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Brain Research

Research Report

Selective transgene expression in cerebellar Purkinje cells and granule cells using adeno-associated viruses together with specific promoters

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ARTICLE INFO

Article history:
Accepted 11 May 2015
Available online 16 May 2015

Keywords:
Adeno-associated virus
Cerebellum
Purkinje cell
Granule cell
Stereotaxic injection
Gene delivery

ABSTRACT

Adeno-associated virus (AAV) is a powerful tool for gene delivery into the brain and has been used for transgene expression in the cerebellar cortex. Although the efficacies of different AAV serotypes to transduce cerebellar Purkinje cells were examined, it has been difficult to achieve cell-type specific transgene expression. Here we used AAV serotype 1 with two specific promoters, namely, Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα) and the minimum region of the $GABA_A$ receptor α6 subunit (GABRα6) promoters, and compared their expression patterns in the cerebellar cortex with the expression patterns of ubiquitous promoters that are often used for AAV-mediated expression. Whereas AAV with ubiquitous promoters, the cytomegalovirus early enhancer/chicken β-actin promoter, and a small fragment of the synapsin-1 gene promoter caused ubiquitous expression in all cerebellar neurons tested, AAV with the CaMKIIα promoter injected into 10-day-old mice enabled selective expression in Purkinje cells. Furthermore, we developed AAV with the GABRα6 promoter, and succeeded for the first time to express the transgene exclusively in granule cells. Fresh cerebellar slices of mice injected with these AAVs were applicable for physiological experiments, such as patch clamp recording, optogenetic imaging, and stimulation. Thus, these AAV vectors are useful tools towards understanding the basic properties of cerebellar neurons or mechanisms of cerebellar functions. Particularly, selective expression in Purkinje or granule cells is useful for analyses using genetically-modified animals, such as knockout mice.

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http://dx.doi.org/10.1016/j.brainres.2015.05.015
0006-8993/

1. Introduction

Adeno-associated virus (AAV) has been considered as a powerful tool for gene delivery in the brain, because of its safety, efficacy, and stability of gene transfer (Lentz et al., 2012). AAV has also been successfully used for cell-type specific expression by combining transgenic animals expressing Cre-recombinase under the control of a cell-type specific promoter (Betley and Sternson, 2011). Another important potential of AAV is the availability of multiple serotypes, which may show different cellular tropism of gene delivery owing to their different capsid proteins (Buning et al., 2008; Kwon and Schaffer, 2008). Thus, AAV is not only a powerful tool for gene delivery, but also has the great potential to achieve cell-type specific gene manipulation. Nevertheless, further efforts must be devoted towards fulfilling such a potential.

Neural circuits in the cerebellar cortex are relatively well organized and are composed of several types of cells, such as Purkinje, granule, Golgi, stellate/basket, and Bergmann glial cells (Reeber et al., 2012; Schilling et al., 2008). Viral vector-mediated gene delivery in Purkinje cells has been performed in various research on neurodegenerative disorders as well as Purkinje cell development or function (Alisky et al., 2000; Broekman et al., 2006; Gibson et al., 2014; Kaemmerer et al., 2000; Kaneko et al., 2011; Najafi et al., 2014; Nguyen-Vu et al., 2013; Takayama et al., 2008; Torashima et al., 2006; Tsubota et al., 2011; Xia et al., 2004). Several serotypes of AAV were also tested, and expression of the transgene in Purkinje cells was observed using AAV serotype 1 (AAV1), 2, 5, or 8 (Hirai, 2008). Although the AAVs used in these studies, in which transgene expression was controlled by a ubiquitous promoter, dominantly transduced Purkinje cells, transgene expression was not completely specific to Purkinje cells. In addition, viral vectors that preferably transduce granule cells, another major neuronal type in the cerebellar cortex, have not been reported to date.

