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Introduction

Pain

Physiological pain is an important survival and protective mechanism designed to warn the animal of danger from potentially injurious stimuli from the external environment. Aspects of the somatosensory nervous system responsible for processing physiologically painful stimuli are functionally and topographically organised. The system operates through a specific set of primary sensory neurones and is exclusively activated by noxious stimuli via peripheral transduction mechanisms. These sensory fibres, or nociceptors, are characterised by small diameter axons with slow conduction velocities. Some nociceptors are unimodal, responding only to thermal, mechanical or chemical stimulus. Many are polymodal, responding to more than one type of noxious stimulus. Nociceptors, with their cell bodies located in dorsal root ganglia (DRGs), encode the intensity, duration and quality of noxious stimulus and, by virtue of the topographical organisation of projections to the spinal cord, the location of the stimulus. The nociceptors terminate in a highly ordered manner in the dorsal horn of the spinal cord, with thinly myelinated Aδ fibres synapsing in laminae I and V and the unmyelinated C fibres in lamina II. These high-threshold sensory fibres diverge to activate second order interneurones and projection neurones in the spinal cord. The activity generated by nociceptor input is transferred, after complex processing in the dorsal horn, either directly, or via brainstem relay nuclei, to the ventrobasal thalamus and then on to the cortex, where the sensation of pain reaches consciousness. Parallel outputs from the dorsal horn also pass to the ventral horn and activate flexor motor neurones responsible for generating the flexor withdrawal reflex and retraction from the harmful stimulus. As a result, both the sensation of physiological pain and activation of the flexion reflex occur in unison.

Over the past 30–40 years our understanding and appreciation of the function and operation of nociceptive circuits and information has transformed dramatically. Firstly with the concept and realisation that nociceptive information reaching the spinal cord dorsal horn is profoundly modified at the level of the primary afferent terminals and projection neurones, and secondly that nociceptive information is extensively modulated by descending projections from higher centres. As such, nociceptive information to the central nervous system is integrated, subject to modification and thus edited before being telegraphed to higher supraspinal centres. Extensive research since has revealed a staggering diversity of mechanisms involved in the modulation and fine-tuning of nociceptive information. It is now clear that pain signalling is a complex and highly plastic process.

Chronic pain, or persistent pain, is readily differentiated from normal physiological pain in that it confers no clear advantage to the organism. Clinical pain is apparent when discomfort and abnormal sensitivity are present and is associated with three primary general features. Firstly, pain which may be dull, burning, or stabbing that is spontaneous. Secondly, pain responses to noxious stimuli are exaggerated (hyperalgesia). Thirdly, pain is produced by normally innocuous stimuli (allodynia). Due to the differing pathophysiology, clinical pain is separated into inflammatory pain, associated with either tissue damage or an acute or chronic inflammatory condition, and neuropathic pain, which results from lesions to the peripheral or central nervous systems.

The current treatment of pain remains highly reliant upon non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, which although effective in the treatment of acute nociceptive pain are poor therapies for several other types of pain. For moderate to severe pain, opiates remain the preferred approach but many chronic pain conditions are refractory to opiate therapy and chronic dosing is associated with severe side effects. Chronic inflammatory pain is mainly treated by NSAIDs which have poor efficacy, and following chronic treatment can lead to serious problems including kidney failure, gastropathy and liver damage. The problems associated with NSAIDs have led to the development of more selective second generation compounds, the cyclo-oxygenase (COX) inhibitors. The review by Sharon Bingham et al., examines the Cyclooxgenase (COX) enzymes and the role their products play in the development and maintenance of pain. Two major COX isoforms, COX-1 and COX-2, are involved in the production of prostanoids and are differentially distributed and differentially regulated in different pain states. These enzymes catalyse the first step in prostanoid biosynthesis, leading to the conversion of arachidonic acid (AA) to the prostanoid precursor prostaglandin H_2 (PGH_2), which is further metabolised into the prostaglandin isoforms PGE_2, PGD_2, PGF_2, PGI_2 or thromboxane A2 (TXA_2). Much of the research into the roles of the COX enzymes in pain has focussed on the action of the downstream prostanoid, PGE_2. The mechanisms by which PGE_2 modulates the pain pathway are complex; with four receptors, coupled to different signal transduction pathways,

