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Aberrant dendritic branching and sensory inputs in the superficial dorsal horn of mice lacking CaMKIIα autophosphorylation

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Superficial dorsal horn neurones undergo marked structural and functional activity-dependent development during the early postnatal period, but little is known about the molecular mechanisms underlying these changes. Calcium signalling, through activation and autophosphorylation of CaMKII, has been shown to play a major role in the maturation of neuronal morphology and connectivity in the cortex. Here, we show that the normal structural and functional development of superficial dorsal horn neurones requires CaMKII autophosphorylation at the Thr286 residue. The dendritic branching of neurones from mice containing a point mutation at this site (T286A) was significantly increased compared with wild-type littermates. This was accompanied by significant increases in receptive field size, recorded from intact preparations. Whole-cell patch clamp recordings of superficial dorsal horn slices revealed a selective deficit in low-threshold A fibre-evoked synaptic input. These results show that CaMKII autophosphorylation is required for the normal development of spinal sensory circuits.

Keywords: CaMKII; Development; Dorsal horn; Spinal cord; Substania gelatinosa; Circuitry; T286A; Plasticity; Receptive field; Sensory processing; Dendrite

Introduction

Neural activity is known to modulate synaptic development, and sensory circuits are shaped by experience during critical periods of early postnatal life (Hensch, 2004). Within the somatosensory system, as primary afferent sensory activity increases over the postnatal period (Fitzgerald, 1985; Koltzenburg et al., 1997), neuronal connections in the superficial dorsal horn of the spinal cord are refined, and dorsal horn cell cutaneous sensitivity, receptive field size (Fitzgerald et al., 1988; Fitzgerald and Jennings, 1999; Fitzgerald, 2005) and cutaneous reflex circuits are tuned (Schouenborg, 2004). Modulation of afferent and synaptic activity during postnatal development by local anaesthetics (Waldenstrom et al., 2003) or NMDA receptor antagonists (Beggs et al., 2002) leads to altered connectivity within the adult dorsal horn, and subsequent changes in behavioural sensitivity, although the underlying mechanisms are unknown.

Here, we have focussed upon lamina II of the superficial dorsal horn as a key site of synaptic refinement in the postnatal period. The neurones in this region are the last spinal neurones to mature and undergo most of their dendritic arborisation postnatally (Bicknell and Beal, 1984). This is accompanied by marked postnatal changes in afferent connectivity (Beggs et al., 2002; Nakatsuka et al., 2000), local excitatory and inhibitory synaptic inputs (Baccei et al., 2003; Baccei and Fitzgerald, 2004) and receptor pharmacology (Pattinson and Fitzgerald, 2004), some of which are activity-dependent (Fitzgerald, 2005).

The effects of neuronal activity on synaptic development are mediated by calcium signalling pathways involving proteins such as the calcium/calmodulin-dependent protein kinase II (CaMKII). The α isoform of CaMKII plays an important role in long-term synaptic plasticity, particularly synaptic potentiation, due to its ability to autophosphorylate in response to calcium activation (Lisman et al., 2002; Thiagarajan et al., 2002). Once in an autophosphorylated state, the kinase is able to translocate to the membrane and mediate changes in synaptic efficacy through phosphorylation and insertion of AMPA receptors (Barria et al., 1997; Poncer et al., 2002; Lisman et al., 2002). The kinase is known to play important roles in development of sensory systems such as the barrel cortex, where genetic adjustments of CaMKIIα autophosphorylation lead to alterations in plasticity and connectivity (Glazewski et al., 2001; Hardingham et al., 2003). One mechanism by which CaMKIIα signalling may modulate connectivity is through maturation of the dendritic tree (see Wong and Ghosh, 2002 for review). Increased CaMKIIα activity leads to reductions in axodendritic morphological complexity (Wu and Cline, 1998), while CaMKIIα inhibition produces aberrant morphologies in *Xenopus* tectal neurones (Wu and Cline, 1998; Zou and Cline, 1999).

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Here, we examine the role of CaMKIIα autophosphorylation in the structural and functional development of lamina II interneurones using mutant mice with a point mutation at site Thr286 (Giese et al., 1998), which prevents autophosphorylation of the kinase and thus eliminates the autonomous activity mode.

Results

Lamina II CaMKIIα expression is upregulated postnatally

CaMKIIα is highly expressed in interneurones of the adult superficial dorsal horn (Zeitz et al., 2004), but its postnatal onset of expression in the spinal cord is not known. Western blot analysis of whole cord, using an antibody raised against both the phosphorylated and unphosphorylated forms of CaMKIIα, demonstrated a 50 kDa band, corresponding to CaMKIIα, at all ages. Protein levels were low at P0, but increased over the first postnatal week to adult levels at P8–14 (Fig. 1A). Immunofluorescence shows that CaMKIIα expression in the adult is concentrated in laminae I and II, with moderate staining in lamina III and the dorsolateral funiculus (Fig. 1B). Little expression was observed in the deeper laminae of the dorsal horn or in the ventral horn, in agreement with previous reports (Zeitz et al., 2004). The pattern of CaMKIIα expression in the neonate was similar to that seen in the adult, with labelling concentrated in the superficial laminae of the cord and no staining seen in the deeper dorsal horn or the ventral horn (Fig. 1C).

