lows a single action potential. In the present study, a threshold tracking technique similar to microneurography has been applied to isolated rat and human nerve preparations to follow the post-spike excitability of thin axons to various stimuli with known effects on membrane potential. We found that (1) some depolarising inflammatory mediators (e.g. ATP or serotonin) produce a reduction or loss of post-spike superexcitability; (2) membrane hyperpolarisation due to blockade of axonal Ih currents produces an enhancement of superexcitability; (3) an elevation of extracellular calcium concentration produces an increase in membrane threshold without a shift in membrane potential and/or an alteration in post-spike superexcitability. In addition, we demonstrate that membrane depolarisation and hyperpolarisation induce opposite post-spike latency shifts (changes in supernormality) in isolated C-fiber segments. Thus, recording of post-spike excitability and/or shifts in latency are sensitive techniques for the detection of various types of neuromodulation in correlation with changes in membrane potential of unmyelinated peripheral axons, and may help to understand observations obtained by microneurography in painful human neuropathies.

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NOVEL DUAL MECHANISM OF ACTION OF THE ANTIEPILEPTIC LACOSAMIDE

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Lacosamide is an investigational drug which shows potent analgesic and anticonvulsant effects in various animal models and is currently being evaluated in phase III clinical trials. The aim of the present experiments was to identify lacosamide's mechanisms of action.

Fishhook experiments with affinity ligands in rat brain homogenates and radioligand binding to recombinant proteins were performed to identify binding partners for lacosamide. In patch-clamp experiments, the influence of lacosamide on voltage-gated sodium channels (VGSC) was investigated.

The fishhook and radioligand binding experiments identified collapsin-response mediator protein 2 (CRMP-2) as a binding partner. Since CRMP-2 is involved in neuronal differentiation and axonal outgrowth, the effects of lacosamide on neurotrophin-induced axonal outgrowth were examined. Lacosamide specifically reduced axon outgrowth induced by neurotrophic factors without effects on basal outgrowth further supporting an interaction of lacosamide with CRMP-2. Detailed electrophysiological studies revealed that lacosamide shifted the voltage-dependence of slow inactivation of VGSCs to more negative potentials thereby regulating the long-term availability of sodium channels. Unlike other analgesics and anticonvulsants targeting the sodium channel (e.g. lidocaine) lacosamide did not influence fast inactivation of VGSCs.

These findings suggest that lacosamide has a novel, dual mode of action. Since slow inactivation of Na-channels is an endogenous mechanism by which neurons reduce stimulated or ectopic hyperactivity, this represents one important molecular mechanism for lacosamide. Given the important role of neurotrophic factors in the pathophysiology of chronic pain, the interaction of lacosamide with CRMP-2 might potentially have disease modifying effects. This, however, remains to be further investigated.

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CHARACTERIZATION OF ENDOGENOUS PKC-EPSILON IN F11 CELL, A MODEL SYSTEM FOR STUDYING PAIN SIGNALLING

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Background and aims. Involvement of PKCe in the development of hyperalgesia is well established. Detailed knowledge of signalling-components involved remains patchy partially due the inaccessibility of primary DRG neurons for biochemical analysis and the lack of an surrogate model system.

Methods/Results. We demonstrate that F11 cells, a DRG neuron-derived cell line, express different PKC isotypes, including a, b, g, e, i and l. In contrast to other PKC isoforms, we find the majority of PKCe to be present in a Triton X100 insoluble complex. Activation of PKCe by exogenous stimuli can be observed by its translocation to the plasma membrane as well as by the partial release from the insoluble complex. The localization of PKCe to the complex is resistant against different detergents as well as the cytoskeleton drugs nocodazole, cytochalasin B, cytochalasin D and acrylamide, disruptors of the microtubule, actin and neurofilament cytoskeleton, respectively. We further show that the PKCe