Table of Contents

1 Introduction

1.1 Pain processing in physiological and pathophysiological conditions

Pain is a complex ability with a high clinical relevance. Between 12 % and 30 % of the population of the European countries are suffering from chronic pain defined as pain lasting more than six months duration and a pain intensity of 5 on a 10-point Numeric Rating Scale (NRS) with 1 = no pain to 10 = the worst pain imaginable (Breivik et al, 2006). From this population nearly 60% had pain for 2 to 15 years. Nearly 50% complain about back pain, 15% about headache (Breivik et al, 2006). Pain is more than the transduction of noxious stimuli, it also includes the processing in thalamic and cortical structures and components of cognitive-affective as well as of vegetative systems. For this reasons the International Association of Study of Pain (IASP) has defined pain as an “unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. This definition includes nociception under acute circumstances, as well as inflammatory pain and the emotional-coloured subjective kind of chronic pain syndromes like complex regional pain syndrome (CRPS), phantom limb pain or somatoform pain states. Nociception includes the transduction of chemical or physical signal by specialised nerve fibres and the conduction through the spinal cord to thalamic nuclei and the central procession of a nociceptive stimulus (Almeida et al, 2004). The nociceptive afferent pain pathway starts at specialised sensory nociceptive fibres, that innervate skin and most other tissues like mucosa, membranes, deep fascias, connective tissue of visceral organs, ligaments and articular capsules, periosteum, muscles, tendons and vessels. The unmyelinated or thinly myelinated nerve fibres are connected to the dorsal horn from their cell bodies in the dorsal root ganglia (DRG) and run with the peripheral nerves to the spinal cord, in case of the trigeminal nociceptors, to the trigeminal sensory nuclei in the brainstem (Almeida et al, 2004). Different types of primary

afferent axons can be classified in terms of diameter, nerve conduction velocity and structure. Aβ-fibres are myelinated and rapidly conducting fibres with a velocity of 30-70 m/s and have a large diameter from 7 to 15 µm. They can be further discriminate into slow adapting (AM SA) and rapid adapting (AM RA) Aβ-fibre types (Zimmermann et al, 2009). Most of these fibres detect innocuous stimuli applied to skin, muscle and joints and thus do not contribute to pain, nevertheless they may be involved in the nociceptive processing and participate in the mechanisms of segmental suppression (Calvino and Grilo, 2005). Most, but not all of these have their capability to react on various types of stimuli in common, i.e. they are polymodal. Aδ-fibres are thinly myelinated, have a conduction velocity of 4-20 m/s and a diameter from 2 to 6 µm. Depending on additional attributes like mechanical threshold and adaption velocity these fibres can further be subdivided into different types. Two main classes of Aδ-fibres can be discriminated, both of them promote the quick perception of "first" pain and triggers the reflectory fugue. About 60% of fibres are slow-adapting mechano-sensitive A-fibres with a mechanical threshold between 1-128 mN and in addition detect heat (~40°C) and intense cold stimuli (<28°C) (Koltzenburg et al, 1997). They are called Mechano-Heat Aδ-fibres (AMH) or Mechano-Cold Aδ-fibres (AMC). The second kind of Aδ-fibres are mechanoreceptors, which respond strong to mechanical stimuli (Mechano Aδ-fibres [AM]) and can be further discriminated fibres with a low (A[LT]M; Von Frey threshold <1-5.7mN) or high (A[HT]M; Von Frey threshold ~5.7-128mN) mechanosensitive threshold. Nearly 85% of all Aδ-fibres found in skin of primates are Mechano-Heat fibres, therefore AMH units . In the muscle activation of Aδ-fibres produces an aching sensation. In comparison to the skin more Aδ-fibres respond to a pH reduction (Xu et al, 2010). Their modalities contain innocuous mechanical, thermal and chemical stimuli to noxious stimuli like painful pressure or ischemia and hypoxia (Julius et al, 2001; Zimmermann et al, 2009; Djouhri and Lawson, 2004). C-fibres are unmyelinated, slowly adapting afferents with an axon diameter from 0.4 to 1.2 µm and a conduction velocity of 0.5 to 2.0 m/s. They propagate the information in a slower way, the "second pain". Depending on the stimuli they react on, they can be classified into different mechanosensitive C-fibres of low threshold (C[LT]M; Von Frey threshold <1-4mN) and high threshold (C[HT]M; Von Frey threshold ~5.7-128mN), thermosensitive C-fibres just reacting of noxious cold (Cold Nociceptors, CC) and noxious heat (CH). Also polymodal mechano-cold nociceptors (CMC) and mechano-heat nociceptors (CMH) or C-fibres, that are insensitive to mechanical and heating stimuli (CH_iM_i), are particular subclasses of C- fibres. They can express specific receptors for chemical noxes, e.g. potassium and hydrogen ions, substance P, acetylcholine, histamine, prostaglandine, serotonine and proteolytic enzymes,

