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Pregabalin action at a model synapse: Binding to presynaptic calcium channel α_2-δ subunit reduces neurotransmission in mice☆

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Abstract

Pregabalin, ((*S*)-3-(aminomethyl)-5-methylhexanoic acid, also known as (*S*)-3-isobutyl GABA, Lyrica™) is approved for treatment of certain types of peripheral neuropathic pain and as an adjunctive therapy for partial seizures of epilepsy both the EU and the USA and also for generalized anxiety disorder in the EU. Though pregabalin binds selectively to the alpha$_2$-delta (α_2–δ) auxiliary subunit of voltage-gated calcium channels, the cellular details of pregabalin action are unclear. The high density of α_2−δ in skeletal muscle fibers raises the question of whether pregabalin alters excitation–contraction coupling. We used the mouse soleus neuromuscular junction from mice containing an artificially mutated α_2–δ Type 1 protein (R217A) as a model to examine the effect of pregabalin. Pregabalin reduced nerve-evoked muscle contractions by 16% at a clinically relevant concentration of 10 μM in wildtype mice. When acetylcholine receptors were blocked with curare, pregabalin had no effect on contraction from direct stimulation of muscle, suggesting a lack of drug effects on contraction coupling. Our data are consistent with pregabalin having no effect on striated muscle L-type calcium channel function. However, in mice expressing mutant (R217A) α_2–δ Type 1, there was no significant effect of pregabalin on nerve-evoked muscle contraction. We propose that pregabalin reduces presynaptic neurotransmitter release without altering postsynaptic receptors or contraction coupling and that these effects require high affinity binding to α_2–δ Type 1 auxiliary subunit of presynaptic voltage-gated calcium channels.
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Keywords: Presynaptic; Calcium channel; Analgesia; Anticonvulsant; Anxiolytic; Transmitter release

1. Introduction

Pregabalin has recently been approved and marketed for the treatment of peripheral neuropathic pain and epilepsy in the European Union and in the USA for neuropathic pain from post-herpetic neuralgia, diabetic neuropathy and as adjunctive treatment for partial seizures. It recently has received approval in EU for treatment of generalized anxiety disorder. It is effective and well tolerated in several large randomized and placebo-controlled clinical trials for each of these indications (Beydoun et al., 2005; Dworkin et al., 2003; Lesser et al., 2004; Pande et al., 2003; Pande et al., 2004; Pohl et al., 2005; Richter et al., 2005; Rickels et al., 2005; Rosenstock et al., 2004).

Pregabalin (Lyrica™) is a close structural relative of gabapentin (Neurontin™), both being alkylated analogues of γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system (Bryans and Wustrow, 1999). However, neither compound interacts with $GABA_A$ or $GABA_B$ receptors in radioligand binding assays, nor does pregabalin alter physiological responses to $GABA_A$ or $GABA_B$ ligands in cultured neurons or recombinant receptor systems, indicating that neither compound interacts with pre- or post-synaptic GABA receptors. Furthermore, pregabalin and gabapentin are not blockers or substrates for GABA transporters (Su et al., 2005) and neither compound alters rat whole-brain GABA concentrations (Errante and Petroff, 2003).

The exact cellular mechanism of action of pregabalin is still unclear, although evidence from several studies suggests that it reduces excitatory neurotransmitter release in a manner similar to the structurally related compound, gabapentin (Cunningham et al., 2004; Dooley et al., 2002; Dooley et al., 2000a; Dooley et al., 2000b; Fehrenbacher et al., 2003; Fink et al., 2002;

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Maneuf et al., 2001) by binding to the $\alpha_2-\delta$ auxiliary subunit (Gee et al., 1996) of voltage-gated calcium channels in brain and spinal cord. Calcium channels purified from native tissues contain the main calcium conducting pore protein (α_1) and also several auxiliary proteins, including a cytosolic β subunit, and transmembrane $\alpha_2-\delta$ and γ subunits (see Arikkath and Campbell, 2003 for a review of calcium channel auxiliary subunits). Calcium channel α_1 subunits are products of 10 separate genes, and these different channels are expressed in a heterogeneous manner in different brain and peripheral tissues (Yu and Catterall, 2004). Most of the various calcium channel α_1 type proteins are thought to associate with a single $\alpha_2-\delta$ protein in vivo.

