Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology & Therapeutics 107 (2005) 139 – 154

Pharmacology & Therapeutics

www.elsevier.com/locate/pharmthera

Associate editor: L. Ballou

COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites

Maria Burian, Gerd Geisslinger*

pharmazentrum frankfurt/ZAFES, Institut für Klinische Pharmakologie, Klinikum der Johann-Wolfgang-Goethe-Universität Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany

Abstract

Despite the diverse chemical structure of aspirin-like drugs, the antinociceptive effect of NSAIDs is mainly due to their common property of inhibiting cyclooxygenases involved in the formation of prostaglandins. Prostaglandins are potent hyperalgesic mediators which modulate multiple sites along the nociceptive pathway and enhance both transduction (*peripheral sensitizing effect*) and transmission (*central sensitizing effect*) of nociceptive information. Inhibition of the formation of prostaglandins at peripheral and central sites by NSAIDs thus leads to the normalisation of the increased pain threshold associated with inflammation. The contribution of peripheral and central mechanisms to the overall antinociceptive action of NSAIDs depends on several factors including the location of the targets of drug action, the site of drug delivery and the uptake and distribution to the site of action.

The present work reviews the data on the regulation and location of cyclooxygenases at central and peripheral sites of the nociceptive pathway and focuses on the role of COX in the generation and maintenance of pain hypersensitivity. Experimental and clinical evidences are used to evaluate the significance of the peripheral and central antihyperalgesic effects of NSAIDs.

Keywords: NSAIDs; Hyperalgesia; Antinociceptive effects

Abbreviations: CFA, Complete Freund's adjuvant; CNS, Central nervous system; COX, Cyclooxygenase; DRG, Dorsal root ganglion; NSAIDs, Non-steriodal anti-inflammatory drugs; PG, Prostaglandin; TTX-R, Tetrodotoxin-resistant sodium channels.

Contents

* Corresponding author. Tel.: +49 69 63017619; fax: +49 69 63017617.
E-mail address: geisslinger@em.uni-frankfurt.de (G. Geisslinger).

0163-7258/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.pharmthera.2005.02.004

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed and widely used drugs in the management of pain, especially pain associated with inflammatory conditions. Despite the wide use of NSAIDs during the last century (the first NSAID, aspirin, was developed in the late 1800s), for a long period only little was known about the mode of action of these drugs. It was not until the 1970s that investigations into the mode of action of aspirin-like drugs were taken over by prostaglandin researchers, who showed an association between prostaglandin production and the action of aspirin-like drugs (Vane, 1964). In 1971, Vane discovered that NSAIDs could inhibit prostaglandin synthesis and proposed that this mechanism was the basis of their pharmacological action (Vane, 1971). This hypothesis was supported by other experimental data demonstrating the presence of prostaglandins at sites of inflammation (Willis, 1969; Di Rosa et al., 1971) and their ability to reproduce some of the cardinal signs of inflammation (Arora et al., 1970). The biomolecular target of NSAIDs was not identified until 1976 when a purified and enzymatically active cyclooxygenase (COX) was isolated from sheep vesicular glands (Hemler & Lands, 1976). It took several years more i.e. until the late 1980s before it was discovered that COX exists in at least two isoforms (Kujubu et al., 1991; Hla & Neilson, 1992), COX-1 and COX-2, and that "classical" NSAIDs are non-selective inhibitors of both isozymes (Xie et al., 1992).

Initially, the principle mode of the antinociceptive action of NSAIDs was considered to be related to their anti-inflammatory action and was thought to be due solely to the inhibition of prostaglandin production at the site of inflammation. This argument has its origin in the experimental observations of Horton (1963), Willis (1969), Juhlin and Michaelsson (1969), Crunkhorn and Willis (1971), Karim (1971) and, particularly, those of Ferreira (1972). By estimating pain in healthy volunteers following the intradermal administration of various inflammatory mediators (histamine, bradykinin and alprosadil (PGE_1)), Ferreira could show that whilst PGE_2 'per se' produced no pain, its presence was essential for the induction of pain by histamine and bradykinin. These findings form the basis of the currently accepted view that PGs are not generally algogenic but act as sensitizing agents for enhancing the nociceptive properties of various inflammatory mediators. Additional evidence for the concept that NSAIDs exert their anti-nociceptive action peripherally comes from tissue distribution data obtained from whole-body autoradiography after administration of various NSAIDs in rats (Brune, 1974). In particular, it was shown that NSAIDs, because of the acidic moiety, can be extensively accumulated in inflamed tissues, where they exert their pharmacological action. At that time, the distribution of NSAIDs in other tissues, in particular in the CNS, was construed only as an explanation for the organ specific toxicity and was not thought to be related to their antinociceptive action (Brune, 1974).

The traditional belief that NSAIDs have exclusively a peripheral mode of action was not challenged until the beginning of the 1990s in the wake of the growing evidence that the anti-inflammatory and the antinociceptive effects of NSAIDs are unrelated (McCormack & Brune, 1991). So, an existence of some central antinociceptive mechanisms, an idea initially put forward by Hazlick as early as 1926 (Jurna, 1997), has been suggested (Ferreira et al., 1978; Carlsson et al., 1988). Although various mechanisms have been proposed to account for central effects of NSAIDs (Bjorkman, 1995), inhibition of prostaglandin synthesis (Abdel-Halim et al., 1978; Ferreira et al., 1978) in the CNS, and particularly in the spinal cord (Jurna et al., 1992; Malmberg & Yaksh, 1992), appears to be a property of all NSAIDs. Furthermore, the finding that both COX isozymes, i.e. COX-1 and COX-2, are expressed in the CNS (Kaufmann et al., 1997) and the observation that experimental induction of peripheral inflammation is associated with an increase in the expression of COX-2 in the spinal cord (Beiche et al., 1996) have been used as evidence supporting a central antinociceptive mechanism of action of NSAIDs (Vasquez et al., 2001; Vanegas, 2002).

This review of the central and peripheral antinociceptive mechanisms of action of NSAIDs mainly focuses on the inhibition of cyclooxygenase-mediated prostaglandin synthesis, as a class-like pharmacological property of NSAIDs. Cyclooxygenase-independent actions of NSAIDs, including cellular mechanisms mediated via interference with transcription factors (Tegeder et al., 2001b) and direct modulation of the activities of various ion channels (Lee et al.,

2003), have so far only been observed with a few NSAIDs and are therefore not discussed here in detail.

The mechanisms and clinical manifestations involved in inflammatory pain, a condition commonly treated with NSAIDs, are dealt with first and this is followed by a discussion on the location and regulation of the main target of NSAIDs, cyclooxygenases at the central and peripheral sites. Biochemical aspects of prostaglandin synthesis are presented and special attention is given to the mechanisms of the prostaglandin-mediated hyperalgesia. The final section deals with the antihyperalgesic action of NSAIDs where attention is given to the currently available experimental and clinical evidence supporting their peripheral and central mechanisms. The contribution of both mechanisms to the overall antinociceptive action of this class of analgesics is discussed.

2. Inflammatory pain

The nociceptive signalling in physiological pain is initiated by activation of the specialized pain receptors (nociceptors), which are polymodal sensory fibres of the primary sensory neurons located in trigeminal and dorsal root ganglia (DRG). Although the nociceptors are able to respond to a wide range of stimulus modalities, different noxious stimuli generally cause activation of a single receptor [e.g. heat activates $TRPV_1$-receptors (Caterina et al., 1999) and mechanical force, P_2X_3 receptors (Nakamura & Strittmatter, 1996)]. The activation of nociceptors generates depolarizing currents (***transduction***), which are conducted to the spinal cord along unmyelinated, slow conducting C fibres and more rapidly conducting Aδ primary sensory fibres (***conduction***). This sensory inflow then activates secondary sensory neurones in the dorsal horn of the spinal cord through synaptic transfer (***transmission***), which project to the cortex via a relay in the thalamus.

