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Short communication

Comparison of the efficacy of five adeno-associated virus vectors for transducing dorsal raphé nucleus cells in the mouse

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ABSTRACT

Background: Delivery of genes to various brain regions can be accomplished using serotype 2 of the adeno-associated virus (AAV). Pseudotype AAV2 vectors, composed of the AAV2 genome packaged in the capsid of an alternative serotype, have increased efficiency of viral transduction. Transduction of pseudotype AAV2 vectors depends on cell type, brain region and stage of development. The dorsal raphé nucleus (DRN) and median raphé provides the majority of serotonin to forebrain regions and are implicated in the pathology and treatment of depression and anxiety. Viral vector technology in combination with stereotaxic surgery in mice provides a means to differentiate gene function in the DRN compared to the median raphé nucleus.
New method: Since AAV transduction efficiency has not yet been characterized for the DRN, we tested if AAV2 pseudotypes are more efficient than a standard serotype (AAV2/2) in transducing DRN cells in adult male mice on a C57BL/6J background.
Results: Although transduction did not differ significantly among vectors by 15 days post-injection, pseudotype AAV2/9 and AAV2/rh.10 vectors achieved significantly greater transduction of the DRN than did AAV2/2 and AAV2/1 vectors by 30 days post-injection. Pseudotypes AAV2/1 and AAV2/5 tended, although not significantly, to transduce DRN cells more efficiently than did AAV2/2.
Comparison with existing methods: At the same titer, all pseudotype AAV tested tended to transduce the DRN more efficiently than standard AAV2/2 serotype at 30 days post-injection.
Conclusions: Our results support the use of pseudotype AAV2/9 and AAV2/rh.10 for studying gene deletion or overexpression in the DRN.

1. Introduction

Of the nine raphé nuclei; the dorsal raphé nucleus (DRN) and median raphé nucleus produce the majority of serotonin to the brain via six ascending projections. Serotonin affects many physiological functions including sleep, appetite, circadian rhythm, autonomic function, fear, and mood, and has been implicated in the pathology and treatment of depression and anxiety disorders (Nutt, 2002). Because both the DRN and the median raphé innervate brain structures implicated in depression and anxiety such as the prefrontal cortex, amygdala, and hippocampus (Hale et al., 2013; Price and Drevets, 2010; Waselus et al., 2011); it is unclear how or if either of these regions contributes to depression and anxiety-related symptoms.

The DRN projects to additional forebrain, mid-brain, and brainstem regions not innervated by the median raphé (Hale et al., 2013) that may serve to distinguish the roles of the DRN and median raphé. For example, the DRN sends additional serotonergic forebrain projections to the nucleus accumbens (Waselus et al., 2011), which is thought to be involved in the anhedonia (loss of pleasure) often observed in depression (Price and Drevets, 2010). The DRN also innervates the hypothalamus (Waselus et al., 2011), which may be involved in the altered sleeping patterns and weight fluctuations observed in depressed patients (Price and Drevets, 2010). The DRN brainstem projections have been shown to control panic and sympathetic nervous system responses, which may be relevant to anxiety disorders (Hale et al., 2013). These additional projection targets suggest that the DRN may be involved in a wider range of functions relevant to depression and anxiety than the median

Abbreviations: DRN, dorsal raphé nucleus; eGFP, enhanced green fluorescent protein; rAAV, recombinant adeno-associated virus; TBS, Tris buffered saline; TBS-T, Tris-buffered saline with 0.3% Triton-X-100.

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raphé. The use of virally transduced gene expression in the mouse DRN will provide a means of rapid gene deletion in vivo to differentiate DRN-specific functions from those of the median raphé and other raphé nuclei.

Recombinant adeno-associated virus (rAAV) was selected for this study because rAAV efficiently transduce non-dividing cells such as neurons (Kaplitt et al., 1994). rAAV also elicit lower immunogenic responses compared to other viral vectors (Burger et al., 2004). In addition, unlike retrovirus or lentivirus vectors, rAAV do not incorporate into the host genome (Chooi et al., 2002), which could lead to mutations that compromise the effects of gene manipulation. Furthermore, since rAAV do not incorporate into the genome, this viral vector also provides a safer option for studying gene deletion or expression compared to other viruses such as retrovirus or lentivirus. Additional advantages of using rAAV vectors include the ability to achieve both region-specific and relatively rapid gene deletion in weeks compared to transgenic mouse models, which may take months to achieve complete gene deletion (Burger et al., 2004). Since serotonin facilitates neuronal division, cell migration, and synaptogenesis during development (Gasper et al., 2003), compensatory effects from prolonged gene deletion could be significant (Burger et al., 2004). Therefore, rAAV vectors offer a relatively safe, rapid approach for region-specific and temporally defined gene manipulation.

The AAV capsid proteins determine the ability of the AAV virus to enter a target cell and consequently transduce, or express, the gene of interest (Chooi et al., 2002). To increase rAAV transduction efficiency, pseudotype rAAVs have been established in which the AAV2 genome is packaged into the capsid of another AAV serotype (Rabinowitz et al., 2002). For example, AAV2/9 denotes that the AAV2 genome carrying the transgene was packaged in an AAV9 capsid.

We used stereotaxic surgery to inject different rAAV vectors into the DRN of mice to determine if pseudotypes of AAV2 are more efficient than the parent serotype (AAV2/2) in transducing DRN cells. The titer, volume, and promoter for the fluorescent tag were the same among all viral vectors, allowing the extent of fluorescent marker expression to be used to determine the transduction efficiency of the different AVV serotypes in the DRN (Holehonnur et al., 2014). To determine the time point with maximal viral transduction, mice were sacrificed at 15 and 30 days post-injection to collect the brains for immunofluorescence imaging.

