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Y5 neuropeptide Y receptor overexpression in mice neither affects anxiety- and depression-like behaviours nor seizures but confers moderate hyperactivity

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ABSTRACT

Neuropeptide Y (NPY) has been implicated in anxiolytic- and antidepressant-like behaviour as well as seizure-suppressant effects in rodents. Although these effects appear to be predominantly mediated via other NPY receptors (Y1 and/or Y2), several studies have also indicated a role for Y5 receptors. Gene therapy using recombinant viral vectors to induce overexpression of NPY, Y1 or Y2 receptors in the hippocampus or amygdala has previously been shown to modulate emotional behaviour and seizures in rodents. The present study explored the potential effects of gene therapy with the Y5 receptor, by testing effects of recombinant adeno-associated viral vector (rAAV) encoding Y5 (rAAV-Y5) in anxiety- and depression-like behaviour as well as in kainate-induced seizures in adult mice. The rAAV-Y5 vector injected into the hippocampus and amygdala induced a pronounced and sustained increase in Y5 receptor mRNA expression and functional Y5 receptor binding, but no significant effects were found with regard to anxiety- and depression-like behaviours or seizure susceptibility. Instead, rAAV-mediated Y5 receptor transgene overexpression resulted in moderate hyperactivity in the open field test. These results do not support a potential role for single transgene overexpression of Y5 receptors for modulating anxiety-/depression-like behaviours or seizures in adult mice. Whether the induction of hyperactivity by rAAV-Y5 could be relevant for other conditions remains to be studied.

1. Introduction

Neuropeptide Y (NPY) has been implicated in a wide range of physiological and pathological conditions, including anxiety, depression, and epilepsy (Redrobe et al., 2002b; Thorsell and Heilig, 2002; Heilig, 2004; Xapelli et al., 2006). Indeed, central administration of NPY in rodents exerts anxiolytic-like effects in the open field, elevated plus maze, and light/dark transition tests (Heilig et al., 1989; Sørensen et al., 2004; Karlsson et al., 2005), and antidepressant-like effects in the forced swim test and learned helplessness paradigm (Redrobe et al., 2002b; Ishida et al., 2007). Likewise, rats overexpressing NPY show anxiolytic-like behaviour (Thorsell et al., 2000), whereas NPY knockout mice display anxiogenic-like behaviour in the elevated plus maze (Palmiter et al., 1998; Karl et al., 2008). Central nervous system effects of NPY are predominantly mediated via the G-protein coupled receptors Y1, Y2, and Y5 (Xapelli et al., 2006) and Y1 receptors appear to be centrally involved in mediating anxiety- and antidepressant-like effects of NPY (Heilig et al., 1993; Ishida et al., 2007; Karlsson et al., 2008). Thus, Y1 antagonists inhibit NPY-induced anxiolytic- and depression-like behaviours (Sajdyk et al., 1999; Kask et al., 2002; Redrobe et al., 2002b; Ishida et al., 2007) whereas Y1 agonists induce anxiolytic-like effects (Heilig et al., 1993). In addition, intracerebroventricular NPY injections and viral vector-mediated hippocampal NPY overexpression fails to induce anxiolytic-like effects in Y1 receptor knockout mice (Karlsson et al., 2008; Lin et al., 2010), and we recently showed that vector-mediated overexpression of Y1 in the hippocampus induces anxiolytic-like effect (Olesen et al., 2012).

In contrast, Y2 receptors seem to induce pro-depressant and anxiogenic-like behaviour. Thus, injections of Y2 agonists cause anxiogenic-like behaviour in the elevated plus maze and social interaction tests (Nakajima et al., 1998; Sajdyk et al., 2002b) whereas Y2 receptor knockout mice exhibit anxiolytic-like behaviour (Redrobe et al., 2003; Tschenett et al., 2003). Furthermore, the

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Y2 receptor antagonist BIIE0246 induces antidepressant-like effect in the forced swim test, while the Y2 agonist PYY_{3-36} enhances depression-like behaviour in olfactory bulbectomized (OBX) rats (Morales-Medina et al., 2011). Some studies have also suggested a role for Y5 receptors in anxiety- and depression-like behaviour. Thus, the Y5 antagonist Lu AA33810 causes anxiolytic- and antidepressant-like effects (Walker et al., 2009) whereas the Y5 receptor agonist [cPP_{1-7},NPY_{19-23},Ala^{31},Aib^{32},Gln^{34}]-hPP induces anxiolytic-like effects (Sørensen et al., 2004). Likewise, the mixed Y5/Y2 preferring agonist NPY_{3-36} displays anxiolytic-like effect, which is blocked by pre-treatment with Y5 receptor antagonist (Sajdyk et al., 2002a).

Numerous studies have documented seizure-suppressant effects of NPY both in vivo and in vitro (Vezzani et al., 1999; Woldbye and Kokaia, 2004; Xapelli et al., 2006). In the hippocampus, these seizure-suppressant effects appear to be predominantly mediated via Y2 receptors (El Bahh et al., 2005), whereas Y1 receptors are thought to promote seizures (Benmaamar et al., 2003; Lin et al., 2006; Olesen et al., 2012). A seizure-suppressant role for Y5 receptors has also been proposed (Woldbye et al., 1997; Marsh et al., 1999; Baraban, 2002; Benmaamar et al., 2005). Seizure-suppressant effects may be particularly relevant outside the hippocampus; for instance, electrical kindling in the hippocampus of Y5 knockout mice as compared to wildtype mice is associated with significantly longer afterdischarge durations in the amygdala, but not in the hippocampus (Woldbye et al., 2005).

