293P MOLECULAR BIOLOGY OF NOCICEPTOR Na CHANNELS

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Voltage-gated sodium channel transcripts present in dorsal root ganglia have been defined functionally, and cloned alpha subunits catalogued by Northern blots, PCR and *in situ* hybridisation. The distribution of such channels is shown below

The neuronal forms type I and II are present, whilst the embryonic type III reappears after axotomy. Both SNS and the TTXs channel PN1 are present at high levels in peripheral neurons. PN-1, type-1, NaCh6 and type II TTX sensitive transcripts occur in descending order of abundance. The atypical sodium channel NaG is expressed predominantly by Schwann cells, but is also found in sensory neurons.

Only the small diameter sensory neuron-specific SNS alpha-subunit is exclusively present in small diameter sensory neurons, together with the recently identified NaN/SNS2 transcript. The destruction of small diameter sensory neurons by capsaicin, which acts predominantly on nociceptors, leads to loss of the SNS and NaN transcripts.

These observations, combined with the resistance to TTX block (IC50 60mM), support the view that SNS underlies the TTXi currents observed in C-fibres and small diameter neurons. A direct approach to determine the functional significance of SNS is to ablate the expression of the channel in a null mutant mouse, and measure the behavioral and electrophysiological consequences. Studies of such null mutant mice demonstrate that all TTXr activity found in sensory neurons in culture is encoded by SNS.

The behavioral correlates of a loss of SNS expression are of major interest in terms of nociceptive processing. There is compensatory upregulation of PN1 in SNS null mutant mice, with increased electrical excitability in C-fibres. Behaviorally, the null mutant mice show deficits in mechanical and thermal pain thresholds. Because of the selective expression and role of SNS in nociceptive neurons, it provides an attractive analgesic drug test.

Name	Gene	Chromosome		Functional
Type I	SCN1a	2	XO3638	Yes
Type II	SCNa2	2	XO3639	Yes
Type IIa				Yes
Type III	SCN3a	2	YOO766	Yes
SM1	SCN4a	11	JN0007	Absent
SM2	SCN5a	9	A33996	Absent
NaCh6	SCN8a	15	U59966	Yes
PN1, HneNa	SCN9a	2	X82835	Yes
SNS	SCN10a	9	X92184	Yes
NaN/SNS2	SCN11a	9	AF059030	Yes
NaG/SCL11	SCN7a	?	YO9164	?

294P MOLECULAR COMPONENTS OF THE MECHANOTRANSDUCER IN NOCICEPTORS

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Almost all sensory neurons of the dorsal root ganglia have receptive fields in the periphery that are mechanosensitive. Nociceptors are notable in that the mechanical stimuli needed to activate them is 2-10 times larger than those needed to activate low threshold mechanoreceptors. We assume that the intrinsic molecular mechanisms used to transduce mechanical stimuli in nociceptors and low threshold mechanoreceptors will be very similar.

Our finding that neurotrophins regulate mechanotransduction has led us to concentrate on elucidating the molecular mechanisms underlying mechanotransduction in mammals. Based on a molecular model of mechanotrans-duction in *C. elegans*, we want to establish whether mammalian homologues of genes essential for touch sensitivity in *C.elegans* (the 'Mec' genes) also function as part of a mechanotransduction complex in mammals. In my laboratory, we have carried out *in situ* hybridization, northern blotting and immunocytochemical studies that have established that some species homologues are appropriately expressed in dorsal root ganglion neurons (DRG).

Two mammalian homologues of MEC proteins are stomatin, an integral membrane protein, and mdeg, a sodium channel (MEC-2, and MEC-4 respectively in *C.elegans*). To test whether such genes are functionally involved in mechanotransduction we have examined the stomatin and mdeg knockout mice for signs that mechanotransduction is impaired.

In addition to these functional studies, we have recently isolated two novel cDNAs encoding new members of the stomatin family and have found that both are highly expressed in mammalian sensory neurons. We also plan to establish whether these novel genes also have a functional role in mechanotransduction. Detailed information about the cellular mechanisms underlying mechanotransduction and the regulation of mechanical sensitivity will be important in understanding sensory disorders leading to pain in humans.

295P NOCICEPTOR RESPONSES TO BRADYKININ AND HEAT

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Pain differs from other sensations in many respects. Primary pain-sensitive neurones respond to a wide variety of noxious stimuli, in contrast to the relatively specific responses characteristic of other sensory systems, and the response is often observed to sensitize on repeated presentation of a painful stimulus, while adaptation is typically observed in other sensory systems.

We investigated the responses of nociceptive neurones to heat, and we found that application of pulses of noxious heat to isolated nociceptive neurones rapidly activates an inward current (Cesare & McNaughton, 1996). The recent cloning of the capsaicin receptor, VR1 (Catarina *et al*, 1997), has given us a clue to the molecular identity of the heat-activated ion channel. Many aspects of the behaviour of the heat-activated and the capsaicin-activated ion channels are similar, suggesting that the two may be synonymous. The capsaicin receptor is activated by heat in a similar manner to the native heat-activated channel, and the single-channel conductances of the two channels are similar (Tominaga *et al*, 1998; Cesare *et al*, 1999a). The response of neurones to capsaicin is enhanced by PKC activation, as is the response to heat (see below) (Vellani *et al*, 1999).