Whilst ***physiological pain*** has a protective function in warning the body of potentially damaging stimuli, inflammatory pain associated with any kind of tissue damage has a pathological character. The specific hallmark of inflammatory pain is ***sensitization***, which is a process involving a reduction in the threshold for activation, an increase in response to a given stimulus and the appearance of spontaneous activity in the nociceptors. It manifests itself clinically as ***hyperalgesia*** (enhanced response to noxious stimuli) or ***allodynia*** (sensation of pain in response to a previously non-noxious stimulus). Both phenomena are an expression of neural plasticity which is a response of the nervous system to the peripheral tissue injury and inflammation (Woolf & Salter, 2000). Sensitization can occur at both the peripheral and central level of nociceptive processing (Woolf, 1983), where prostanoids are potent sensitizing agents involved in the modulation of transduction and transmission of nociceptive information.

3. Cyclooxygenase-prostanoid cascade in nociceptive processing

3.1. Prostaglandin synthesis

Despite the diverse chemical structure of aspirin-like drugs, the antinociceptive effect of NSAIDs is mainly due to their common property of inhibiting cyclooxygenases involved in the formation of prostanoids. Prostanoids are formed by most cells and act as autocrine and paracrine lipid mediators. They are not stored but are synthesized de novo from membrane-released arachidonic acid mobilized by phospholipases (PLA_2) when cells are activated by mechanical trauma, cytokines and growth factors, etc. Conversion of arachidonic acid to prostanoid endproducts is carried out by cyclooxygenases (COX), also known as prostaglandin H synthase (PGHS), at two different sites on the enzyme. Arachidonic acid is initially cyclized and oxidized to the endoperoxide PGG_2 at the cyclooxygenase site of the COX and the product then reduced to a second endoperoxide PGH_2 at the peroxidase site (Garavito & deWitt, 1999). Subsequent formation of prostaglandin end products from PGH_2 depends on the presence of specific synthases that produce the functionally important prostanoids PGD_2, PGE_2, PGF_2, PGI_2 (prostacyclin) and TXA_2 (thromboxane).

These prostaglandins may undergo facilitated transport out of the cell by prostaglandin transporters (PGT) and other carriers and exert autocrine or paracrine actions on a family of prostaglandin receptors on the cell membrane that bind PGE_2 (EP_1, EP_2, EP_3, EP_4 receptors), PGD_2 (DP_1, DP_2 receptors), PGF_2 (FP receptor), PGI_2 (IP receptors) and TXA_2 (TP_α and TP_β receptors). Prostaglandin receptors, with the exception of DP_2 which is a chemoattractant receptor, belong to three clusters within a distinct subfamily of a G-protein-coupled superfamily of receptors. The "relaxant" receptors IP, DP_1, EP_2 and EP_4 in one cluster signal via Gs-mediated increases in intracellular cyclic adenosine monophosphate (cAMP). The "contractile" receptors EP_1, FP and TP form a second group that signals via Gq-mediated increases in intracellular calcium. The EP_3 receptor is regarded as an "inhibitory" receptor that couples to Gi and decreases cAMP formation. The differential expression of the synthases and prostaglandin receptors are determinants of the prostanoid-production profile within various cells.

3.2. Biochemistry of cyclooxygenases (COX)

COX exists as two isoforms, COX-1 and COX-2. It has recently been shown that the COX-1 protein family consists of at least four different mRNA variants derived from the COX-1 gene. These include COX-1 itself, COX-3 and the two truncated partial pCOX-1 sequences, pCOX-1a and pCOX-1b (Chandrasekharan et al., 2002; Dinchuk et al., 2003). Apart from COX-1, only the COX-3 transcript, which contains the catalytic structure of COX-1 and retained

intron 1, has been shown to possess cyclooxygenase activity in dogs. However, on the basis of nucleotide sequence data obtained in humans, the existence of COX-3 has been called to question by at least two independent research groups (Dinchuk et al., 2003; Schwab et al., 2003).

COX-1 and COX-2 are membrane-associated enzymes with a molecular weight of 71 kDa and an amino-acid sequence similarity of 63% (Vane et al., 1998; Hawkey, 1999). The COX-2 gene is a small immediate early gene (8.3 kb), whereas COX-1 originates from a much larger (22 kb) gene (Garavito et al., 2002; Vane et al., 1998).

The identification of two isoforms of COX in the early 1990s offered the simple and attractive hypothesis. It was believed that COX-1, being found in almost all cells, is the constitutive "house-keeping" enzyme responsible for production of basal "beneficial" PGs, vital for protecting the stomach through production of mucus and the maintenance of renal blood flow. Furthermore, COX-2, which has low expression or is undetectable in most cells but increases dramatically in a variety of pathological conditions, was proposed to be an inducible enzyme responsible for the "pathological" production of PGs during inflammation and in cancerous states. However, experience gained from the introduction of selective COX-2 inhibitors showed that the mechanisms inherent in the above hypothesis were more complicated than initially thought since evidence was accruing that COX-1 and COX-2 both have physiological and pathological roles (Garavito et al., 2002). A notable exception thus concerns the COX isoforms in the CNS and the localization, regulation and involvement of these in nociception will be discussed in the subsequent section.

3.3. Cyclooxygenase (COX) and prostanoids at peripheral sites

COX-2 is the predominant isoform expressed in injured tissue and a main source of prostanoids during inflammation. This evidence comes from various experimental models, including the subcutaneous air pouch (Appleton et al., 1995) and carrageenan-induced pleurisy in the rat (Tomlinson et al., 1994). In these models, polymorphonuclear leucocytes, phagocytosing mononuclear cells and fibroblasts show an increased expression of COX-2 and PGE_2 is the most abundant prostanoid found in the injured tissue. However, it is important to note that the quantity of prostanoid produced and the profile of prostanoid production during the inflammatory reaction can change. For example, in the carrageenan-induced pleurisy model, elevated PGE_2 levels are only observed during the early stages of inflammation, whereas the production of PGD_2 is most pronounced during the final stages of the response (Gilroy et al., 1999). Thus, the quantity and variety of prostanoids produced during inflammation and which can contribute to peripheral sensitization are determined by the nature and the activation state of the cells present in the inflammatory lesion.

Although the induction of COX-2 expression by inflammatory stimuli is mainly responsible for the high prostanoid levels at the site of inflammation, the role of COX-1 in modulating the inflammatory response should not be overlooked. The involvement of COX-1 in the inflammatory response is supported by experimental findings showing an increase in COX-1 in circulating monocytes on exposure to LPS (McAdam et al., 2000) and the early production of prostaglandins in stimulated mast cells (Reddy & Herschman, 1997). There are also results from studies in mice deficient in the expression of COX-1 or COX-2 which indicate that COX-1 has a unique role in the initiation of certain inflammatory responses, for example, in the arachidonic acid-induced inflammation of the ear and in the subdermal air pouch model (Langenbach et al., 1995, 1999).

Both COX isoforms are involved together in the inflammatory reaction and may contribute to peripheral sensitization where the earliest prostanoid response is dependent on COX-1 and COX-2 becomes a major source of prostanoid production as inflammation progresses.

