockout' mice: Sex differences and implications for the treatment of attention deficit hyperactivity disorder

Ashley J. Porter, Katharine Pillidge, Ewelina M. Grabowska, S. Clare Stanford*

Department of Neuroscience, Physiology & Pharmacology, University College London, Gower St, London WC1E 6BT, UK

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Abstract
Mice lacking functional neurokinin-1 receptors (NK1R−/−) display behavioural abnormalities resembling attention deficit hyperactivity disorder (ADHD): locomotor hyperactivity, impulsivity and inattentiveness. The preferred ligand for NK1R, substance P, is metabolised by angiotensin converting enzyme (ACE), which forms part of the brain renin angiotensin system (BRAS). In view of evidence that the BRAS modulates locomotor activity and cognitive performance, we tested the effects of drugs that target the BRAS on these behaviours in NK1R−/− and wildtype mice. We first tested the effects of the ACE inhibitor, captopril, on locomotor activity. Because there are well-established sex differences in both ADHD and ACE activity, we compared the effects of captopril in both male and female mice. Locomotor hyperactivity was evident in male NK1R−/− mice, only, and this was abolished by treatment with captopril. By contrast, male wildtypes and females of both genotypes were unaffected by ACE inhibition. We then investigated the effects of angiotensin AT_1 (losartan) and AT_2 (PD 123319) receptor antagonists on the locomotor activity of male NK1R−/− and wildtype mice. Both antagonists increased the locomotor activity of NK1R−/− mice, but neither affected the wildtypes. Finally, we tested the effects of captopril on the performance of male NK1R−/− and wildtype mice in the 5-choice serial reaction-time task (5-CSRTT) and found that ACE inhibition prevented the impulsivity of NK1R−/− mice. These results indicate that certain behaviours, disrupted in

*Corresponding author. Tel.: +44 20 7679 3731.
E-mail address: c.stanford@ucl.ac.uk (S.C. Stanford).

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ADHD, are influenced by an interaction between the BRAS and NK1R, and suggest that ACE inhibitors could provide a novel treatment for this disorder.

1. Introduction

Male mice with functional ablation of the *Nk1r* gene, which encodes the substance P-preferring NK1 receptor (NK1R−/−), express locomotor *hyperactivity* in several experimental contexts (Fisher et al., 2007; Herpfer et al., 2005; Yan et al., 2010). In the 5-choice serial reaction-time task (5-CSRTT), a procedure that is used to evaluate cognitive performance, NK1R−/− mice also express more omissions (*inattentiveness*) and more premature responses (*motor impulsivity*) compared with their wildtypes (Dudley et al., 2013; Yan et al., 2011). Hyperactivity, inattentiveness and impulsivity are diagnostic criteria for attention deficit hyperactivity disorder (ADHD). On this basis, and supported by evidence from human genetic studies (Sharp et al., 2014), we have proposed that polymorphism(s) of the *TACR1* gene (the human equivalent of the mouse *Nk1r* gene) could be associated with increased risk of developing ADHD.

Studies in vitro have shown that substance P is degraded by angiotensin converting enzyme ('ACE': peptidyl dipeptidase A; EC 3.4.15.1; Skidgel et al., 1984), which forms part of the brain renin angiotensin system (BRAS). It is still not certain that ACE metabolises substance P in vivo (Mitchell et al., 2013) and, in any case, ACE is not the only peptidase that metabolises this peptide (Oblin et al., 1988). Nevertheless, a substantial body of evidence indicates that the BRAS regulates both locomotor activity and executive function (for recent review, see: Wright and Harding, 2011). For instance, ACE inhibitors improve performance in several preclinical screens of learning and memory, such as the Morris water maze and tests of active/passive avoidance (e.g., Barnes et al., 1992; Nikolova et al., 2000). ACE inhibitors also enhance cognitive performance in hypertensive patients and healthy controls, as well as in patients with dementia (Croog et al., 1986; Currie et al., 1990; Rozzini et al., 2006). Moreover, histochemical markers indicate that the BRAS is distributed across neuronal networks that have been strongly implicated in ADHD and motor control. For example, both ACE and angiotensin (AT) receptors are densely expressed within the basal ganglia, in regions such as the dorsal striatum, globus pallidus and substantia nigra (Strittmatter et al., 1984; Chai et al., 1987; Allen et al., 1992).

We reasoned that if ACE degrades substance P in vivo, then inhibition of this enzyme would reduce locomotor activity of wildtypes but would not affect NK1R−/− mice because they lack functional NK1R. Even if substance P fragments bind to and activate other sites, inhibition of ACE should modify the locomotor activity of wildtype and NK1R−/− mice in different ways. To test this possibility, we compared the locomotor activity of male NK1R−/− mice and their wildtypes in a light/dark exploration box (LDEB) following administration of the ACE inhibitor, captopril. Unlike many ACE inhibitors, this compound penetrates the brain in its active form (Geppetti et al., 1987; Ranadive et al., 1992). A caveat to this experiment was prompted by reports that ADHD, especially of the predominantly hyperactive/impulsive subtype, is more common in boys than girls (Waddell and McCarthy, 2012). There is also a report suggesting sex differences in ACE activity, which is reduced by oestrogen (Komukai et al., 2010). In light of this evidence, we compared the effects of captopril on the locomotor activity of both male and female NK1R−/− mice and their wildtype counterparts.

Contrary to our prediction, treatment with captopril reduced the locomotor activity of male NK1R−/− mice but did not affect that of male wildtypes, or female mice of either genotype. Given that ACE is better known for converting the (presumed) inactive precursor, angiotensin I, to the active product, angiotensin II (AngII), an obvious possibility is that this behavioural response to captopril could be due to a deficit in angiotensin II production. If so, this response should be mimicked by drug antagonism of AngII (type 1 (AT_1) and/or type 2 (AT_2)) receptors, which are expressed by neurones and glial cells in subcortical regions, including the striatum (Allen et al., 1992). To investigate this proposal, we compared the locomotor response of the two genotypes after treatment with either a selective AT_1 receptor antagonist (losartan) or AT_2 receptor antagonist (PD 123319).

Finally, there is extensive evidence that the BRAS modulates cognitive performance. For instance, several early studies suggested that captopril could have nootropic actions in rodents (e.g., Earley et al., 1989; Mondadori and Etienne, 1990; see: Wright and Harding, 2011). Against this background, a third experiment compared the effects of captopril on the cognitive performance and response control of male NK1R−/− and wildtype mice in the 5-CSRTT. We used this protocol because NK1R−/− mice have previously demonstrated both increased *premature responses* (an index of one form of impulsivity) and increased *omissions* (an index of inattentiveness) in this test (Dudley et al., 2013; Yan et al., 2011). Furthermore, it has been concluded, from a battery of studies measuring impulsivity in ADHD patients, that premature responses are "…the most sensitive measures for discriminating ADHD from control children" (Rubia et al., 2007). Consequently, the measurement of *premature responses* by NK1R−/− mice in the 5-CSRTT has strong translational relevance for ADHD research.

2. Experimental procedures

These experiments were authorised under the UK Animals (Scientific Procedures) Act 1986 and received approval from the local Animal Welfare and Ethical Review Body (UCL). This report complies with the ARRIVE guidelines for reporting of animal experiments.

2.1. Animals

All the animals were from inbred colonies maintained at University College London. NK1R−/− mice and their wildtype counterparts