too. In the human skin 45% of CMH-, 24% CH_iM_i-, 13% of CM- and 6% of CH-C-fibres are found. Other C-fibres, called “silent C-fibres” are just responsive after the sensitization by a tissue injury (Zimmermann et al, 2009; Schmidt et al, 1995; Julius et al, 2001; Garry et al, 2004). The cranial primary afferent fibres reach the brain stem through the cranial nerve pairs V, VII, IX and X, while the thoracolumbar and sacral primary afferent fibres reach the spinal cord via the dorsal root of the spinal nerves. Their central processes end in the dorsal horn within the grey matter of the spinal cord. They surround the outermost layer of the dorsal horn and penetrate perpendicularly the gray matter to reach the superficial Rexed laminae I-II or to descend to the deep Rexed laminae V, VI, VII and X. In the gray matter, that consists of neuron cell bodies and glial cells, the axons form synapses with second-order neurons in the above-mentioned laminae. The second-order neurons can be discriminate into three groups addicted to the afferent signals they receive and the response pattern in case of a noxious stimulus. The specific nociceptive neurons are located primary in the outermost layers of the dorsal horn, i.e. laminae I and II and can be secondary found in the layers V and VI. They receive their information just by Aδ- and C-Fibres and respond to high-intense mechanical stimulation, heat and the polymodal stimulus of C-Fibres. Their receptive fields are punctiform and show a kind of somatotopic organisation mainly in lamina I. The nonnociceptive neurons react on peripheral stimuli like low-intensive mechanical and thermal irritation transmitted by Aβ- and Aδ-fibres. They take indirectly place in segmental suppression mechanisms but not in integrating the nociceptive information. The third group are called Wide Dynamic Range (WDR) neurons. Their cell bodies are located mainly in lamina V, but also in the outermost layers as well as in laminae IV and VI. They respond to mechanical, thermal and chemical stimuli coming from nonnociceptive as well as from nociceptive fibres, i.e. they respond to low- and high-intensity peripheral stimuli coming from Aβ-, Aδ- and C-fibres. In contrary to the nociceptive neurons their firing frequency increases linearly or exponentially with ascending strength of the stimulus. One WDR neuron can receive afferent fibres from different tissues like skin and muscles, i.e. that their receptive field varies explicitly. The WDR-neurons receive input from large-diameter fibres (Aα- and Aβ -fibres) as well as from small-diameter fibres (Aδ- and C-fibres). While both fibre qualities activate direct the WDR-neurons, the large-diameter fibres activate additionally inhibitory interneurons, whereas excitatory interneurons are activated by small-diameter fibres (Schaible and Grubb, 1993; Millan, 1999). This “Gate Control Theory” (published by Melzack and Wall 1965), explains on one hand why pain in one tissue can create pain in another part of the human body (e.g. pain in shoulder and left arm by myocardial infarction)

and explains on the other hand why pain is suppressed by a innocuous counter irritation (e.g. suppressing hand pain by rubbing the fingers) (Calvino and Grilo, 2005; Almeida et al, 2004). However this is controversially discussed because Melzack and Wall assumed a linkage between sensitization of spinal cord neurons and nociception but till now a direct relationship between spinal cord sensitization and pain perception has not been demonstrated (Cervero, 2009; Craig, 2002; Craig, 2003). The conveyance between the first- and the second order neurons is mainly triggered by a release of numerous neurotransmitters, just a few of them act directly on the postsynaptic part. Under physiological conditions excitatory amino acids like glutamate, aspartate, calcitonin gene-related peptide (CGRP) and substance p are known to be the main nociceptive transmitters in the spinal cord. Other neurotransmitters like vasoactive intestinal peptide (VIP), cholecystokinin (CCK) or neurotensin as well as glutamate, aspartate and substance p, are released from interneurons of the dorsal horn to modulate the afferent nociceptive signal, but also inhibitory peptides like Gamma-amino-butyric acid (GABA), glycine or enkephaline plays a role in that modulation (Riedel et al, 2001) . The concourse of primary afferent sensory fibres with descending efferent inhibitory tracts and interneurons from the grey matter in the dorsal horn of spinal cord form the setting where inhibitory, excitatory or modulatory influences vary the primary nociceptive signal. Important for the modulation are the ionotropic glutamate receptors as an excitatory system, the opioid receptors as an inhibitory system and the nitric oxid (NO)/ cyclic guanosine monophosphate (cGMP) system, that can partially have nociceptice and antinociceptive effects (Schmitko et al, 2009; Riedel et al, 2001; Zamponi et al, 2008). Ionotropic glutamate receptors, including AMPA-, kainate- and NMDA-receptors are ligand-gated cation-channels which bind glutamate (and aspartate on NMDA-receptors) and after gating are permeable for K^+, Na^+ and Ca^{2+}. While the AMPA- and kainate-receptors mediate fast synsaptic transmission under normal conditions, NMDA-receptor is blocked by a Mg^{2+}-Ion. Depolarisation of the membrane lets the Mg^{2+}-ion dissociate from the channel and allows cation influx. NMDA-receptor activation is required for long-term potentiation (LTP), which enhances synaptic transmissions efficiency and evokes a stronger response to stimuli that normally do not evoke pain or an increase of the response to stimuli that normally evoke pain (Baumbauer et al, 2009). The rising intracellular postsynaptic Ca^+- concentration Ca^{2+} - dependent signaling pathways including activation of protein kinases, phospholipase C (PLC), nitric oxide synthase (NOS) and members of the mitogen-activated protein kinase family (MAPK), including extracellular signal regulated kinase (ERK) (Sandkühler, 2007, Schmidtko et al, 2009; Miclescu et al, 2009). Activation of NO synthase (NOS) for example, catalyses the

