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Research report

Neuropeptide Y suppresses absence seizures in a genetic rat model

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Abstract

Evidence from studies in rodents and humans support an anti-seizure action of neuropeptide Y (NPY) in focal, acquired epilepsy. However, the effects of NPY in generalized genetic epilepsy remain unexplored. In this study, adult male Genetic Absence Epilepsy Rats of Strasbourg (GAERS) were implanted with extradural electrodes and an intracerebroventricular (icv) cannula. Six and 12 nmol NPY or vehicle was administered icv in a random order ($n = 6$), and the effect of NPY on seizure activity quantitated from a 90-min EEG recording. A rapid onset and sustained seizure suppression was observed following NPY treatment compared to vehicle, with both 6 and 12 nmol NPY having a significantly decreased mean percentage time in seizure (5.7 ± 1.4% and 5.0 ± 1.7% vs. 15.8 ± 3.4%) and mean number of seizures per minute (0.5 ± 0.1 and 0.4 ± 0.1 vs. 1.1 ± 0.1). There was no significant difference between the degree of seizure suppression after 6 and 12 nmol NPY. The results of this study demonstrate that NPY suppresses absence seizures in GAERS. This suggests that NPY modulates pathological oscillatory thalamocortical activity and may represent a new therapeutic approach for the treatment of generalized epilepsies.

Theme: Disorders of the nervous system
Topic: Epilepsy: human and animal models

Keywords: Generalized epilepsy; GAERS; Thalamocortical; Absence seizures; NPY

1. Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide that is widely and abundantly expressed in the mammalian CNS [1]. NPY is a member of the pancreatic polypeptide family, which also comprises peptide YY (PYY) and pancreatic polypeptide (PP). The three peptides share structural similarities and activate multiple receptors. To date, five G protein coupled NPY receptors have been cloned [17]; these are linked to the inhibition of adenylate cyclase and the regulation of intracellular calcium levels. NPY is co-localized with several other neurotransmitters and is a critical inhibitory regulator of neuronal excitability. Evidence for the involvement of NPY as an endogenous anticonvulsant, and possibly antiepileptogenic, agent in *acquired focal epilepsies* (e.g. temporal lobe epilepsy, TLE) has been steadily accumulating from both in vivo and in vitro studies in animals and man.

One of the few phenotypic alterations observed in the NPY knockout mouse is increased susceptibility to chemically induced seizures [5]. More recently, transgenic mice with reduced NPY gene expression were shown to have increased mortality following kainic acid-induced seizures [4]. Conversely, transgenic overexpression of NPY reduced the susceptibility to seizures induced by both kainic acid and electrical stimulation [27]. Importantly, NPY has also been shown to inhibit acquired limbic epileptogenesis by delaying electrical hippocampal kindling in rats, both when administered by infusion into the hippocampus [19] and also with enhanced endogenous hippocampal expression via the

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local application of recombinant adeno-associated viral vectors [20]. NPY expression is increased following acute seizures induced by kainic acid injection and repeated electroconvulsive shocks in rats [9,11], as well as following electrical and chemically induced kindling [13,15]. NPY is also selectively increased in galanin receptor knockout mice that manifest spontaneous seizures [6]. Support for a role for NPY in human epilepsy is provided by the observation of increased NPY expression in temporal lobe tissue from patients who underwent surgery for medically refractory focal seizures [7]. In vitro experiments on rat hippocampal slices demonstrated NPY suppression of glutamate release [8], and this has subsequently been shown to occur via the regulation of calcium currents [16,22]. Similar effects have also been found in human hippocampal tissue [14].