Over the past years, gene therapy has appeared as a potential future alternative to current treatments for several brain disorders (Manfredsson and Mandel, 2010; LeWitt et al., 2011). In animal models of epilepsy, including kainate-induced seizures, recombinant adeno-associated viral (rAAV) vector-mediated NPY overexpression in the hippocampus or piriform cortex have been shown to induce seizure-suppressant effects (Richichi et al., 2004; Lin et al., 2006; Foti et al., 2007; Sørensen et al., 2009; Noe et al., 2010). Our group recently showed that rAAV-mediated hippocampal overexpression of Y2 receptors suppresses while overexpression of Y1 receptors promotes kainate-induced seizures (Woldbye et al., 2010; Olesen et al., 2012). As for anxiety-like behaviour, rAAV-mediated overexpression of NPY in amygdala (Thorsell et al., 2007) or of NPY or Y1 receptors in the hippocampus (Lin et al., 2010; Olesen et al., 2012) confers anxiolytic-like effect in rodents. The aims of the present study were to explore effects of gene therapy using the Y5 receptor. Accordingly, we studied effects of rAAV-mediated Y5 overexpression on anxiety- and depression-like behaviours as well as kainate-induced seizures in adult mice. The viral vector was injected into both the hippocampus and amygdala to potentially optimise the effects of Y5 gene therapy since the NPY system in both brain regions have been implicated in emotional behaviour and seizures (Xapelli et al., 2006; Dumont et al., 2009).

2. Methods

2.1. Animals

To determine the efficacy of the novel vector for inducing overexpression of Y1 receptors, we used adult male Balb/c mice (vector control experiment: B&K, Sweden, 25–30 g). This experiment was part of a larger control experiment with Balb/c mice for testing several different viral vectors. After confirming efficacy of the vector, we used adult male NMRI mice (behavioural experiment: Taconic M&B, Denmark, 30–40 g) since we have more experience with this mouse strain in our behavioural tests (Christiansen and Woldbye, 2010; Christiansen et al., 2011; Olesen et al., 2012). The animals were housed in groups of 2–4 per cage with a 12-h light/dark cycle and access to food and water ad libitum. The mice were allowed to acclimatise for at least 7 days before being subjected to experiments performed in accordance with the Danish Animal Experimentation Inspectorate and the Swedish Animal Welfare Agency guidelines, approved by the local Ethical Committee for Experimental Animals.

2.2. rAAV vector injections

The rAAV vectors (GeneDetect, Auckland, New Zealand) derived from a mixture of serotypes 1 and 2, encoding the full-length cDNA of the mouse Y5 receptor (rAAV-Y5, stock solutions: 1.2×10^{12} genomic particles/ml; Y5 Gene Accession No. AF049329) and empty vector (rAAV-Empty, stock solution: 1.2×10^{12} genomic particles/ml). The transgenes were subcloned into a rAAV expression cassette consisting of the neuron-specific enolase promoter (NSE), woodchuck post-transcriptional regulatory element (WPRE), and a bovine growth hormone polyA signal (bGHpA) flanked by rAAV inverted terminal repeats (Richichi et al., 2004; Woldbye et al., 2010).

As described previously (Olesen et al., 2012), the mice were anesthetised by intraperitoneal (ip) injection of s-ketamine (80 mg/kg; Pfizer Inc., NY), xylazine (15 mg/kg; Sigma–Aldrich, St. Louis, MO), and a solution containing temgesic (0.06 mg/kg, Schering-Plough, DK), rimadyl (5 mg/kg, Pfizer ApS, DK) and baytril (10 mg/kg, Bayer Healthcare, Germany), and were placed into a stereotaxic frame (Kopf Instruments, USA). A volume of 1 μl viral vector suspension was infused through drill holes in the skull unilaterally into the dorsal hippocampus (vector control experiment: anterior–posterior (AP) −1.9 mm, medial–lateral (ML) +1.2 mm, dorsal–ventral (DV) −2.2 mm) or bilaterally into the hippocampus and amygdala (behavioural experiment: AP −2.9 mm, ML ± 3.0 mm, DV −2.8 mm; mid-hippocampal level, and AP −1.40 mm, ML ± 2.52 mm, DV −4.13 mm; amygdala; Paxinos and Franklin, 2001) through a glass pipette (0.1 μl/min) that was left in place for an additional 3 min after the injection. Reference points were bregma, midline, and dura for the AP, ML, and DV axis, respectively. A dorsal hippocampal level was used for vector injections in the control experiment because it was part of laboratory standard coordinates for testing of different transgenes in the hippocampus. In contrast, a mid-hippocampal level was used for the behavioural experiment in order to ensure transgene expression in more ventral parts of the hippocampus believed to be central for emotional and affective behaviour (Fanselow and Dong, 2010). Viral vector stock solutions were diluted 1:2 with sterile phosphate-buffered saline (PBS) before use. Postoperatively, each animal received a subcutaneous (sc) injection of isotonic saline (0.5 ml) to improve recovery.

2.2.1. Confirmation of rAAV-Y5-mediated transgene overexpression

In control mice not subjected to behavioural testing, rAAV-mediated Y5 receptor overexpression was confirmed 3 weeks post-injection by studying Y5 receptor binding and functional Y5 receptor binding (see below). In animals subjected to behavioural testing, consistent levels of rAAV-mediated Y5 transgene overexpression were confirmed 8 weeks post-injection by studying Y5 receptor mRNA levels (in situ hybridisation) and functional Y5 receptor binding (see also below).

2.3. Behavioural tests

Animals treated with rAAV-Y5 or rAAV-Empty vectors ($n = 11$ per group) were subjected to behavioural testing in the following order: open field, elevated plus maze, tail suspension, forced swim, SHIRPA, and light/dark tests, and kainate-induced seizures. All tests were performed with 3-day intervals, except the tail suspension