2. Materials and methods

2.1. Animals

All animal use was approved by the Institutional Animal Care and Use Committee of Albany Medical College and followed the standards of the NIH *Guide for the Care and Use of Animals* (Institute of Laboratory Animal Resources, 2010). Experiments used male mice on a pure C57BL/6J background with a floxed glucocorticoid receptor gene from our colony (Vincent et al., 2013). Mice were housed on a 12 h light/12 h dark cycle (lights on at 7:00 a.m.) with ad libitum access to rodent chow.

2.2. Stereotaxic injection

Mice were injected with recombinant AVV2 vectors expressing the fluorescent marker, enhanced green fluorescent protein (eGFP) obtained from the Gene Therapy Center and Vector Core of the University of Massachusetts Medical School (Worcester, MA). All mice were 2–3 months old at the time of surgery. Mice were anesthetized with 125 mg/kg ketamine and 12.5 mg/kg xylazine, ip. Anesthetized mice were placed in Kopf model 922 tapered mouse ear bars and mounted in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was anesthetized with bupivicaine (Abbott Laboratories, Abbott Park, IL). The skull was then exposed, and bregma and lambda were visualized with a dissecting microscope (PZMIII-AAC; World Precision Instruments, Sarasota, FL). A digitizer (Stoelting, Wood Dale, IL) attached to the micromanipulator of the stereotaxic apparatus was used to locate coordinates from bregma or lambda. Burr holes were drilled in the skull using a Dremel drill equipped with a 0.75 mm carbide bit (Stoelting). 500 nl of virus was injected at 10 nl/s through 33 ga. stainless steel beveled tubing (Vita Needle, Needham, MA) attached by PE 20 tubing (Fisher, Pittsburgh, PA) to a 2 μl Hamilton Syringe (Fisher). rAAV virus stocks were diluted on the day of surgery to 6×10^{12} genomic copies/ml with sterile 1× Dulbecco's phosphate buffered saline (Cellgro, Manassas, VA). For each injection, we administered 500 nl of AAV at a titer of 6×10^{12} genomic counts/ml into the DRN using the following coordinates from the bregma: anteroposterior, −4.5 mm; mediolateral, 0.0 mm; dorsoventral, −3.5 mm. The injector was left in place 9 min before being withdrawn at 0.1 mm/10 s to permit diffusion of the virus and to minimize backflow of the virus after needle retraction. After the scalp incision was sutured, 1 mg/kg indomethacin ip (Alexis Biochemicals, San Diego, CA) in 10 ml/kg of sterile-filtered 0.1 M sodium carbonate was provided for analgesia. In addition, 0.001 M indomethacin (Sigma Aldrich, St. Louis, MO) solution in 0.1 M sodium carbonate (Fischer) was provided as a separate drinking fluid for 3 days after surgery. Mice were individually housed after surgery. Mice were sacrificed 15 and 30 days after surgery to collect the brains for immunohistochemistry (see below).

2.3. Recombinant adeno-associated virus vectors

Recombinant adeno-associated vectors (rAAV) expressing eGFP under the control of the a promoter containing the cytomegalovirus immediate – early (CMV IE) enhancer and 260 bp (nucleotides −1261 to −1001) of the chicken β-actin 6 promoter were packaged and provided by the Gene Therapy Center and Vector Core of the University of Massachusetts Medical School, Worcester, MA (Gao and Sena-Esteves, 2012). The AAV2 genome was packaged into AAV capsids from AAV1, AAV5, AAV9, and AAVrh.10, generating recombinant pseudotypes that will hereafter be referred to as AAV2/1, AAV2/5, AAV2/9, and AAV2/rh.10.

2.4. Immunocytochemical analysis of GFP expression

Mice were given lethal injections of sodium pentobarbital (100 mg/kg, ip) and perfused intracardially via a peristaltic pump (Harvard Apparatus, Holliston, MA) with 0.9% saline and then 4% paraformaldehyde. Brains were stored after perfusion fixation in 4% paraformaldehyde overnight at 4 °C and then cryoprotected in 30% sucrose at 4 °C (Vincent et al., 2013). Within two weeks of cryoprotection, coronal sections (25 μm) were cut using a cryostat (Leica, Buffalo Grove, IL), and distributed sequentially over the wells of a 6-well tissue culture plate (Corning Incorporated Life Sciences, Lowell, MA). Free-floating sections were stored in cryoprotectant solution (50% 0.05 M Tris buffered saline (pH 7.5), 30% ethylene glycol, 20% glycerol) in the 6-well tissue culture plate at −20 °C.

Coronal brain sections were washed in Tris-buffered saline (TBS) with Triton (TBS-T; 0.01 M Tris pH 7.5, 0.15 NaCl, 0.3% Triton-X-100), blocked for 30 min at room temperature with 3% normal goat serum (Vector Laboratories, Burlingame, CA) in TBS (0.01 M Tris buffered saline, 0.15 NaCl), and incubated overnight at 4 °C in chicken anti-GFP (1:500, Invitrogen, cat. no. A-10262). On day 2, sections were washed in TBS-T buffer, incubated for 1 h at room temperature in Alexa-488 goat anti-chicken in TBS (1:400, Invitrogen), and washed in TBS-T buffer. The sections were mounted on