3.4. Cyclooxygenase (COX) and prostanoids in CNS

In contrast to the situation in the periphery, both COX-1 mRNA and COX-2 mRNA as well as COX-1 and COX-2 proteins are expressed constitutively in neurons and non-neuronal elements of cervical and lumbar sections of the spinal cord (Beiche et al., 1996). COX-2 appears to be the predominant isoform and is present in neurons of all laminae, particularly the superficial layers, motoneurons and non-neuronal cells such as astrocytes (Goppelt-Struebe & Beiche, 1997; Willingale et al., 1997; Beiche et al., 1998). COX-1 expression at the spinal level is not extensive and can only be found in the cytoplasm of glial cells. Dorsal root ganglion (DRG) cells contain mainly COX-1 which is located in the cytoplasm, nuclear membrane and axonal processes of small- and medium-sized neuronal cell bodies (Willingale et al., 1997). The presence of COX-2 mRNA in DRGs, however, has also been reported (Inoue et al., 1999).

Although both isoforms are constitutively expressed in the spinal cord, there is a significant difference in the way in which they are regulated. Whereas COX-2 expression can be induced above the constitutive level by several experimental conditions, the same stimuli cause no significant increase in COX-1 mRNA. Thus, an increase in COX-2 mRNA is seen in DRG cell cultures stimulated with IL-1β (Inoue et al., 1999); COX-2 expression in the spinal cord of experimental animals is increased after intraspinal injection of exogenous IL-α (Tonai et al., 1999) and after induction of spinal cord injury associated with increased levels of endogenous IL-α and IL-1β (Resnick et al., 1998; Tonai et al., 1999).

The up-regulation of spinal COX-2 also takes place during peripheral inflammation. In experimental animals, the induction of neuronal and non-neuronal COX-2 in ipsi- and contralateral portions of the cervical and lumbar cord

has been observed after hindpaw injection of several irritants such as carrageenan (Hay & de Belleroche, 1997; Ichitani et al., 1997; Ebersberger et al., 1999; Maihofner et al., 2000), zymosan (Maihofner et al., 2000; Tegeder et al., 2001a) and complete Freund's adjuvant (CFA) (Beiche et al., 1996, 1998a; Hay & de Belleroche, 1998; Samad et al., 2001). The up-regulation is paralleled by a substantial elevation in basal and evoked PG, mostly PGE_2, in the cerebrospinal fluid and hyperalgesic behaviour of the animals. In contrast to the events which take place during the inflammatory response in peripheral tissue where both phospholipase A_2 (PLA_2), another rate-limiting step in prostaglandin synthesis, and COX-2 contribute to the increased production of prostaglandins, in the spinal cord there is no increase in the activity of PLA_2 (Saunders et al., 1999; Samad et al., 2001). Thus, COX-2 in the spinal cord appears to have a major role in the elevation of PGE_2 during peripheral inflammation and is mainly responsible for the central hypersensitivity observed with inflammation. However, this does not exclude COX-1 in the spinal cord also being a source of spinal PGs in peripheral inflammation and, indeed, this has been demonstrated in COX-2-deficient knockout mice (Ballou et al., 2000). Recent findings have also shown that COX-1 plays an important role in spinal nociceptive processing and sensitization after surgery (Zhu et al., 2003) and that the expression of COX-1 in the spinal cord changes in a time- and laminar-dependent manner after nerve injury (Zhu & Eisenach, 2003). This suggests that the inhibition of spinal COX-1 may be useful in the treatment of post-operative and neuropathic pain.

A significant increase in COX-2 mRNA is also observed in supraspinal structures of the CNS including the pons, ventral midbrain, hypothalamus, thalamus and areas of cortex limited to somatosensory processing. These changes resemble the up-regulation of COX-2 in the spinal cord which occurs in response to peripheral inflammation (Samad et al., 2001) and are associated with an increase in PGD_2 in cerebrospinal fluid, a major prostaglandin found in rodent brain homogenates (Abdel-Halim et al., 1978).

An important question concerns the nature of the linkage between peripheral inflammation and the up-regulation of COX-2 in the CNS where both neuronal and humoral mechanisms may be important. On the neuronal side, prolonged activity in small primary afferents generated by local inflammation results in afferent-evoked release of glutamate/aspartate and substance P in the spinal cord leading to activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NK-1 (neurokinin-1) receptors and this is followed by activation of NMDA (*N*-methyl-D-aspartate) receptors in dorsal horn neurones (Dickenson et al., 1997). Stimulation of these receptors may produce a synaptic activity-dependent increase in the transcription of a variety of enzymes, including spinal COX-2. On the humoral side, peripheral inflammatory reactions associated with tissue injury result in the release of pro-inflammatory cytokines such as TNF-α and IL-1β, which can enhance the transcription of COX-2. Samad et al. (2001) addressed this question in a study of the COX-2 expression in the spinal cord in animals with a complete sensory and motor blockade of sciatic nerve conduction carried out prior to inducing hind-paw inflammation with CFA. Neural blockade produced a slight but not complete attenuation of the COX-2 mRNA induction in the lumbar spinal cord and electrical stimulation at C-fibre-strength of the sciatic nerve increased the induction of spinal COX-2 by much smaller extent than that produced by inflammation. These findings and the knowledge that the transcription of COX-2 in the spinal cord is delayed (COX-2 up-regulation is at maximum 6 h after the induction of inflammation) lead Samad et al. to favour a non-neuronal mechanism of central COX-2 induction mediated by IL-1β. It has, indeed, been shown that the intrathecal injection of IL-1β causes the up-regulation of COX-2 and there is a significant reduction in COX-2 mRNA levels following the intrathecal administration of IL-1β receptor antagonists and interleukin-converting enzyme (ICE) inhibitor. The current view is that circulating IL-1β, which is released at peripheral sites after injury and cannot pass the blood–brain barrier, induces COX-2 and specific PGE_2 synthases (mPGES) in the cell lining of the barrier. This is followed by the entry of de novo synthesized PGE_2 into the brain and cerebrospinal fluid and prostanoid receptor activation in neurons and microglia.

3.5. Mechanisms of prostaglandin-mediated hyperalgesia

Prostaglandins are potent sensitizing agents, which are able to modulate multiple sites in the nociceptive pathway enhancing transduction (*peripheral sensitizing effect*) and transmission (*central sensitizing effect*) of nociceptive information. PGE_2 is thought to be a principle mediator of the hypersensitivity (Daher & Tonussi, 2003) but several prostanoids show similar effects (Ferreira & Lorenzetti, 1981; Hamilton et al., 1981).

The hyperalgesic properties of prostaglandins in the periphery have been demonstrated in human and experimental studies. The subcutaneous injection of a small dose of PGE_2 in human volunteers produces long-lasting hyperalgesia to mechanical and chemical stimuli although PGE_2 per se does not produce spontaneous pain (Ferreira, 1972). Monoclonal antibodies against PGE_2 inhibit hyperalgesia in carrageenan-induced rat paw edema and reduce the nociceptive dorsoflexion response to the intraperitoneal injection of phenylbenzoquinone in mice (Mnich et al., 1995). In vitro, PGE_2 is able to significantly sensitize the canine testicular polymodal receptors of sensory C-fibre receptors to heat and bradykinin by acting on EP_2 and EP_3 receptors, respectively (Mizumura et al., 1987; Kumazawa et al., 1996).