Utilizing cell-type specific promoters is not a favorable option for creating cell-type specific AAVs, owing to the limitation of its packaging capacity of 5 kb. On the other hand, small fragments of specific promoters have been used for AAVs (Guo et al., 2014; Johansen et al., 2010; Kügler et al., 2003), and hence this may be one way for achieving cell-type selective expression of molecules in a given area of the brain. However, AAVs with specific promoters have not been systematically tested in the cerebellum to date. Furthermore, understanding the expression patterns of molecules expressed in the cerebellum by AAV may expand the possibilities of their use in combination with Cre transgenic mice.

In this study, we used two AAV1s with specific promoters and two AAV1s with ubiquitous promoters, to compare the expression patterns of molecules transduced by these AAVs in the cerebellar cortex. Our results demonstrated that, although all of these AAVs successfully transduced cells in the cerebellar cortex, their cellular selectivity of transgene expression varied. The AAV vectors used in this study are useful tools for basic research and may have the potential for application to clinical research.

2. Results

2.1. Broad expression patterns of transgenes driven by the CAG or sSyn promoter of AAV injected into the cerebellum

To investigate the efficacy of various promoters in expressing transgenes in cells of the cerebellar cortex, we used AAV1 vectors that drive GFP expression under the control of four different promoters, cytomegalovirus early enhancer/chicken β-actin (CAG), a short fragment of the synapsin-1 gene promoter (sSyn), Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα), and the minimum region of the $GABA_A$ receptor α6 subunit promoter (GABRα6) (Fig. 1A; AAV1-promoter-GFP). The AAV vectors, with estimated titers of 10^{12}–10^{13} vector genome copies, were stereotaxically injected into cerebellar lobe IV–V of 5 to 6-week-old or 9 to 11-day-old mice (Fig. 1B). At 10 to 14 days after injection, the mice were fixed, and cerebellar slices of the vermis, where GFP expression was detected (Fig. 1C), were subjected to immunohistochemistry using antibodies of marker proteins. Because GFP expression was mostly observed in lobe IV–V (Fig. 1D), analyses were performed in lobe IV–V of 6 to 8-week-old or 3 to 4-week-old mice, which we defined as adult or young mice, respectively, unless otherwise stated.

We first examined the expression of GFP driven by AAV1 with ubiquitous promoters. The CAG promoter as well as other ubiquitous promoters, such as cytomegalovirus or elongation factor-1α promoter, have been used for AAV-mediated transduction of cerebellar Purkinje cells (Broekman et al., 2006; Kaemmerer et al., 2000; Kaneko et al., 2011; Najafi et al., 2014; Nguyen-Vu et al., 2013; Xia et al., 2004). Consistently, we also confirmed that AAV1 with the CAG promoter (AAV1-CAG) successfully drove GFP expression in Purkinje cells of adult mice, which were visualized by staining with a calbindin antibody (Fig. 2A). However, GFP expression was also apparent in other types of neurons, including stellate/basket and granule cells, which were stained by antibodies of parvalbumin and neuronal nuclear antigen (NeuN), respectively (Fig. 2A). GFP expression was also detected in Golgi cells (Fig. 2A), which were identified as large somatostatin-positive cells located in granule cell layers (Geurts et al., 2001). In addition, GFP fluorescence was apparent in glial cells and their processes, which were stained by a glial fibrillary acidic protein (GFAP) antibody (Fig. 2A and D). The CAG ubiquitous promoter resulted in transgene expression in all the cell types tested, suggesting that the AAV1 used in this study has broad cellular tropism in the cerebellar cortex of adult mice.

The sSyn promoter coupled with AAV has been used to confer exclusive neuronal expression *in vitro* and *in vivo* (Kügler et al., 2003). Because there are several types of neurons in the cerebellar cortex, we tested whether AAV1 with the sSyn promoter (AAV1-sSyn) has any selectivity or preference of gene expression among cerebellar neurons. In adult mice, GFP expression was observed in all neurons of the cerebellar cortex tested, namely, Purkinje, granule, stellate/basket, and Golgi cells (Fig. 2B). In contrast to AAV1-CAG (Fig. 2D), AAV1-sSyn did not result in transgene expression in glial cells (Fig. 2B, GFAP), as expected from previous studies in other