production of NO from the amino acid L-arginine. In the spinal cord the most important NOS isoform in development and maintenance of inflammatory and neuropathic pain is the neuronal NOS isoform (nNOS), that is created by neuronal cells located in the inner lamina II sending their fibres to the laminae I-III. But also the inducible NOS isoform (iNOS) could be part of processing pathological pain. NO, as a soluble gaseous molecule, easily diffuses out of the cell body and can act as a paracrine and autocrine transmitter. One of the targets of NO is the NO-sensitive guanylate cyclase (NO-GC), which forms from Guanosine-5'-triphosphate (GTP) the second messenger cyclic guanosine monophosphate (cGMP).

NO-GC can be found in neurokinin 1 (NK_1) receptor positive projection neurons in lamina I as well as in inhibitory interneurons in lamina II and III. While the projection neurons, that are important for the ascending conduction of pain, the basal tone in pain circuits and in creating of the LTP, get directly sensitised by cGMP dependent pathways, the inhibitory interneuron loose the ability of inhibition through the increase of cGMP (see Fig. 1). The effect of NO-GC to sensitise projection neurons and to delay the inhibitory influence of interneuron seems to be one mechanism that leads to the so-called central sensitization, another mechanism could be triggered by NO directly, leading to an enhance release of substance p and calcitonin-gene related peptide from C-fibre terminals. Animal experiments

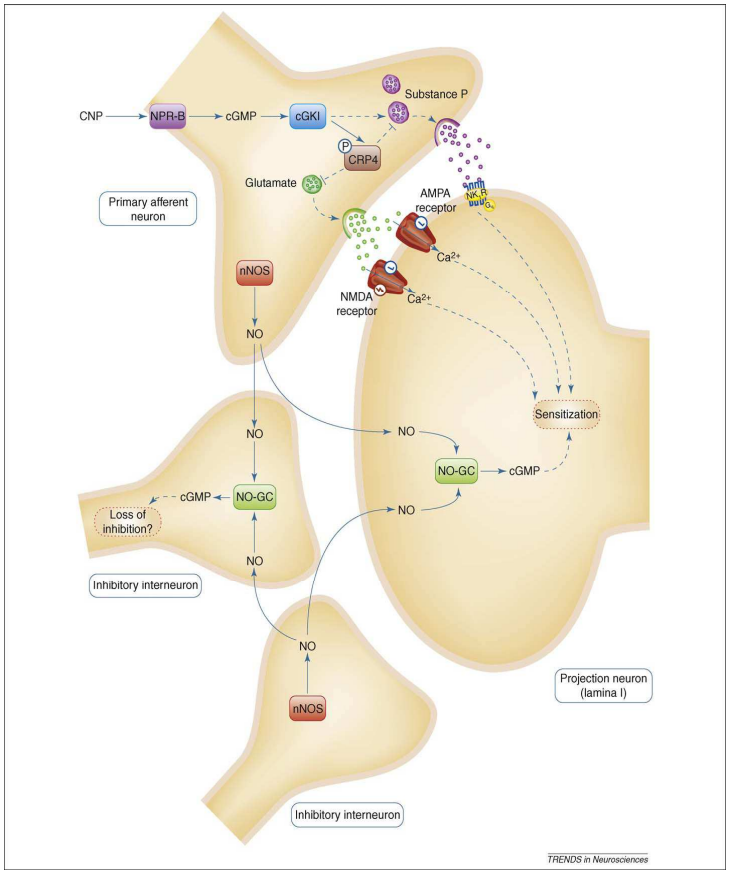

Fig. 1: Suggested NO and cGMP signaling pathways during spinal pain sensitization. In the dorsal horn of the spinal cord, **NO-GC** is localized to inhibitory interneurons and to lamina I projection neurons that express the substance P receptor NK1 (**NK1-R**). In some inhibitory interneurons and in <5% of primary afferent neurons **nNOS** is expressed. nNOS is activated and upregulated, during inflammatory and neuropathic pain, in inhibitory interneurons and in primary afferent neurons. The increased NO production leads to activation of NO-GC and subsequent cGMP production in NO-GCexpressing neurons. In contrast to NO-GC, cGMP-dependent protein kinase I (**cGKI**, a isoform) is mainly expressed in primary afferent neurons. It seems, that during nociceptive processing cGKI is activated by cGMP produced by the guanylyl cyclase natriuretic peptide receptor B (**NPR-B**) upon stimulation by C-natriuretic peptide (**CNP**). An inhibitory role in the creation of inflammatory pain has the Cysteine-rich protein 4 (**CRP4**) and it is a downstream effector of cGKI. A possible contribution of CRP4 to nociceptive processing might be an inhibitory effect of CRP4 on pronociceptive pathways under resting conditions and the cessation of this inhibitory effect during the processing of pain sensitization that includes phosphorylation of CRP4 by cGKI. Solid and dashed lines indicate direct and indirect interactions (from Schmidtko et al. 2009).