The central hyperalgesic properties of prostanoids have been established mainly in experimental studies with intrathecal administration of PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxB_2 which evoke thermal and mechanical hyperalgesia

(Uda et al., 1990; Minami et al., 1992, 1994) and mimic the central sensitization associated with peripheral inflammation (Vanegas & Schaible, 2001). These effects appear to be mediated by several subtypes of prostanoid receptors (Tilley et al., 2001) including EP_1 (Stock et al., 2001), EP_3 (Minami et al., 2001) and IP receptors (Murata et al., 1997). The observation that IP, EP_1 and EP_3 receptors are co-expressed in peripheral and central structures involved in the nociceptive pathway is consistent with this hypothesis (Oida et al., 1995).

3.5.1. Peripheral sensitization

The main mechanism involved in the hyperalgesic action of prostaglandins is traditionally considered to be due to a sensitizing effect on primary afferent nerves (Davies et al., 1984). The molecular targets associated with this mode of action, however, have only recently been identified where both direct and indirect mechanisms of the hyperalgesic action of PGs have been discussed. The direct effects are mediated by the action of prostaglandins on EP/IP receptors and modulation of ion channels in primary afferents. The indirect effects are directed towards enhancing the sensitivity of sensory neurons to noxious agents such as heat and bradykinin and also assumed to contribute significantly to the PG-mediated peripheral sensitization (Fig. 1).

When PGs act via prostaglandin receptors, they decrease the activation threshold of a specific subclass of voltage-gated sodium channels i.e. TTX-R Na^+ (tetrodotoxin-resistant sodium channels) (Gold et al., 1996a) which are

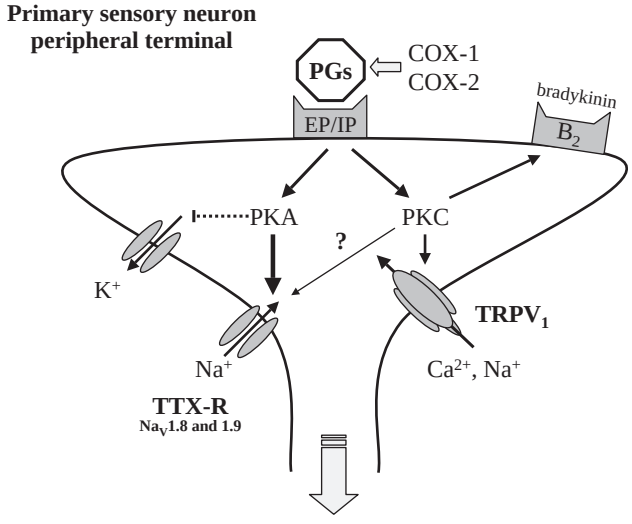

Fig. 1. Molecular mechanisms of the sensitizing action of prostaglandins in peripheral terminals of the primary sensory neurons. Prostaglandins contribute to peripheral sensitization by several mechanisms, including activation of TTX-R Na+ channels as well as inhibition of voltage-gated potassium currents. Prostaglandins also possess indirect effects e.g. enhancement of the sensitivity of sensory neurons to bradykinin by acting on B_2-receptors and to capsaicin by acting on $TRPV_1$-receptors. Activating and inhibiting pathways are depicted by solid and slashed lines, respectively. PGs=prostaglandins; TTX-R=tetrodotoxin-resistant sodium channels; TRPV1=vanilloid receptors; PKA=protein kinase A; PKC=protein kinase C.

expressed in nociceptors and are involved in the transduction of nociceptive information. These channels are believed to contribute significantly to the firing-rate and duration of the action potential in small-diameter sensory neurons (Baker & Wood, 2001). PG-mediated modulation of the TTX-R Na^+-current involves phosphorylation of the TTX-R channels following activation of the protein kinase A (PKA) pathway (England et al., 1996). Protein kinase C (PKC) is also thought to be involved but the exact role of the enzyme is not clear (Gold et al., 1998). Which of the two known TTX-R Na^+-channel subtypes within DRG ($Na_v1.8$ or $Na_v1.9$) are targeted by prostaglandins and under what conditions this occurs has also yet to be determined. Behavioural analysis of $Na_v1.8$-deficient mice revealed modest deficits in acute sensation to noxious stimuli and a delayed onset of inflammatory thermal hypersensitivity, although the maximal response was equivalent to that in wild-type animals (Akopian et al., 1996). Such observations support the involvement of $Na_v1.8$ in hypersensitivity resulting from tissue injury and indicate that this subtype of TTX-R Na^+-channels is a target for prostanoids. However, redundancy of voltage-gated Na^+ channel subtypes in nociceptor function has not been ruled out.

It is known that PGE_2 and PGI_2 can sensitize primary afferent neurons by inhibiting voltage-gated potassium currents and this mechanism could contribute to neuronal excitability (England et al., 1996; Nicol et al., 1997). This modulation, like the enhancement of the TTX-R sodium current described above, also results from the activation of protein kinase A (Evans, et al., 1999) and leads to an increase in membrane resistance, a decrease in the firing threshold and an increase in the number of action potentials per stimulus. PGE_2 may also inhibit a potassium current by increasing intracellular calcium in capsaicin-sensitive primary afferent neurons (Gold et al., 1996b).

In addition to the actions described above, prostaglandins can also enhance the sensitivity of sensory neurons to excitatory chemical agents such as bradykinin acting via B_2 receptors (Cui & Nicol, 1995) and capsaicin acting via $TRPV_1$ receptors (Lopshire & Nicol, 1998).

3.5.2. Central sensitization

PGE_2 is released within the spinal cord after peripheral nociceptive stimulation. Results from in vivo spinal microdialysis and sampling of cerebrospinal fluid show an increase in PGE_2 following i) acute activation of small afferents using intraplantar injection of formalin and application of heat (Malmberg & Yaksh, 1995; Scheuren et al., 1997); ii) chronic inflammation following the injection of carrageenan in a knee joint and intraplantar injection of FCA and zymosan (Ebersberger et al., 1999; Guhring et al., 2000; Samad et al., 2001); and iii) intrathecal administration of substance P, NMDA and kainate (Yang et al., 1996; Hua et al., 1999) and the systemic administration of cytokines (Samad et al., 2001). These increases in the release of PGE_2 have functional relevance since they are typically blocked

by the intrathecal and systemic administration of COX inhibitors.

Although specific binding sites for PGE_2 (Kawamura et al., 1997) as well as proteins and mRNA of all four known subtypes of PGE_2 receptors (EP_1–EP_4) have been detected in the dorsal horn of the spinal cord, the exact molecular and cellular elements involved in PGE_2-mediated central hyperalgesia have not yet been fully elucidated. The effects of prostaglandins are thought to occur from an action on both presynaptic and post-synaptic membranes of the primary afferent synapse in substantia gelatinosa of the spinal cord (Fig. 2). An action of PGE_2 on prostanoid receptors at the presynaptic membrane may cause enhancement of nociception by facilitating the spinal release of the excitatory neurotransmitter glutamate (Nishihara et al., 1995) and neuropeptides (substance-P and/or calcitonin gene-related peptide) (Nicol et al., 1992; Hingtgen & Vasko, 1994) from primary afferent C-fibre terminals, as has been demonstrated in DRG cells and isolated spinal cord tissue. These effects are mediated by an increase in the inward calcium current.

At the post-synaptic level, PGE_2 can directly activate deep neurons in the dorsal horn via EP-2-like receptors, thus enhancing the transmission of nociceptive responses (Baba et al., 2001). Recent evidence indicates that PGE_2 may also facilitate the transmission of nociceptive input by blocking inhibitory glycinergic neurotransmission to dorsal horn neurones (Harvey et al., 2004) by activation of EP_2-like receptors, cholera-toxin-sensitive G-proteins and cAMP-dependent protein kinase in the inhibitory neurons (Ahmadi et al., 2002).