Taken together, these data are strongly supportive of a role for NPY following acute provoked seizures and in focal acquired epilepsy, particularly limbic epilepsy. What has been less certain is the importance of this neurotransmitter in genetic generalized syndromes, such as absence epilepsy. Given that the generalized epilepsies differ greatly in their pathogenesis and neuronal circuitry (i.e. thalamocortical vs. limbic), the effect of NPY needs to be specifically investigated in models of these epilepsies. Some data are available that suggest that NPY may have a role in regulating thalamocortical circuitry. NPY is released from the reticular thalamic nucleus (nRT) neurons during electrical stimulation-induced rhythmic oscillatory thalamocortical activity in non-epileptic rat brain slices [26]. However, only one previous study has investigated the role of NPY in a whole animal model of genetic generalized epilepsy, the Spontaneously Epileptic Rat (SER)—a double mutant strain that has tonic convulsions in addition to absence-like seizures [21]. Increased NPY expression was found in several brain regions (striatum, amygdala, hippocampus and mesolimbic system) in homozygote SERs compared with heterozygotes, but thalamic regions were not studied. To our knowledge, no previous study has investigated the effects of NPY on seizure expression in models of generalized genetic epilepsy. In this study, we investigated the effect of injections of exogenous NPY on seizure expression in one of the best validated animal models of generalized genetic epilepsy, the Genetic Absence Epilepsy Rats of Strasbourg (GAERS) [3].

GAERS are an inbred Wistar line, which, after 8–13 weeks of age, exhibit spontaneous absence-like seizures while in a quiet waking state [3]. The animals manifest no other neurological deficits. During seizures, the animals have arrest of activity, often associated with some mild clonic activity of neck muscles and whisker twitching. The electroencephalogram (EEG) during seizures demonstrates well formed 5–8 Hz generalized spike and wave discharge (SWD), which is morphologically similar to human absence epilepsy (with a faster frequency). The seizures in GAERS demonstrate a similar pharmacoresponsiveness to antiepileptic drugs to that seen in human absence epilepsies and thus represent a useful approach to explore the underlying mechanisms of generalized epilepsy.

2. Material and methods

2.1. Animals

Male animals (NEC and GAERS) were obtained from a breeding colony housed in the Ludwig Institute of Cancer Research/Department of Surgery animal house at the Royal Melbourne Hospital. All animals were aged between 12 and 15 weeks at the time of surgery. Following surgery, the animals were housed in individual cages and fed a diet of normal rat chow and water. All procedures performed were approved by the Ludwig Institute of Cancer Research/Department of Surgery Animal Ethics Committee (AEC #03/11) and were in accordance with NHMRC guidelines on the use of animals for scientific research.

2.2. Surgeries

Rats were anesthetized with ketamine and xylazine (7.5 mg ketamine and 1.0 mg xylazine/100g body weight, ip). Five extradural screw electrodes were implanted through burr holes (three to the left and two to the right of the midline: two anterior and three posterior to the bregma). Additionally, an icv catheter was implanted 1.5 mm to the right and 1.0 mm posterior to the bregma using stereotactic frame guidance (Kopf™), with the head positioned at an angle of 90° to the implantation trajectory. A 22 gauge guide cannula and injecting needle (Plastics One™) were lowered into the hole to a depth of 3–3.5 mm ventral to the dura, until it entered the right lateral ventricle (confirmed by drawback of CSF in the injecting line). Dental cement (Vertex™) was used to hold all components in position before the rat was removed from the frame. The injecting needle was removed from the guide cannula, which was sealed with an obturator. Three days after surgery, animals received 1 nmol angiotensin II (Auspep, Melbourne, Australia) icv, and their water intake was observed, in order to verify the correct placement of the cannula. In animals where the icv catheter was correctly placed, excessive drinking induced by the angiotensin was demonstrated immediately and observed for 30 min. The animals were handled on a daily basis to accustom them to the investigator and minimize handling associated stress.

2.3. NPY injections and EEG recordings

Injections of NPY commenced after a 7-day post-surgery recovery period. The studies took place in a well-lit, generally quiet room with the rats in their home cages. In order to limit any diurnal variability, serial studies were performed at the same time of the day for each animal. Wires were attached to the electrodes by gold crimp pins to