with intrathecal application of NO-donors suggest, that the NO-dependent pathways are not involved in acute pain perception but play a major role under pathophysiological pain conditions like inflammatory or neuropathic pain (Schmidtko et al, 2009; Riedel et al, 2001; Miclescu et al, 2009). After the modification of the incoming sensitive information in the gray matter the output from the dorsal horn to higher centres in the brain is carried by spinal projection neurons along ascending pathways located in the white matter´s lateral funiculi of the spinal cord. All of these ascending pathways project to other parts of the brain, communicate therefore with different functional systems and trigger a specific kind of reaction. The main afferent pain pathway is the spinothalamic tract. Fibres of WDR neurons, specific nociceptive neurons (SN neurons) and nonnociceptive neurons traverse the spinal cord, decussate in the same spinal segment to the contralateral site and form the spinothalamic tract. This pathway transmits information about noxious, thermal and rough mechanical stimulation to thalamic core region and can be classified into three forms of afferences. The ventral spinothalamic tract, also called monosynaptic neospinothalamic pathway, projects directly to nuclei of the lateral part of the thalamus and is involved in the sensory–discriminative component of pain. The dorsal spinothalamic tract, the multisynaptic paleospinothalamic pathway, transmits to nuclei of the posterior medial and intralaminar thalamic complex. He takes part in motivational-affective aspects of pain. The monosynaptic spinothalamic pathway at the end is also involved in the affective component of a painful experience and projects to the thalamic medial central nucleus. Other ascending pathways are the spinoreticular tract, that afferent fibres direct to the precerebellar nucleus in

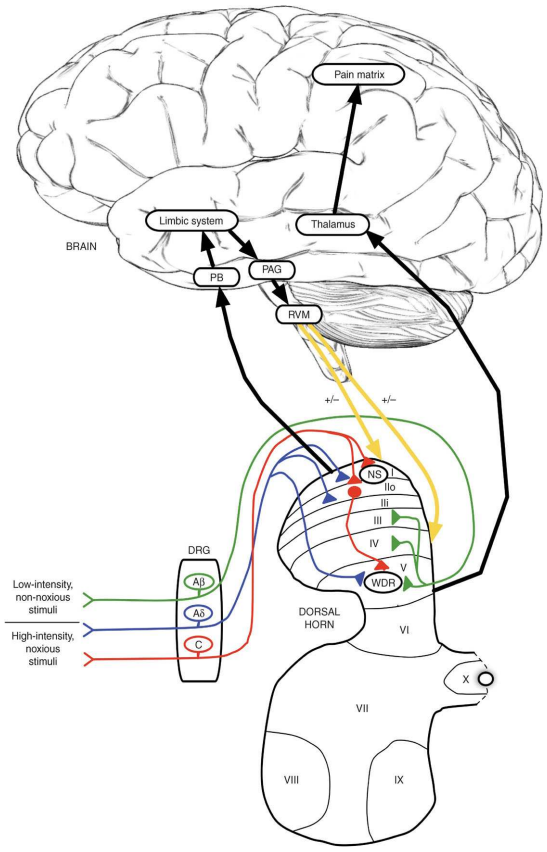

Fig. 2: Pain pathways from periphery to brain. Incoming peripheral impulses are transmitted by Primary afferent fibres (**Aα-, Aδ-, and C-fibres**), through the dorsal root ganglion (**DRG**) into the dorsal horn of the spinal cord. Nociceptive specific (**NS**) cells are mainly located in laminae I–II, whereas most wide dynamic range neurons (**WDR**s) are found in deeper lamina V. Lamina V neurones mainly form the spinothalamic tract and project to the thalamus. The Thalamus as a central relay station projects to various cortical regions, like primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices.
These areas are summarized to the 'pain matrix'. Projection neurones from lamina I form mainly the spinoparabrachial and the spinomesencephalic tract, who project to areas such as the parabrachial nucleus (**PB**) and periaqueductal grey (**PAG**) and such pathways are affected by limbic areas. From here descending from brainstem nuclei such as the rostral ventromedial medulla (**RVM**) are activated and modulate spinal processing (from D'Mello and Dickenson, 2008).