4. Central and peripheral antihyperalgesic effects of non-steriodal anti-inflammatory drugs (NSAIDs)

NSAIDs are a disparate group of weakly acidic, highly protein-bound compounds having the common pharmacological property of inhibiting prostaglandin biosynthesis (Smith et al., 1994; Kirtikara et al., 2001). This inhibition results from a variety of effects on cyclooxygenases including the irreversible inactivation of COX (e.g. by aspirin) and reversible competitive inhibition of COX (e.g. with ibuprofen). NSAIDs usually normalise the increased pain threshold associated with inflammation rather than elevate a normal pain threshold and thus their antinociceptive action is more accurately described as *antihyperalgesic* than as *analgesic*.

The existence of a central component in the antinociceptive action of NSAIDs demonstrated by Ferreira et al. (1978) is supported by the finding of a significant dissociation between the anti-inflammatory and antinociceptive effects of aspirin-like drugs (Brune et al., 1991; McCormack & Brune, 1991). Quantification of this dissociation has been carried out using a dissociation index which is defined as the mean potency of the NSAID in an in vivo model of inflammation (i.e. ED_{50} required to inhibit carrageenan-induced edema) divided by the mean potency of the agent in an in vivo model of pain (i.e. ED_{50} required to reduce the number of writhings after intraperitoneal injection of phenylbenzoquinone) (McCormack & Urquhart, 1995). The magnitude of this dissociation with various NSAIDs can be visually assessed by plotting the index

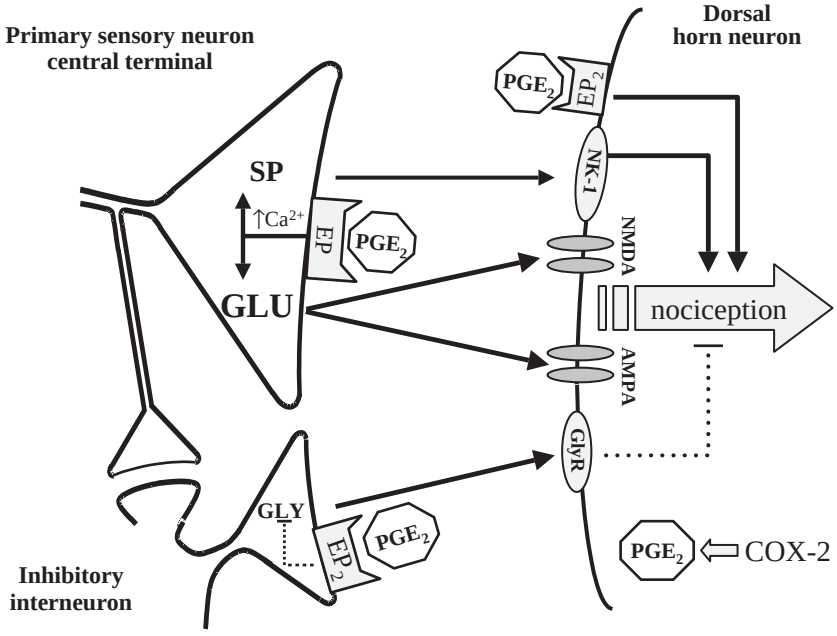

Fig. 2. Molecular mechanisms of the sensitising action of PGE2 in dorsal horn PGE_2 exerts antihyperalgesic effects by acting on pre- and post-synaptic membranes of the primary afferent synapse. The activation of the presynaptic EP receptors leads to facilitated spinal release of glutamate and neuropeptides resulting in enhanced nociceptive processing. At the post-synaptic level, PGE_2 can directly activate deep dorsal horn neurons via EP-2-like receptors located on dorsal horn neurons and block inhibitory glycinergic neurotransmission by activation of EP-2-like receptors in the inhibitory neurons. Activating and inhibiting pathways are depicted by solid and slashed lines, respectively. GLU=glutamate, GLY=glycine, SP=substance P.

against a clinical measure of the antihyperalgesic efficacy of the NSAID determined in the post-dental extraction pain model (i.e. mean placebo-corrected 4-h sum of pain intensity differences) and this dissociation parameter may reflect the net effect of the interaction between COX-dependent peripheral and central sites of action (the possibility that additional mechanisms of action, other than an inhibition of PG-synthesis are involved, cannot be ruled out). Using these quantification methods, the NSAIDs ketoprofen, ketorolac and bromfenac had particularly large dissociation indices suggesting that the contribution of non-peripheral antinociceptive mechanisms in the action of these drugs may be of particular significance.

From the pharmacological point of view, the relative contribution of central and peripheral mechanisms to the overall antinociceptive action of an NSAID depends on:

- site of drug delivery (e.g. systemic, local–peripheral, epidural, spinal intracerebro-ventricular) and
- uptake and distribution from the site of drug delivery, as determined by factors such as physical and chemical properties of the NSAID, specific transport mechanisms, local and systemic blood flow and tissue barriers to drug permeation such as the blood–brain barrier.

The particular role of these factors is discussed in the subsequent sections.

4.1. Intrathecal delivery of non-steriodal anti-inflammatory drugs (NSAIDs) in behavioural studies in animals

An accepted approach for establishing that NSAIDs alleviate pain by acting on the CNS is to measure antinociceptive activity after direct application to spinal and supraspinal tissues and this approach has gained particular importance in behavioural studies in animals. The intrathecal application of NSAIDs has been tested in models of acute short-term and long-term inflammatory pain, including formalin-induced flinching (the so-called formalin test), carrageenan-induced thermal hyperalgesia, zymosan-induced thermal hyperalgesia and CFA (Table 1).

The formalin test is a classical model of acute inflammatory pain. Subcutaneous injection of formalin into the hind paw of the animal induces a stereotypical biphasic response, consisting of an early, short-lasting painful response occurring about 3 min after injection, followed by a prolonged period of tonic/persistent pain after 20–30 min. The first phase results from a direct activation of C-fibres due to the peripheral stimulus, whereas the second phase involves a period of sensitization associated with acute inflammation. The precise origin (i.e. central or peripheral) of the second phase is a subject of debate (Tjolsen et al., 1992; Yashpal et al., 1998), therefore, its inhibition with systemically administered NSAIDs (Hunskaar & Hole, 1987; Geisslinger et al., 1994; Yashpal & Coderre, 1998) may have both peripheral and central components. However, a significant reduction in nociceptive behaviour after intrathecal application of NSAIDs at doses that are inactive systemically is clear evidence for a central nociceptive mode of action. The rank order of potency and ID_{50} on intrathecal delivery is: indomethacin $\geq$ flurbiprofen > ketorolac $\geq$ zomepirac < *S*(+)-ibuprofen $\geq$ ibuprofen (racemic) > acetylsalicylic acid > acetaminophen (Malmberg & Yaksh, 1992). Intrathecal application of *R*(−)-flurbiprofen, which is only a very weak COX-inhibitor, also shows antinociceptive activity (Brune et al., 1991; Geisslinger et al., 1994; Malmberg & Yaksh, 1994) suggesting that the central mode of action of *R*-flurbiprofen is independent of COX-inhibition (Geisslinger et al., 1994).