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being identified for PGE_2: EP1, EP2, EP3 and EP4. Prostanoids influence inflammatory and immune responses, and sensitise and augment pain. Thus, non-steroidal anti-inflammatory drugs are effective in relieving many of the signs and symptoms of inflammatory diseases including pain via inhibition of COX enzymes.

Although patients with inflammatory or neuropathic pain may have similar symptoms, the underlying mechanisms are different. Following the development of models of these various forms of pain along with the "molecular revolution," it is now transpiring that there are clear and distinct changes in gene expression that occur during the development of painful conditions, including changes in gene expression associated with intracellular signal cascades, ion channels regulating intrinsic electrical excitability, ligand-gated ion channels, neurotransmitters, inflammatory agents and G-protein coupled receptors at all levels of the neuraxis. This has not only led to a greater appreciation of the complexity of nociceptive information processing and the extraordinary degree and scope of plasticity within the pathways but also to identification of novel putative molecular targets for therapeutic intervention into clinical pain states.

One family of such targets are voltage-gated calcium channels (VGCCs). These channels are crucial for the control of neurotransmitter release and neuronal excitability. This functionally diverse group of ion channels are distributed throughout the central and peripheral nervous systems. The review by Valentin Gribkoff gives an overview of data supporting a pivotal role for these channels in pain pathways. VGCCs exist in two main physiological classes based on their voltages of activation: low-threshold T-type VGCCs and high-threshold L, N, P/Q and R channels, referring to their typical ranges of activation voltage. There are three subfamilies, L-type (Ca_v1), N, P/Q and R-type (Ca_v2), and T-type (Ca_v3), based on the structural similarity of the α_1 (pore-forming) subunit and their pharmacological characteristics. All VGCCs are composed of a characteristic single α_1 subunit, of which 10 isoforms have been identified, and a combination of several other $\alpha_2\delta$, β and in some cases γ subunits. Auxiliary subunit composition and the environment of the channel changes during development, during normal activity and in response to injury and disease, including physiologic nociception and chronic/neuropathic pain.

The $\alpha_2\delta$ accessory subunit of the voltage-dependent calcium channel complex is the subject of the review by Yannick Maneuf et al. The importance of this molecular entity is highlighted by the fact that considerable evidence is now accumulating to support the concept that $\alpha_2\delta$ is the target of the neuropathic pain treatment, Gabapentin (Neurontin). The $\alpha_2\delta$ family consists of four genes, *$\alpha_2\delta$-1* being the first identified and existing as five tissue-specific splice variants. $\alpha_2\delta$ proteins are synthesised as pre-proteins that undergo extensive post-translational modifications. The membrane targeting signal is proteolytically removed whilst further cleavage generates a small C-terminal fragment (δ) that remains attached to the larger (α_2) fragment by a disulphide bridge. It is thought that the δ subunit forms a single transmembrane spanning segment which anchors the extracellular α_2 subunit to the calcium channel complex. Three further $\alpha_2\delta$ genes have been identified, *$\alpha_2\delta$-2*, *$\alpha_2\delta$-3* and *$\alpha_2\delta$-4*. *$\alpha_2\delta$-1* and *$\alpha_2\delta$-2* bind gabapentin. In chronic pain states the expression of $\alpha_2\delta$ increases and these changes correlate with hyperalgesia. *$\alpha_2\delta$-1* expression is enhanced in sensory and spinal dorsal horn neurones of neuropathic pain models, including increased expression at presynaptic terminals of DRGs. Thus, modulation of presynaptic functions associated with increased *$\alpha_2\delta$-1* and/or calcium channels may form the molecular basis for therapeutic intervention into these pain states.