There are two different genes (CACNA2D1, CACNA2D2 or $\alpha_2-\delta$ Types 1 and 2, respectively) that each encode closely-related $\alpha_2-\delta$ proteins that have high affinity binding sites for gabapentin and pregabalin (Hobom et al., 2000; Klugbauer et al., 2003). Two other $\alpha_2-\delta$ isoforms (CACNA2D3, CAC NA2D4) appear to lack these high affinity binding sites (Marais et al., 2001; Qin et al., 2002). Several studies suggest that the major isoform of $\alpha_2-\delta$ in neocortex, spinal cord dorsal horn and skeletal muscle is $\alpha_2-\delta$ Type 1 (Bian et al., 2006; Cole et al., 2005). Generation of a knockout mouse strain that lacks protein expression of $\alpha_2-\delta$ protein would be a good test of the contribution of this binding site to pregabalin pharmacology. However, knockouts of $\alpha_2-\delta$ Type 1 are lethal in mice at birth (J. Offord, unpublished observations) and spontaneous genetic mutant mice that are functional knockouts of $\alpha_2-\delta$ Type 2 have frequent seizures, ataxia, tremors and short lifespan (Brill et al., 2004), which compromises their use as an experimental tool. To avoid the difficulties with $\alpha_2-\delta$ knockout mice and to investigate the contribution of $\alpha_2-\delta$ drug binding to pharmacology of pregabalin, a genetically-modified mouse was generated with an arginine-to-alanine mutation at amino acid 217 in the $\alpha_2-\delta$ Type 1 protein gene sequence (GenBank accession no. NM_009784, Mus musculus, voltage-dependent calcium channel alpha$_2$–delta subunit (Type 1)) (Bian et al., 2006; Bramwell et al., 2004; Li et al., 2003). This mutation reduced [^{3}H]-pregabalin binding in forebrain, without altering the abundance of $\alpha_2-\delta$ protein expression, or causing any obvious changes in mouse phenotype or behavior (Bian et al., 2006).

The $\alpha_2-\delta$ Type 1 subunit is expressed at high levels in skeletal, cardiac and vascular smooth muscle and in the brain (Angelotti and Hofmann, 1996) and can associate with several different α_1 subunit isoforms. Heterologous studies in which $\alpha_2-\delta$ subunits have been co-expressed with various calcium channel α_1 subunits show that $\alpha_2-\delta$ increases the expression level of functional calcium channels in the membrane, and alters the voltage dependence and kinetics of calcium currents (Arikkath and Campbell, 2003). It has been reported that gabapentin binds to $\alpha_2-\delta$ protein from brain and skeletal muscle with similar kinetics and high affinity (K_d=38 nM in brain and 29 nM in skeletal muscle (Gee et al., 1996; Suman-Chauhan et al., 1993)). However, the effects of high-affinity $\alpha_2-\delta$ drug ligands on skeletal muscle function have not been previously examined. The high density of $\alpha_2-\delta$ protein in skeletal muscle fibers (Gee et al., 1996) therefore raises questions of whether pregabalin alters skeletal muscle physiological processes such as excitation–contraction coupling (ECC) or if its actions are limited within the neuromuscular junction and other synapses. Using the mouse neuromuscular junction as a model system, this study tests the hypotheses that pregabalin-induced reduction in neurotransmission requires high-affinity binding to the $\alpha_2-\delta$ protein and that pregabalin does not alter skeletal muscle excitation–contraction coupling.

2. Materials and methods

2.1. R217A mutant mice

A strain of mutant mice with the R217A mutation to $\alpha_2-\delta$ Type 1 that was previously shown to reduce [^{3}H]gabapentin binding in a recombinant system (Wang et al., 1999), has been fully described in a separate communication (Bian et al., 2006). Briefly, a polymerase chain reaction fragment with the R217A mutation to the $\alpha_2-\delta$ Type 1 gene was generated from a mouse genomic DNA library. This fragment was introduced, through homologous recombination, into a yeast strain carrying a mouse genomic library. A clone containing a portion of the $\alpha_2-\delta$ Type 1 genomic DNA with the mutation was placed into a neo cassette to generate the targeting construct used to create the mutant mice. This targeting vector was introduced into 129/SvEvBRD embryonic stem cells via electroporation and homologous recombination. Selected clones were implanted into blastocytes derived from C57Bl/6 albino mice and transferred into C57Bl/6 pseudo-pregnant dams. The offspring chimeras were bred with C57Bl/6 mice. The pups with the mutation were then bred with 129/SvEv mice to generate a hybrid heterozygous colony that was inbred to obtain individually genotyped mice (either homozygous R217A mutant or homozygous wildtype) used in this study.

Animal care and surgical procedures were conducted in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. The American Association for the Accreditation of Laboratory Animal Care accredited housing facilities. An internal animal use committee approved all experimental procedures.

2.2. In vitro isometric tension measurements

Mice (male about 2–3 months) were anesthetized and killed by decapitation. The soleus muscle and nerve were removed and placed into oxygenated (95% O2 and 5% CO2) Tyrode's solution (in mM; NaCl 126, KCl 3.5, MgSO4 1, CaCl2 2, NaHCO3 26, Na2HPO4 1.25, glucose 10) at 35 °C for 1 h. The distal muscle tendon was securely fastened with silk thread and tied to an isometric force transducer (Radnoti). The nerve was taken up into a polyethylene suction pipette for nerve electrical stimulation (0.5 ms, 10–20 μA). Bipolar wire electrodes were used for direct muscle stimulation (0.5 ms, 100–1000 μA) in the presence of the cholinergic receptor blocker, curare. Indicated drugs were added to oxygenated superfused solutions. Tension responses (in mg) were digitized and recorded using Clampex 9 software (Axon Instruments).

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