The central antihyperalgesic effects of NSAIDs have been extensively studied in the modified tests of acute pain, where the nociceptive stimuli (thermal or mechanical) are applied to that part of the body (e.g. paw) which is inflamed following subcutaneous injection of an irritant such as carrageenan, zymosan and CFA. In experiments of this type

Table 1
Intrathecal delivery of NSAIDs in behavioural studies in animals

Experimental pain model	Drug	Effect on nociceptive behaviour	Reference
Flinching after formalin injection (formalin test, phase 2)	Indomethacin	Inhibition	(Yamamoto & Nozaki-Taguchi, 1996)
	Ketorolac	Inhibition	(Malmberg & Yaksh, 1993)
	Indomethacin; flurbiprofen; ketorolac; zomepirac; *S*(+)-ibuprofen; ibuprofen (racemic)	Inhibition	(Malmberg & Yaksh, 1992)
	R-ibuprofen	No effect	
	S-ibuprofen	Inhibition	(Dirig et al., 1997)
Carrageenan-induced thermal hyperalgesia	Indomethacin	Inhibition	(Yamamoto & Nozaki-Taguchi, 1996)
	S(+)-ibuprofen	Inhibition	(Dirig et al., 1998)
Zymosan-induced thermal hyperalgesia	Indomethacin	Inhibition	(Guhring et al., 2000)
Injection of Freund's complete adjuvant (CFA)	*S*(+)-ibuprofen	Inhibition	(Seybold et al., 2003)
	R(−)-ibuprofen	No effect	

it could be shown that the inflammation produced by the subcutaneous injection of carrageenan into the paw is associated with a significant decrease in the nociceptive threshold to heat (Hargreaves et al., 1988) and this decrease correlated with an increased expression of COX-2 in the spinal cord and other regions of the CNS (Ichitani et al., 1997). Intrathecal application of indomethacin (Yamamoto & Nozaki-Taguchi, 1996) and *S*(+)-ibuprofen (Dirig et al., 1998) could block the carrageenan-induced thermal hyperalgesia. Similar effects of intrathecally applied indomethacin have been observed in the zymosan model (Guhring et al., 2000) in which the drug attenuated the mechanical and thermal hyperalgesia induced by the intraplanar injection of zymosan (Meller & Gebhart, 1997). The intrathecal application of NSAIDs was also effective in the long-term inflammatory pain induced by intraplantar injections of CFA (Stein et al., 1988). In this model, (*S*)-ibuprofen, but not (*R*)-ibuprofen, attenuated the flexor reflex dose-dependently and did not exert this effect in untreated rats (Seybold et al., 2003).

The above observations show convincingly that intrathecally administered NSAIDs are effective in reducing behavioural hyperalgesia in animal models of inflammatory pain and these findings are additional support for the hypothesis that the CNS is an additional site of action for the antinociceptive effects of NSAIDs.

4.2. Central and peripheral antinociceptive mechanisms of non-steriodal anti-inflammatory drugs (NSAIDs) in human studies

Several approaches have been used to assess central and peripheral mechanisms of action of NSAIDs in humans. Human experimental pain models, in particular, have proved to be important tools in the assessment of the pharmacological effects of NSAIDs. There are only few human models available, which are sensitive to NSAIDs and nearly all of them relate to nociception in skin. Cutaneous injury leads to alterations in thermal and mechanical hypersensitivity of the damaged tissue ("primary hyperalgesia") and to mechanical hypersensitivity in the skin surrounding the injury ("secondary hyperalgesia") (Meyer et al., 1994). It is believed that primary hyperalgesia is mostly mediated by peripheral mechanisms and that secondary hyperalgesia is produced by central sensitization (Torebjork et al., 1992; Vanegas, 2004). Thus, experimental evaluation of these two forms of hyperalgesia following the administration of NSAIDs may provide a further insight into the sites of action of aspirin-like drugs. In practice, this approach is hardly possible. The main limiting factor in such studies is that not all experimental models permit a clear differentiation between primary and secondary hyperalgesia and the observed pain hypersensitivity may have both central and peripheral origins. For example, hyperalgesia in pinching models, where pain is induced by the repeated stimulation of the interdigital web between the middle and ring fingers, is supposed initially to be due to peripheral sensitization, however, with continuation of the pinching stimulus, central components are also involved (Growcott et al., 2000). In the "freeze lesion" model, where hyperalgesia is produced by a short-lasting freezing of skin, the observed hypersensitivity is dominated by peripheral mechanisms but it is suspected that there is an additional contribution due to spinal sensitization (Kilo et al., 1995). In fact, of all the human experimental pain models where sensitivity to NSAIDs has been established (only double-blind, randomized, placebo-controlled studies are included; Table 2) the assessment of drug effects on primary and secondary hyperalgesia has been only performed in the cutaneous burn injury model (Petersen et al., 1997). In this model, ibuprofen reduces pain induced by motor brush stimulation within the area of secondary hyperalgesia but has no effect on the responses to pinpricks and stroking in this area making it difficult to confirm the involvement of central mechanisms using this technique.

Recently, we have described a pharmacokinetic–pharmacodynamic approach for assessing the relative contribution of central and peripheral mechanisms in the antihyperalgesic

Table 2
Human studies showing antihyperalgesic effect of NSAIDs in experimental pain models*

Model	Stimulus type	Number of subjects	Medication	Reference
Pinch model	Mechanical	12	Aspirin 1000 mg; aspirin 1500 mg	(Forster et al., 1988)
Pinch model	Mechanical	22	Ibuprofen 800 mg; dipyrone 1000 mg; paracetamol 1000 mg	(Forster et al., 1992)
Pinch model	Mechanical	24	Ibuprofen 1200 mg; ibuprofen 2400 mg	(Kilo et al., 1995)
Freeze lesion	Mechanical; thermal			
Laser induced pain	Argon laser	10	Ibuprofen 400 mg; ibuprofen 800 mg	(Nielsen et al., 1990)
Freeze lesion	Mechanical	12	Diclofenac 100 mg	(Burian et al., 2003)
UVB model	Mechanical; thermal	21	Ibuprofen 600 mg	(Bickel et al., 1998)
Pinch model	Mechanical			
UVB model	Thermal	32	Ibuprofen 800 mg	(Sycha et al., 2003)
Pinch model	Mechanical	20	Ibuprofen 600 mg	(Petersen et al., 1997)
Cutaneous burn injury				

* Only double-blind, randomized, placebo-controlled studies are included.

effect of diclofenac in the experimental model of "freeze lesion". We carried out algesimetric measurements of the induced hyperalgesia, whilst monitoring the peripheral drug concentrations at the site of injury using intradermal microdialysis following the application of topical diclofenac (65 mg) versus oral diclofenac (93 mg) (Burian et al., 2003). The doses of the two formulations were selected so as to achieve similar diclofenac concentrations at the site of injury. We could show that oral diclofenac was markedly superior to diclofenac applied topically despite the fact that the tissue concentrations at the site of injury were almost equal. Since the systemic concentrations produced by topically applied diclofenac were negligible, we concluded that a non-peripheral, presumably central, component was also involved in the antinociceptive effect of orally administered diclofenac. The relative contribution of the central component accounted for approximately 40% of the total analgesic efficacy of oral diclofenac.