Voltage-dependent sodium channels are similarly crucial for nociceptive information processing and involved in long-term chronic pain states, including inflammatory and neuropathic pain. Their role in these processes and their potential as both current and future targets for therapeutic intervention is the subject of the review by Marc Rogers and colleagues. The review focuses on sodium channels in neuropathic pain. In the neuropathic state, spontaneous activity in sensory neurons, referred to as ectopic discharge, is increased. This increased, inappropriate spontaneous activity is associated with a reduction in firing threshold and remodelling of sodium channel conductances through changes in sodium channel gene expression, sodium channel trafficking or phosphorylation of sodium channel proteins. Sodium currents are functionally divided by their sensitivity to the toxin tetrodotoxin (TTX). TTX-sensitive (TTX-S) and resistant (TTX-R) currents are differentially expressed, have been identified in dorsal root ganglion neurons and are modified in models of neuropathy. TTX-S currents represent multiple subtypes of sodium channel reflected by the diversity of α subunits and the range of inactivation kinetics in different fibre types. TTX-R currents are associated with nociceptive fibres and four types have been identified. Amongst the family of voltage-gated sodium channels, several are linked to neuropathic pain: Nav1.3, Nav1.7, Nav1.8 and Nav1.9. The Nav1.3 channel mediates a TTX-S current and its expression is up-regulated in DRG in a range of neuropathic pain models. Nav1.7 channels are almost exclusively expressed in DRG, in small C-fibre nociceptors. These channels underlie a fast TTX-sensitive current and their properties and distribution in sensory nerve endings suggests a major role in transmitting painful stimuli. Nav1.7 expression along with its four splice variants are reduced in models of neuropathic pain, whereas Nav1.7 protein expression increases in a model of diabetic neuropathy. Nav1.8 in nociceptors is reduced in human patients and in most, *in vivo* models of neuropathic pain, as is Nav 1.9. However, in various neuropathic models, there is significant redistribution of Nav1.8 channels which may mediate spontaneous firing in uninjured C-fibres and contribute to on-going pain.

Of the ligand-gated ion channels and sensory receptors involved in pain pathways, the capsaicin receptor Transient Receptor Potential Vanilloid 1 (TRPV1, Vanilloid Receptor 1, or VR1) is a developing target for the treatment of pain and is reviewed by David Imke and Narender Gavva. TRPV1 is a ligand-gated ion channel that binds capsaicin, the pungent chemical found in chili peppers, and is believed to transduce the sensations of noxious heat and pain. TRPV1 shows polymodal activation responding to vanilloids, such as capsaicin, elevated temperatures and acidic conditions. Putative endogenous ligands for TRPV1 include endocannabinoids,

N-arachidonoyldopamine, lipoxygenase products of arachadonic acid and polyamines. TRPV1 shows a unique expression profile in cell bodies of subsets of sensory neurons of the peripheral nervous system including DRG, trigeminal ganglion (TG), and nodose ganglion (NG). In the DRG and TG, TRPV1 expression is relatively restricted to the small- to medium-sized neurons constituting putative nociceptors. Direct and indirect mechanisms finely tune TRPV1 activity. For example, sensitivity to capsaicin is enhanced by sub-threshold concentrations of protons and small increases in temperature. Inflammatory mediators can also indirectly modulate or sensitize the receptor. Therefore, under inflammatory conditions primary activators and secondary modulators are able to interact with TRPV1 suggesting a crucial site of convergence and integration for pain generating stimuli. Furthermore, the ability of these modulators to render TRPV1 in a tonically active state may contribute to the underlying pathology of chronic pain.

