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Activity-dependent volume transmission by transgene NPY attenuates glutamate release and LTP in the subiculum

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ABSTRACT

Neuropeptide Y (NPY) gene transduction of the brain using viral vectors in epileptogenic regions can effectively suppress seizures in animals, and is being considered as a promising alternative treatment strategy for epilepsy. Therefore, it is fundamental to understand the detailed mechanisms governing the release and action of transgene NPY in neuronal circuitries. Using whole-cell recordings from subicular neurons, we show that in animals transduced by recombinant adeno-associated viral (rAAV) vector carrying the NPY gene, transgene NPY is released during high-frequency activation of CA1-subicular synapses. Released transgene NPY attenuates excitatory synaptic transmission not only in activated, but also in neighboring, non-activated synapses. Such broad action of transgene NPY may prevent recruitment of excitatory synapses in epileptic activity and could play a key role in limiting the spread and generalization of seizures.

Introduction

One of the most prominent novel strategies to interfere with neurological disease processes is considered to be a gene therapy approach based on viral vectors to transfer genes of interest into the brain. In this regard, NPY gene transduction of brain tissue has attracted particular interest due to its potential to regulate and perhaps ameliorate neurological conditions, in particular epileptic disorders (McCown, 2004; Noe' et al., 2007). It has been demonstrated that delivery of recombinant adeno-associated viral (rAAV) vector carrying the NPY gene into the rat hippocampus or piriform cortex can effectively suppress acutely intracerebroventricular and intrahippocampal (Richichi et al., 2004), as well as intra-peritoneal (Foti et al., 2007) kainic acid-induced limbic seizures, respectively. More recently, hippocampal rAAV–NPY vector-based gene therapy was shown to suppress the frequency of spontaneous seizures in chronically epileptic rats (Noè et al., 2008). The mechanisms underlying such seizure-suppressant effects of transgene NPY are not well understood. Particularly, under which circumstances transgene NPY is released, and whether and how it acts on synaptic transmission within the area of viral vector transduction is not known. These questions are of fundamental importance not only for the implementation of such gene therapy approach in clinical trials with patients, but also for our general understanding of how transgene neuropeptides may act in the brain.

In the normal brain, exogenously applied NPY has been shown to modulate inhibitory (Bacci et al., 2002) and excitatory synaptic transmission (Haas et al., 1987) in cortical and hippocampal regions, respectively. The most thoroughly described action of NPY in the central nervous system is its pronounced inhibitory effects on excitatory synaptic transmission in the hippocampal formation (Colmers and Bleakman, 1994). NPY has been shown to decrease glutamate release (Colmers et al., 1988; Klapstein and Colmers, 1993) by inhibiting presynaptic, voltage-dependent Ca^{2+}-channels in glutamatergic terminals in CA1 (Qian et al., 1997).

In the present study, we explore under which circumstances transgene NPY is released from afferent synapses impinging onto subicular pyramidal neurons, and investigate how it modulates excitatory synaptic transmission and plasticity in these synapses. The subicular pyramidal neurons serve as main relay stations for outgoing efferents of CA1 pyramidal cells (O'Mara et al., 2001), and therefore may control spreading of seizure activity from the hippocampal formation. Moreover, some clinical and experimental evidence suggests that temporal lobe epileptic (TLE) activity could be even initiated in the subiculum (Stafstrom, 2003, 2005). In contrast to the CA1 and CA3 areas of the hippocampus, there is relatively little neuronal loss and insignificant reactive gliosis in the subiculum of patients with TLE (Fisher et al., 1998; Dawodu and Thom, 2005).

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Therefore, the preserved subiculum in TLE could offer an attractive target area for gene transfer in order to suppress spreading of epileptic activity from the hippocampal formation.

Results

Transgene NPY expression

Viral transduction and transgene expression in brain slices, prepared three weeks after rAAV–NPY vector administration, was examined by immunohistochemistry. In these animals, high levels of transgene NPY immunoreactivity were detected throughout the hippocampal formation including CA1, CA3, dentate gyrus, dentate hilus and proximal subiculum (Fig. 1A). Dense transgene NPY expression was particularly observed within the inner molecular layer and hilus of the dentate gyrus, and also within the mossy fiber terminals in CA3 and pyramidal cell layer of CA1. Maximal expression of transgene NPY was observed within two weeks after rAAV–NPY vector injection, and it remained stable for at least three months (data not shown). Transgene NPY expression was selective to neurons,