A further approach for confirming that NSAIDs possess central antihyperalgesic activity is the assessment of their action on nociceptive reflexes. This is conducting parallel measurements of the subjective pain threshold and the objective nociceptive flexion reflex threshold of the biceps femoris in response to the transcutaneous electrical stimulation of the ipsilateral sural nerve and skin in the distal receptive field of the nerve (Willer & Bathien, 1977). A significant increase of the reflex threshold after intake of a drug versus placebo suggests involvement of the CNS. This method has been used to verify the presence of a central component in the action of indomethacin (Guieu et al., 1992), ketoprofen (Willer & Harrewyn, 1987) and ibuprofen (Sandrini et al., 1992). The central effects of ketoprofen, in particular, have been evaluated in healthy subjects and paraplegic patients with complete spinal section due to trauma (Willer et al., 1989). In contrast to normal subjects, where ketoprofen injection results in a rapid and significant increase in the threshold for the nociceptive reflex, there was no significant change in the nociceptive threshold in paraplegic patients, indicating that the observed central effects of ketoprofen depend, at least in part, on the involvement of supraspinal structures.

Nociceptive reflexes can be also induced in orbicularis oculi by electrical stimulation of the cornea and supraorbital nerve. In this neurophysiological model, piroxicam significantly suppresses the corneal and blink reflexes and this action is not reversed by naloxone (Fabbri et al., 1992). Other observations on such centrally-mediated, non-opioid, actions of NSAIDs have been reported in the case of piroxicam and lysine acetylsalicylate (Ferracuti et al., 1994).

The central and peripheral analgesic effects of NSAIDs have also been examined in clinical models of pain. The assessment of the relative contribution of central and peripheral mechanisms with such models is difficult because diagnostic tools for identifying specific mechanisms of pain in patients are not available (Woolf & Max, 2001). For example, post-operative and joint pain may involve both peripheral and central mechanisms (Schaible, 2004; Schaible et al., 2002) which means that the individual contribution of the two components after systemic administration of an NSAID cannot be determined. On the other hand, measurement of the peripheral action of an NSAID in these models is possible after local application. Romsing et al. (2000) have conducted a systematic review of 16 randomized, controlled, double-blind studies in a total of 844 patients. The efficacy of locally applied NSAIDs (intra-articular injections, components of intravenous regional anaesthesia (IVRA) and intra-wound at the surgical site) was compared to that after systemic administration and placebo in post-operative patients (Romsing et al., 2000). The review provided evidence for a clinically relevant peripheral antinociceptive action in regard to the intra-articular NSAIDs, whereas the data for IVRA and wound infiltration of NSAIDs in post-operative pain were inconclusive and additional data from further large-scale placebo-controlled trails are needed.

A recently published meta-analysis has shown that topically applied NSAIDs in patients with osteoarthritis have a clinically relevant peripheral antinociceptive action (Lin et al., 2004). Topically applied NSAIDs have been superior to placebo in the first two weeks of treatment, when peripheral mechanisms producing pain may be of special importance in such patients.

The effects of aspirin-like drugs have been extensively studied in pain following dental extraction (wisdom tooth removal). Dental pain after extraction is a sensitive and reproducible model for studies on analgesic drugs suitable for use in relatively minor surgery (Schou et al., 1998). Gordon et al. (2002) determined analgesia in relation to the level of prostanoids at the site of injury following the systemic and local administration of NSAIDs in order to determine the relationship between prostanoid levels and analgesia (Gordon et al., 2002). The intramuscular administration of 30 mg ketorolac tromethamine produced parallel decreases in pain and the PGE_2 and TxB_2 levels at the surgical site. However, injection of smaller doses of the drug (a 1-mg intramuscular dose, or a 1-mg submucosal dose at the extraction site) produced analgesia without a detectable effect on peripheral PGE_2 levels, suggesting that non-peripheral, presumably central, mechanisms are responsible for the observed analgesic effect. The authors concluded that central mechanisms involved in NSAIDs-mediated analgesia are much more sensitive to the effects of NSAIDs than peripheral mechanisms.

The ability of NSAIDs to alleviate pain by an action on CNS has been confirmed in patients with intractable pain due to various types of cancer. In these patients, the intrathecal administration of small doses of lysine–acetylsalicylate (equivalent to acetylsalicylic acid 500 μg/kg) brought rapid and prolonged pain relief. These results were confirmed in a more extended study with 60 cancer patients (Pellerin et al., 1987).

4.3. Influence of pharmacokinetic factors on central and peripheral mechanisms of action of non-steriodal anti-inflammatory drugs (NSAIDs)

There is no close correlation between the plasma concentrations of NSAIDs and their clinical efficacy. The major pharmacokinetic determinant of the antinociceptive action is the extent of their distribution to central and peripheral sites (Day et al., 1988).

4.3.1. Peripheral site

The joints of the body are major peripheral sites of action of NSAIDs in rheumatic patients and the degree of uptake into synovial fluid is of considerable clinical interest. The trans-synovial transport is a relative slow diffusion process governed partly by the pharmacological characteristics of the individual NSAID and partly by the properties of the joint and joint space (Day et al., 1999). The simultaneous assessment of concentrations of NSAIDs in plasma and the synovial fluid compartment has been studied after single and chronic administrations of NSAIDs. The results reveal that NSAIDs can be divided into two groups according to their pharmacokinetic behaviour (Netter et al., 1989). The first type comprises those NSAIDs with short or intermediate plasma elimination half-life (e.g. salicylates, ibuprofen, indomethacin, diclofenac). Their concentration in synovial fluid may exceed those in plasma. The second group includes NSAIDs with a long plasma elimination half-life (e.g. phenylbutazone, piroxicam, meloxicam) (Bannwart et al., 2001). The concentrations of these drugs in synovial fluid are lower than in plasma; their *free* concentrations, however, do not appear to differ significantly from each other (Lapicque et al., 2000).

In spite of the extensive data on the concentrations of the various NSAIDs at peripheral sites, the extent to which these correlate with the peripheral antinociceptive response has not been assessed in detail. Such studies are difficult from a pharmacokinetic point of view because the data obtained cannot be interpreted adequately without information on the protein binding in peripheral tissues and this is especially true for drugs which are bound there to a lesser extent than in plasma. The disposition of the drug and the formation of active metabolites and enantiomers (not only the parent drug) must also be taken into account.

4.3.2. Central site

In order to exhibit a central antinociceptive action, NSAIDs must enter the CNS from the bloodstream. Optimum delivery of drug molecules into the CNS is hindered by the blood–brain barrier (BBB) which tightly controls the exchange of substances between the blood and the CNS. The penetration of NSAIDs into spinal and supraspinal structures, a process believed to occur mainly by passive diffusion, varies from drug to drug (Davison et al., 1961; Bannwarth et al., 1989) and is thought to be limited by the binding capacity of the drug to plasma proteins (Ochs et al., 1985). It is noteworthy, however, that the fraction of drug entering the brain through the BBB is not only the unbound fraction but may also include a part of the bound fraction dissociated from the protein in the perfusate (Tanaka & Mizojiri, 1999). This phenomenon has been demonstrated for isoxicam, tenoxicam and meloxicam by using the carotid injection technique in rats where both the bound and unbound fractions of the drugs were available for transfer into the brain. (Jolliet et al., 1997). Results of binding studies with a special polymeric formulation of ketoprofen have shown that the binding of ketoprofen to plasma proteins is not the major factor governing the exchange between blood and the CSF (Matoga et al., 2002).

An important factor affecting the disposition of NSAIDs is the degree of ionisation and therefore pH. NSAIDs with low ionisation will show higher lipid solubility and better penetration into the CNS (Matoga et al., 1999). However, the degree of lipophilicity alone does not correlate well with the ability to diffuse into CNS and other physicochemical properties of the drug such as molecular size are important. This has been particularly demonstrated for arylpropionic acid NSAIDs using quantitative structure–activity relationship (QSAR) analysis (Pehourcq et al., 2004).