the lateral reticular formation and to the medial pontobulbar reticular formation and seems to take part in the neurovegetative and motivational-affective responses to pain or the spinomesencephalic tract that projects to the periaqueductal grey (PAG) and the deep layers of the superior colliculus. Dependent on the location where the axons reach the PAG they enhance the afferent nociceptive stimulus (on the dorsal part of PAG) or, if they project to the ventral part, activate inhibitory mechanisms responsible for the inhibition of the afference of this same pathway. Stimulation of this tract appears to regulate aversive behaviour as well as autonomic, cardiovascular, motivational, and affective responses. The ascending pathway leading towards the hypothalamus respond to noxious and innocuous stimulation coming from muscles, tendons, joints, skin and viscera and seems to communicate with the autonomic nervous system and to participate in neuroendocrine autonomic alert responses of somatic and visceral origin of the painful experience (Almeida et al, 2004). While the spinoparabrachial tract, leading towards the parabrachial nucleus is attributed to autonomic, neuroendocrine and affective responses to pain, it also seems to be involved in transmitting of visceral pain ascribable to the inflammatory process and thermal stimuli at noxious levels. But there is also evidence that even the lemniscal system, that do not rate among the ascending pain pathways, can transmit visceral pain information, especially under inflammatory conditions (Almeida et al, 2004; Paleček, 2004). The central relay station between the incoming spinal signals and higher cortical areas is the thalamus, who receives, interconnects and transmits the ascending spinal information to various parts of the cortex. Two functional different pain systems can be distinguish in the thalamus, known as the lateral and the medial nuclear complex, that both seem to have different tasks in the transfer and processing of the pain perception. The lateral nuclear complex consists of the ventroposterolateral (VPL), ventroposteromedial (VPM) and ventroposteroinferior (VPI) nuclei of the thalamus and forms the lateral system of ascending pathways, getting information

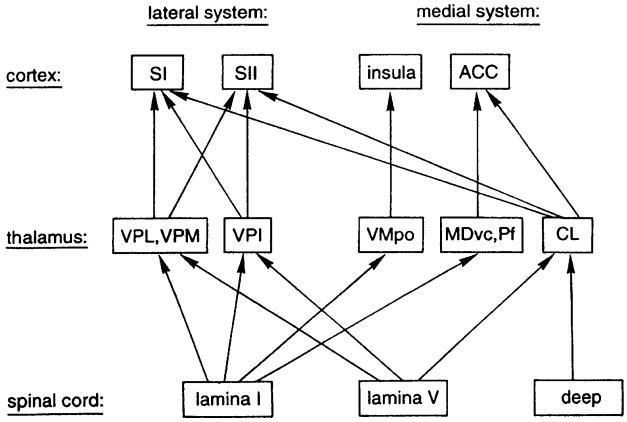

Fig. 3: Cortical areas that receive information from the simplified spinothalamic tract. The projection to the insula is deemed to be a pathway between the lateral and medial systems, because it has sensory-discriminative as well as cognitive-evaluative purpose in pain sensation.
Cortico-cortical connections are not shown (from Brooks and Tracey, 2005).
ACC: anterior cingulate cortex; **CL**: centrolateral nucleus;
MDvc: ventrocaudal part of medial dorsal nucleus;
Pf: parafascicular nucleus; **SI**: primary somatosensory cortex;
SII: secondary somatosensory cortex;
VMpo: posterior part of ventromedial nucleus;
VPI: ventral posterior inferior nucleus;
VPL: ventral posterior lateral nucleus;
VPM: ventral posterior medial nucleus.

mainly from the spinothalamic tract, that means from the dorsal horn laminae I and V. They project to the first somatosensory cortex (S1) and the second somatosensory cortex (S2), whereby the S1 handles incoming noxious information on topographic and intense aspects, while the S2 processes painful stimuli under temporal conditions (Almeida et al, 2004; Brooks and Tracey, 2005; Tracey, 2005).The medial nuclear complex, on the other hand, includes the ventrocaudal part of medial dorsal nucleus (MDvc), the parafascicular nucleus (Pf), the centrolateral nucleus (CL) and the posterior part of ventromedial nucleus (VMpo), as well as the Posterior nuclei (PO). While the fibres of the MDvc, the Pf and the CL reach the Anterior cingulate cortex, involved in affective, cognitive-evaluative procession of pain, the group of posterior nuclei, i.e. the VMpo and the PO, project to the insula, that encodes the intensity, the laterality of painful and non-painful thermal stimuli, but also seem to take part in affective pain processing, and take place between the medial and the lateral system of thalamus (Almeida et al, 2004; Brooks and Tracey, 2005). The system of the above-mentioned subcortical and cortical structures are called the "pain-matrix", but it is just a simplified model, because pain perception also involves other parts of the brain, e.g. the limbic system, mainly the amygdala, that receives fibres from the insula, and the prefrontal cortex, as well as the posterioparietal area, that is interconnected to S1 and S2. This complex relay system explains the different components of pain perception like attentional and emotional mechanisms, motivational–affective components as well as the interlink to behaviour, learning and memory (Almeida et al, 2004; Brooks and Tracey, 2005). Precisely because the perception and modulation of pain is a highly regulated physiological process, involving many parts of the peripheral and central nervous system and with effect on many different achievements of the human body, it is important to control not only excitatory, but as well inhibitory influences on pain processing.