Our knowledge and understanding of another crucial ligand-gated family of receptors, the glutamate receptor family, has also expanded dramatically in recent years. David Bleakman and colleagues review the role of glutamate receptors in physiological pain processing and persistent pain states. The authors focus on their expression patterns and roles in nociceptive pathways and hence their potential as targets for pharmacological intervention strategies. Ionotropic glutamate receptors are separated into α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors, named after selective ligands. The AMPA receptor family is comprised of four genes $GLU_{A1\text{-}4}$ (GluR1-4 or GluRA-D) and the expressed ion channel assemblies of four subunit proteins give rise to homomeric or heteromeric structures. Multiple receptors, isoforms and splice variants render the potential for AMPA receptor constructs, incredibly diverse. The NMDA receptor ion channel is a tetrameric structure generated from up to seven genes coding for seven subunits: GLU_{N1}, GLU_{N2A}, GLU_{N2B}, GLU_{N2C}, GLU_{N2D}, GLU_{N3A} and GLU_{N3B}. Multiple splice variants of the GLU_{N1} receptor subunit exist. Peripheral noxious stimuli, tissue injury or nerve damage leads to an enhanced long-term level of synaptic transmission in dorsal horn neurons of the spinal cord. This key plastic process, referred to as central sensitization, is a feature of glutamatergic pathways both at spinal and supraspinal nociceptive regions. Central sensitization underlies increases in responsiveness of dorsal horn neurons, reductions in pain threshold (allodynia), amplification of pain responses (hyperalgesia) and spread of pain sensitivity to non-injured areas. Central sensitization involves phosphorylation-induced alterations in the biophysical properties of NMDA and AMPA receptors and/or promotion of AMPA receptor trafficking to the post-synaptic membrane. AMPA receptors allow NMDA receptor function by depolarization and relief of the voltage-dependent Mg^{2+} block of NMDA receptors, a mechanism likely important for the establishment and maintenance of pain states. However, metabotropic glutamate receptors acting pre- or post-synaptically also modulate the induction and/or maintenance of central sensitization. The mGlu receptors are the products of eight genes (*mGlu1-8*) belonging to the larger family 3 (or C) class of G-protein—(guanine-nucleotide-binding protein) coupled receptors. Three groups of mGlu receptors are recognized based upon amino acid sequence homology. Group I includes mGlu1 and mGlu5 receptor subtypes and their associated alternatively spliced variants, including mGlu1a,b,c,d and mGlu5a,b which primarily couple to Gq/G11 G-proteins to stimulate phospholipase C. Group II consists of mGlu2 and mGlu3 receptors and group III subclass is comprised of mGlu4, mGlu6, mGlu7 and mGlu8 receptors, including isoforms mGlu7a,b and mGlu8a,b,c receptors. Groups II and III receptors primarily couple to inhibition of adenylyl cylcase via Gi/Go G-proteins. Kainate receptor subunits have a structure similar to AMPA subunits and the family is comprised of five genes, in two subfamilies. GLU_{K1} and GLU_{K2} (KA1 and KA2) show higher affinity for kainate than the $GLU_{K5\text{-}7}$ (GluR5-7) subfamily. Kainate receptors primarily have an excitatory role post-synaptically on excitatory neurons. Within nociceptive afferent pathways, GLU_{K5} kainate receptors appear to be of particular importance. Multiple glutamate receptor subtypes are differentially expressed on pre- and post-synaptic elements within the ascending and descending nociceptive pathways. Whilst crucial in physiological pain processing, changes in gene and/or protein expression of specific glutamate receptors in animal models of persistent pain have begun to identify their potential as substrates for treating chronic pain.

What has become clear over recent years is that pain and nociceptive information processing in higher vertebrates is a complex and plastic phenomena. Extensive research continues to further our understanding and knowledge of this fundamental and crucial behaviour. The mechanisms by which such information processing changes under a range of physiological and pathophysiological conditions has begun to reveal novel therapeutic targets and strategies for pharmacological intervention into debilitating conditions associated with pain states. This small collection of articles aims to highlight only some of those developments.

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