The current activated by heat is markedly increased by bradykinin, by a mechanism involving activation of protein kinase C (Cesare & McNaughton, 1996). We find that of the five PKC isoforms present in sensory neurones only one, PKC-ε, is translocated to the cell membrane by bradykinin (Cesare *et al*, 1999b). Further evidence for a specific role of PKC-ε in sensitization comes from the observation that the heat response is sensitized when a constitutively active PKC-ε is incorporated into heat-sensitive neurones. Conversely, BK-induced sensitization is suppressed by a specific peptide inhibitor of PKC-ε. We conclude that PKC-ε is principally responsible for sensitization of the heat response in nociceptors by bradykinin, and that this isoform may be an attractive target for therapeutic intervention in the control of pain.

Chronic pain states such as neuralgia and phantom limb pain are a considerable clinical problem, as they are usually refractory to relief by conventional analgesics. We have investigated whether nerve injury and NGF might cause an unregulation of the expression of receptors for bradykinin (BK), a potent endogenous peptide involved in pain signalling (Lee *et al.*, 1999). B_2 receptor expression was not detected by PCR in freshly isolated ganglia, nor in ganglia cultured in the absence of NGF, but prominent expression was seen after 3d culture in NGF. The related neurotrophins BDNF, NT-3 and NT-4 were ineffective, but weakly enhanced expression was seen with GDNF (all at 100 ng/ml). Expression was blocked by the high-affinity trkA receptor antagonist K252a. Enhancement of neuronal-specific B_2 expression by NGF was confirmed by *in situ* hybridisation. After nerve crush a similar enhancement of B_2 receptor expression was seen, peaking after 7 days as the nerve regenerated into the peripheral stump. Expression fell again after 14 days, at a time when reinnervation of muscles takes place. Conditioned media prepared by incubating injured nerve had a similar effect in upregulating B_2 expression, and the activity was blocked by anti-NGF antibodies. These experiments show that NGF is the main factor responsible for upregulating B_2 receptor expression after nerve injury *in vivo*.

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296P MULTIPLE TROPHIC FACTOR INFLUENCES ON NOCICEPTIVE SYSTEMS.

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In addition to their clear developmental role in regulating nociceptor number and phenotype, it has recently become clear that the adult nociceptive system continues to be sensitive to a variety of trophic factors. There is now increasing evidence that these effects represent more than an interesting pharmacology and that the endogenous production of some factors have important regulatory roles. Three factors in particular have been studied: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF)

About one-half of the nociceptors in dorsal root ganglia express receptors for NGF (trkA and p75). NGF given exogenously produces a rapid onset pain and hyperalgesia in man and animals. One mechanism appears to be the sensitisation of peripheral nociceptive terminals. Much of this sensitisation appears indirect, depending on products released from mast cells, sympathetic terminals and neutrophils. In addition to these peripheral effects, the retrograde transport of NGF in trkA-expressing nociceptors controls the expression of a host of genes in these cells, ranging from neuropeptides (e.g. substance P), ion channels (e.g. sodium channels) and receptors (e.g. VR1). NGF is up-regulated in a variety inflammatory conditions and models, and 'antagonism' of NGF in these states reduces some abnormal pain sensitivity. The evidence suggests NGF is a peripheral inflammatory mediator of pain.

BDNF is constitutively expressed in some trkA-expressing nociceptors. NGF (given exogenously or produced in inflammatory states) dramatically up-regulates BDNF expression in virtually all trkA-expressing nociceptors. BDNF in these cells is transported anterogradely to the central terminals of nociceptors where some is packaged in dense core vesicles. It seems likely that BDNF will be released with activity from nociceptors under inflammatory conditions.

We have found that exogenous BDNF increases spinal nociceptive reflexes and induces fos expression in dorsal horn neurones. Functionally, we find that sequestering BDNF with intrathecal trkB-IgG reduces spinal reflex excitability measured electrophysiologically, and behaviourally reverses the some of the hyperalgesia induced by NGF. Thus, growing evidence suggests that BDNF is a central mediator of central sensitisation.

Nociceptors in the DRG that do not express trkA/p75 all have receptor components for GDNF family members (i.e. either GFRα1 and/or GFRα2 binding proteins and the tyrosine kinase RET). These nociceptors are sensitive to capsaicin, but do not express neuropeptides such as substance P or CGRP (or BDNF). However, they (unlike other DRG cells) express the purinoreceptor $P2X_3$ and the enzyme TMP. They also selectively bind the lectin IB4 and have distinct central termination patterns in lamina II_i.

We have not observed any changes in nociceptive responses in animals given peripheral or intrathecal GDNF. However, we have found that GDNF can rescue specifically these cells from many of the effects of axotomy – such as injury-induced down-regulation of IB4 binding, TMP and $P2X_3$ expression. We also find that exogenous GDNF largely reverses the mechanical allodynia that develops in animals subjected to a either a partial sciatic nerve or L5 spinal nerve ligation. GDNF also reduces the degree of spontaneous activity that develops in these damaged afferents. We therefore conclude that GDNF has strong neuro-protective actions on damaged sensory neurones and may be therapeutically useful in treating neuropathic pain.