These inhibitory influences, called the antinociceptive system, can modify the incoming noxious signal on every step of the ascending pathway, i.e. primary at the first order synapse in the spinal cord and secondary at the second order synapse in the brainstem. It seems, that the strongest antinociceptive modification occurs on spinal cord level, involving different interneuron and using endogenous opioid peptides and other biogenic amines like γ-amino-butyric acid (GABA). Some different influences can weaken the incoming peripheral information. One of the spinal segmental antinociceptive control mechanism is called the diffuse noxiuos inhibitory control (DNIC) and exists for nonspecific nociceptive neurons at all spinal segmental level. It involves a spinal-medullary-spinal feedback loop using serotoninergic pathways and is triggered by a heterotopic stimulation, i.e. a painful stimulus

outside the receptive field of the inhibited neuron, e.g. to a distinct part of the body. When stronger painful stimulus is applied to one part of the body, it activates the DNIC, that leads to an inhibition of the nonspecific nociceptive neurons, even when they receives nocious signals. The DNIC is the explaination, that pain from one site covers pain from another part of the body, the so called counter irritation (Calvino and Grilo, 2004; Butler and Finn, 2009). Descending inhibitory influences to the dorsal horn project mainly from nuclear groups located in the brainstem. The Focus of these inhibitory pathways are the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM), including the nucleus raphe magnus (NRM). The PAG receives inputs from the spinal cord, higher cortical centres like frontal and insular cortex as well as from diencephalic structures, e.g. the hypothalamus. Its fibres project via glutamatergic pathways to the RVM, but also to the parabrachial nucleus,and the nucleus tractus solitarius. Activation of the RVM leads via serotonergic mechanisms to an inhibitory influence on level of the dorsal horn, while the PAG activates also the locus coeruleus and locus subcoeruleus, who inhibit the dorsal horn of the spinal cord by noradrenergic transmitted projections using a2-noradrenergic receptors in gray matter layers II and IV (Riedel et al, 2001; Calvino and Grilo, 2004; Butler and Finn, 2009). It is crucial to understand, that the perception of pain is a far complex process, involving more than transduction of chemical, mechanical and thermal stimuli. As important the physiological ascending and descending pathways and the ability of modulation of a nociceptive stimulus are, as strong are other more psychological environments. Pain perception depends on geographical, sociocultural and affective circumstances as well as on an individual´s psychological status (Calvino and Grilo, 2004).

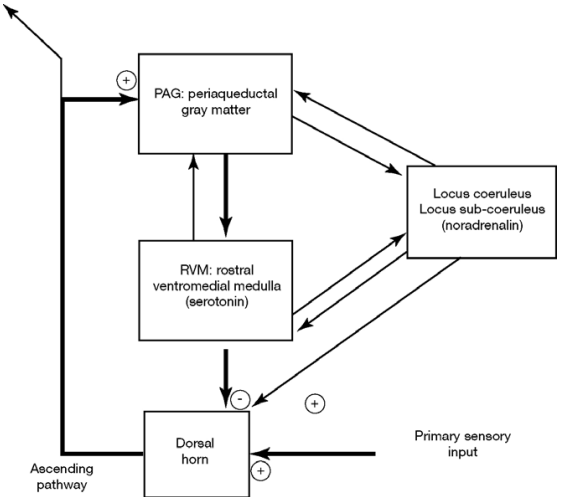

Fig. 4: Spinal-medullary-spinal negative feedback loop underlying an endogenous analgesic system called into play by nociceptive stimuli (from Calvino and Grilo, 2004).

1.2 Inflammatory pain and his mediators

Every kind of injury or infection is attended by a sequence of physiological processes that has the task to limit the releasing factor and to initiate the repair mechanisms of the tissue. This sequence is called inflammation and causes a change in the structure and function of the tissue. The inflammatory reaction is characterized by the five cardinal signs swelling (tumor), heat (calor), redness (rubor), pain (dolor) and loss of function (functio laesa). The first phase of inflammation is triggered by mediators released from damaged cells including ions like H^+ and K^+, as well as histamine, bradykinin, serotonin, ATP and nitric oxid. These substances active the nociceptive nerve terminals directly via different receptors, e.g. the acid-sensing ion channels (ASICs) for H^+, ionotropic purinoreceptors for ATP and the Bradykinin $B_{1/2}$-receptors, or sensitise the fibres for other stimuli via activation of second messenger cascades that takes influence on ion channels like the tetrodotoxin-resistant sodium channels (TTX-R) or the multimodal transducer channel TRPV1.

Nociceptor sensitisation is characterised as a decrease of activation thresholds and an increasing action potential frequency evoked during suprathreshold stimulation (Schaible and Richter, 2004; Kidd and Urban, 2001). Later phases of the inflammatory reaction are characterized on one hand by an activation of different inflammatory pathways, like the cyclooxygenase pathway and on the other hand by recruitment of different immune cell types, that contribute to pathological pain. Mainly three cell types takes part in perpetuation of the inflammatory cascade, i.e. mast cells, that store different pain mediators, activated macrophages and neutrophil granulocytes, the first immune cells that immigrate from blood to the tissue.