CaMKIIα protein levels are unchanged in T286A mutant spinal cord

In order to test the role of CaMKIIα autophosphorylation in the development of lamina II cells, we examined a mouse which had a point mutation at the T286 site (T286A) of the CaMKIIα gene. This prevents the kinase from autophosphorylating and thus from remaining active after a transient calcium stimulus (Giese et al., 1998). The mutation has previously been shown to produce a reduction in total CaMKIIα protein levels in neocortical areas of the brain (Hardingham et al., 2003), but not in others (Giese et al., 1998). Here, we carried out western blot analysis of whole lumbar spinal cord homogenates and found that the levels of CaMKIIα protein were not significantly different between the T286A mutant and the wild-type mice (Fig. 1D).

CaMKIIα autophosphorylation controls dendritic growth in lamina II

Lamina II cell morphology in adult T286A mutant and wild-type mice was examined by filling cells from parasagittal slices with biocytin. The slices were then fixed and coverslipped, and images captured at a number of different focal planes in order to visualise all dendritic processes. A total of 15 cells were analysed from 8 wild-type mice and 13 cells analysed from 6 mutant mice. Quantitative analysis revealed increased lamina II cell dendritic arborisation in T286A mutants compared to their wild-type littermates (Figs. 2A and B). The number of dendritic branches was significantly higher in the mutant (26.3±4, versus 16.7±2; $p<0.05$), which in turn led to a significantly larger total dendritic length (1599±174 μm versus 1132±100 μm; $p<0.05$). The diameter of the cell body (T286A: 53.2±4.4 μm; wild type: 47.5±3.1 μm), the number of primary dendrites emanating from the soma (T286A: 3.38±0.25; wild type: 3.35±0.28) and the total dorsoventral and rostrocaudal extents (wild type: 148± 17 μm× 295±27 μm and T286A: 174±33 μm×364±44 μm) did not differ between the two groups. The gross cytoarchitecture of the dorsal horn laminae (examined using Nissl staining), and the termination pattern

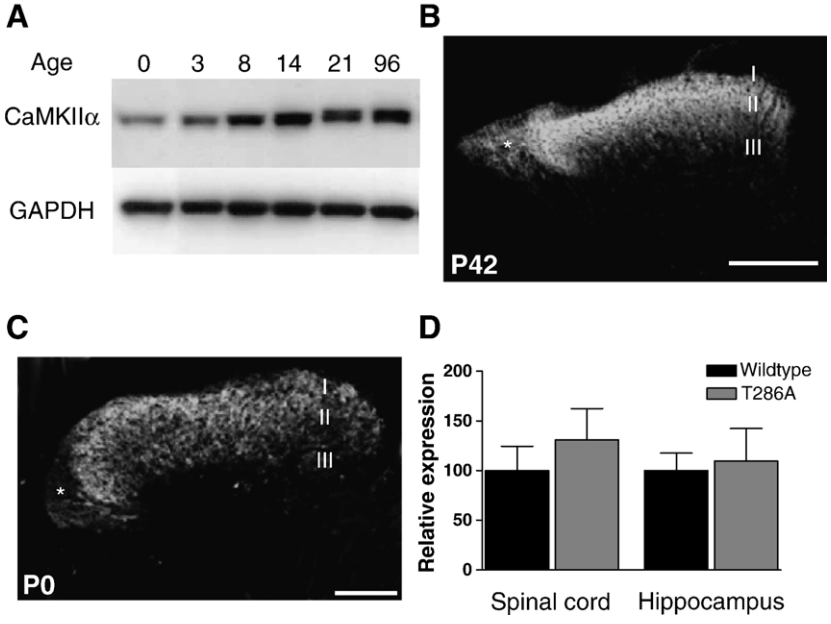

Fig. 1. CaMKIIα is expressed in the superficial dorsal horn of the spinal cord from birth. (A) Western blot analysis of cord homogenates shows low expression levels at birth and a subsequent upregulation to a peak at P14. Equal amounts of protein were loaded in each lane, as normalised by GAPDH. (B) Immunofluorescent analysis of adult cords shows high levels of protein in the superficial dorsal horn and the dorsolateral funiculus (asterisk). Numerals denote Rexed's laminae. Scale bar=100 μm. (C) CaMKIIα protein was present in the same regions of the cord at P0. Scale bar=25 μm. (D) Quantification of western blots in the spinal cord and hippocampus of wild-type and T286A mutant reveals no significant differences in protein content between genotypes ($p>0.4$, $n=6$).