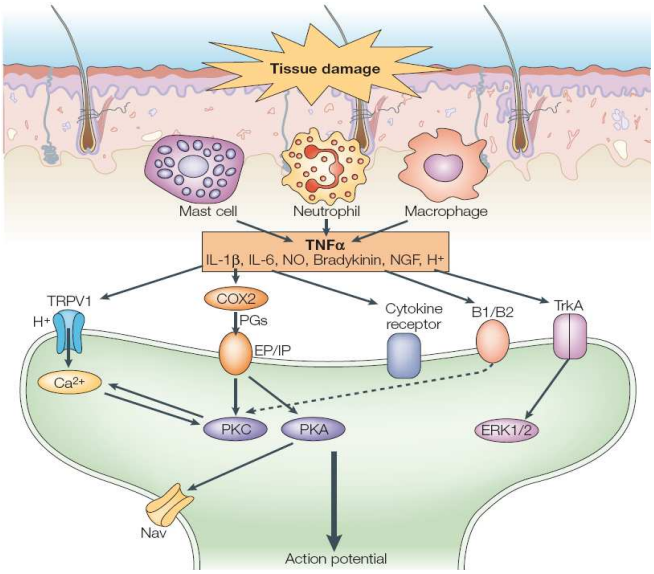

Fig. 5: After tissue damage, different immune cells are activated. They release various immune mediators like tumour necrosis factor-α (**TNFα**), interleukin-1β (**IL-1β**), interleukin-6 (**IL-6**), nitric oxide (**NO**), bradykinin, nerve growth factor (**NGF**) and protons. They act directly on nociceptors or indirectly through the release of other mediators, like prostanoids. The intracellular cascades that are activated in nociceptors by these mediators, activate or sensitize these neurons. (from Marchand et al, 2005)
COX2: cyclooxygenase 2; **B1/B2**: bradykinin receptor;
EP/IP: prostanoid receptor;
ERK1/2: extracellular signal-regulated kinase 1/2;
Nav: voltage-activated sodium channel; **PGs**: prostaglandins; **PKA/PKC**: protein kinase A/C; **TrkA**: tyrosine receptor kinase A;

They release pro-inflammatory cytokines like tumour necrosis factor-α (TNFα), interleukin 1β (IL1 β), Interleukin 6 (IL6), nerve growths factor (NGF) and prostaglandins. Each of these cytokines can create thermal and mechanical hyperalgesia and each of them act on specialised receptors expressed on nociceptive terminals. They influence the prostaglandin-synthesis by an upregulation of key enzymes as well as different receptors like the transient receptor potential vanilloid subfamily, member 1 (TRPV1) by activation of second messenger cascades like activation of protein kinase A (PKA) or PKC (Sommer and Kress, 2004). The NGF production, for example, is increased by TNFα and IL1β and acts via his tyrosine kinase receptor A (TrkA). Activation of the NGF-TrkA-complex leads to phosphorylation and sensitization of TRPV1 receptors and modulates after retrograde transport to the nucleus the expression of nociceptive genes, like CGRP, substance P, TRPV1, purinoreceptors, the bradykinin receptor B_2 and the voltage-gated sodium channel $NaV_{1.8}$ (Kidd and Urban, 2001; Schaibler and Richter, 2004; Marchand et al, 2005; Gold and Flake, 2005). As above mentioned the cyclooxygenase pathway plays a pivotal role in sensitising nociceptive fibres and in conveying hyperalgesia and allodynia. Three Cyclooxygenase isoforms are know, COX-1, COX-2 and COX-3. While COX-1 and COX-2 are coded on different chromosomes and located in different cell compartments, COX-3 seems to be a splice variant of COX-1, that is predominantly expressed in the CNS and can be inhibited by the analgesic and antipyretic drug Paracetamole (Ayoub et al, 2006). Cyclooxygenases (COX) catalyses the first step in prostanoid biosynthesis by converting arachidonic acid (AA) to the prostanoid precursor Prostaglandine H_2 (PGH_2). This precursor will be metabolized into different prostaglandin isoforms like prostaglandin E_2 (PGE_2), D_2 (PGD2), F_2 (PGF2), prostacyclin (PGI_2) or thromboxane A_2 (TXA_2). Two Cyclooxygenase isoforms are know,

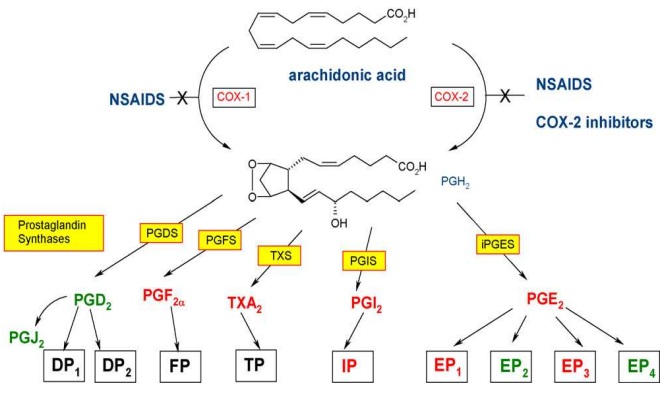

Fig. 6: The prostanoid cascade. Arachidonic acid is catalysed to PGH_2 by activity of cyclooxygenase, and prostaglandin or thromboxane synthases convert PGH_2 into biologically active products (from Bingham et al, 2006).

COX-1 is constitutively expressed and involved in physiological processes like cytoprotection in the GI tract, platelet function and renal perfusion (Vane et al, 1998), while COX-2 is the inducible isoform that is quickly upregulated under inflammatory conditions by inflammatory cytokines like IL1β or TNFα. An important role for pain sensitisation plays the prostaglandin PGE_2 (Samad et al, 2001)

Upon intrathecal injection PGE_2 causes sensitization of spinal neurons to heat and mechanical stimulation, by inhibition of postsynaptically located glycine receptors (Zeilhofer and Zeilhofer, 2008). It interacts with G-protein coupled EP receptors. $EP_{2/4}$ receptors are G_s-protein coupled receptors that increase via cAMP the activity of PKA and this increases the open probability at TTX-resistant sodium channels (Burian and Geisslinger, 2005). Sensitisation of $NaV_{1.8}$ channels occur via IP-receptors and activation of PKCε (Narumiya et al, 1999; Bingham et al, 2006; Gold and Flake, 2005).

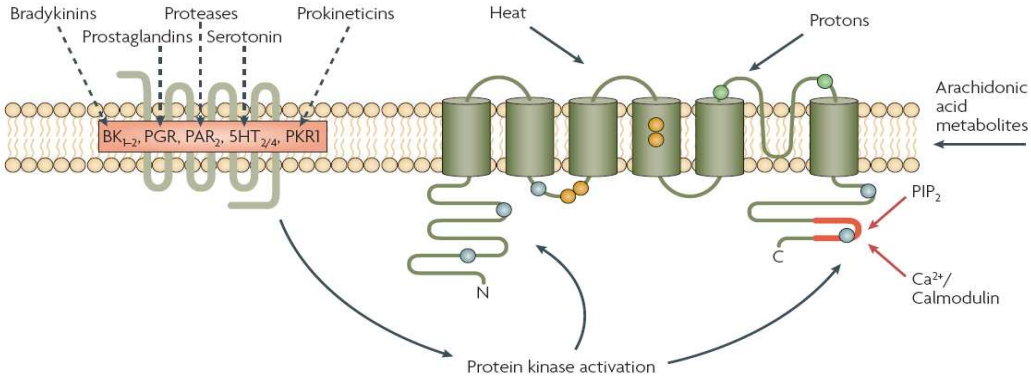

Fig. 7: Simplified summary of TRPV1 signal integration in peripheral nociceptor terminals. Solid arrows indicate TRPV1-sensitizing stimuli. The red arrows indicate negative regulation by phosphatidylinositol 4,5-bisphosphate (PIP2), calcium and calmodulin. Pro-inflammatory mediators and their receptors, that mediate the sensitization of TRPV1 are shown on the left. These sensitize TRPV1 through proteinkinase (PKC/PKA) activation, as well as increased arachidonic acid metabolite production and PIP2 hydrolysis do.
Coloured circles represent amino-acid residues that have been identified to be important in particular functions: orange: vanilloid binding (Y511, S512, L547, T550); blue: protein kinase phosphorylation sites (S116, T370, S502, T704, S800); and green: low-pH activation (E600, E646). The red line indicates the carboxy-terminal domain of TRPV1, which has been shown to interact with PIP2 and calmodulin (from Szallasi et al, 2007).

The TRPV1 thermoreceptor is apparently one of the main targets of different cytokines and pro-inflammatory mediators under pathological pain conditions and seems to be essential for thermal hyperalgesia and allodynia (Davis et al, 2000, Moriyama et al, 2005). The TRPV1 is member of a receptor family called "transient receptor potential", unspecific cation ion channels that react on changes of temperature. Some of the TRP family members respond to nonnocious heat like the TRPV3 and TRPV4, others are activated by cooling and menthol (TRPM8) or by painful cold (TRPA1). TRPV1 and TRPV2 both react on noxious heat with temperature above 42°C, but TRPV1 is the only channel that responds as well to capsaicin, the active substance in chili peppers (8-methyl-N-vanillyl-6-nonenamide) as to acid and its activation leads to an unspecific inward current of Na^+ and Ca^{2+} -ions into the cell (Willis, 2006). Especially nociceptive Aδ- and C-fibres express TRPV1 It exhibits a dynamic activation threshold, that can be increased by different inhibitory substances, but also decreases under inflammatory conditions. The list of mediators, who sensitise the TRPV1-channel, is long and includes bradykinin, NGF, prostanoids like PGE_2 and PGI_2, ATP and

adenosine. Generally a dephosphorylation of TRPV1 by protein phosphatases desensitises the channel, i.e. increases the activity threshold, while a protein-kinase-dependent phosphorylation lowers the threshold and facilitates the activity. PKA and PKC, especially PKCε, a diacyl glycerol (DAG)-dependent, Ca^{2+} -independent isoform of PKC, phosphorylates the TRPV1 channel and holds therefore a central position in initiation and maintenance of an enhanced response to nocious stimuli. Some cannabinoids and morphins on the other hand prevent the sensitisation of TRPV1 by blocking the above mentioned protein kinases (Gold and Flake, 2005; Szallasi et al, 2007; Willis Jr, 2009

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