In contrast to the pharmacokinetics of NSAIDs in peripheral sites (i.e. synovial fluid), the concentrations of NSAIDs in the human CNS have not been extensively studied and there are two main reasons for this: a) the CSF compartment cannot easily be sampled and b) NSAIDs are extensively protein-bound, so that accurate estimation of their concentration in CSF requires specific and very sensitive analytical methods (Bannwarth et al., 1989). Therefore, only few data on concentration of NSAIDs in CNS in humans are available. Bannwarth et al. (1990) have shown that indomethacin given intramuscularly rapidly crosses the blood–brain barrier and can be detected in CSF 0.5 h after administration (Bannwarth et al., 1990) where the level may exceed that in plasma. Similar findings have been reported following the intramuscular administration of ketoprofen (Netter et al., 1985) and pirprofen (Zecca et al., 1988) with levels in the CSF detectable as early as 15 min after injection. In the case of orally administered ibuprofen, CSF concentrations rise rapidly and can exceed the unbound plasma levels of the drug within 90 min (Bannwarth et al., 1995). All these NSAIDs are characterised by a rapid distribution within CNS due to their high lipid solubility (Bannwarth et al., 1989). The analgesia produced by these drugs may show a more marked central component than other NSAIDs. Attempts to correlate their CSF concentrations with central analgesic activity, however, have not yet been made.

5. Conclusion

NSAIDs are potent antinociceptive agents, whose efficacy in reducing pain is widely recognized in various

pain conditions, including post-surgical pain and chronic pain associated with arthritis and cancer. Although NSAIDs have long been used in clinical practice, opinion on the mechanism of their antihyperalgesic action remains controversial. According to published findings, it appears that the inhibition of prostaglandin synthesis by NSAIDs takes place at the site of peripheral inflammation and in the CNS indicating that both peripheral and central mechanisms are involved in the antinociceptive action of these drugs. The relative contribution of peripheral and central COX-dependent mechanisms to the overall antinociceptive action depends on the particular NSAID used, the site of drug delivery and the pharmacokinetic characteristics of the drug especially those determining its penetration to the sites of action (i.e. access to peripheral and spinal COX).

Acknowledgment

The work of the authors is supported by the Deutsche Forschungsgemeinschaft (DFG 695/2-1) and the BMBF 01EM0103.

The authors are grateful to Dr. B.G. Woodcock for the valuable editorial assistance with the manuscript.

References

Abdel-Halim, M. S., Sjoquist, B., & Anggard, E. (1978). Inhibition of prostaglandin synthesis in rat brain. *Acta Pharm Toxicol (Copenh) 43*, 266–272.

Ahmadi, S., Lippross, S., Neuhuber, W. L., & Zeilhofer, H. U. (2002). PGE_2 selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. *Nature*, 34–40.

Akopian, A. N., Silvilotti, L., & Wood, J. N. (1996). A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature 379*, 257–262.

Appleton, I., Tomlinson, A., Mitchell, J. A., & Willoughby, D. A. (1995). Distribution of cyclooxygenase isoforms in murine chronic granulomatous inflammation: implications for future anti-inflammatory therapy. *J Pathol 176*, 413–420.

Arora, S., Lahiri, P. K., & Sanyal, R. K. (1970). The role of prostaglandin E1 in inflammatory process in the rat. *Int Arch Allergy Appl Immunol 39*, 186–191.

Baba, H., Kohno, T., Moore, K. A., & Woolf, C. J. (2001). Direct activation of rat spinal dorsal horn neurons by prostaglandin E2. *J Neurosci 21*, 1750–1756.

Baker, M. D., & Wood, J. N. (2001). Involvement of Na^+-channels in pain pathways. *Trends Pharmacol Sci 2001*, 27–31.

Ballou, L. R., Botting, R. M., Goorha, S., Zhang, J., & Vane, J. R. (2000). Nociception in cyclooxygenase isozyme-deficient mice. *Proc Natl Acad Sci U S A 97*, 10272–10276.

Bannwarth, B., Netter, P., Pourel, J., Royer, R. J., & Gaucher, A. (1989). Clinical pharmacokinetics of nonsteroidal anti-inflammatory drugs in the cerebrospinal fluid. *Biomed Pharmacother 43*, 121–126.

Bannwarth, B., Netter, P., Lapicque, F., Pere, P., Thomas, P., & Gaucher, A. (1990). Plasma and cerebrospinal fluid concentrations of indomethacin in humans: relationship to analgesic activity. *Eur J Clin Pharmacol 38*, 343–346.

Bannwarth, B., Lapicque, F., & Pehourcq, F. (1995). Stereoselective disposition of ibuprofen enantiomers in human cerebrospinal fluid. *Br J Clin Pharmacol 40*, 266–269.

Bannwart, B., Bertin, P., Pehourcq, F., Schaeverbeke, T., Gillet, P., Lefrancois, G., et al. (2001). Piroxicam concentrations in plasma and synovial fluid after a single dose of piroxicam-beta-cyclodextrin. *Int J Clin Pharmacol Ther 39*, 33–36.

Beiche, F., Brune, K., Geisslinger, G., & Goppelt-Struebe, M. (1998a). Expression of cyclooxygenase isoforms in the rat spinal cord and their regulation during adjuvant-induced arthritis. *Inflamm Res 47*, 482–487.

Beiche, F., Klein, T., Nusing, R., Neuhuber, W., & Goppelt-Struebe, M. (1998b). Localization of cyclooxygenase-2 and prostaglandin E2 receptor EP3 in the rat lumbar spinal cord. *J Neuroimmunol 89*, 26–34.

Beiche, F., Scheuerer, S., Brune, K., Geisslinger, G., & Goppelt-Struebe, M. (1996). Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. *FEBS Lett 390*, 165–169.

Bickel, A., Dorfs, S., Schmelz, M., Forster, C., Uhl, W., & Handwerker, H. O. (1998). Effects of antihyperalgesic drugs on experimentally induced hyperalgesia in man. *Pain 76*, 317–325.

Bjorkman, R. (1995). Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol: experimental studies in the rat. *Acta Anaesthesiol Scand Suppl 103*, 1–44.

Brune, K. (1974). Biodistribution of salicylates: a clue to the understanding of some effects and side effects. *Agents Actions Suppl 2*, 163–177.

Brune, K., Beck, W. S., Geisslinger, G., Menzel-Soglowek, S., Peskar, B. M., & Peskar, B. A. (1991). Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition. *Experientia 47*, 257–261.

Burian, M., Tegeder, I., Seegel, M., & Geisslinger, G. (2003). Peripheral and central antihyperalgesic effects of diclofenac in a model of human inflammatory pain. *Clin Pharmacol Ther 74*, 113–120.

Carlsson, K. H., Monzel, W., & Jurna, I. (1988). Depression by morphine and the non-opioid analgesic agents, metamizol (dipyrone), lysine acetylsalicylate, and paracetamol, of activity in rat thalamus neurones evoked by electrical stimulation of nociceptive afferents. *Pain 32*, 313–326.

Caterina, M. J., Rosen, T. A., Tominaga, M., Brake, A. J., & Julius, D. (1999). A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature 398*, 436–441.

Chandrasekharan, N. V., Dai, H., Roos, K. L., Evanson, N. K., Tomsik, J., Elton, T. S., et al. (2002). COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A 99*, 13926–13931.

Crunkhorn, P., & Willis, A. L. (1971). Cutaneous reactions to intradermal prostaglandins. *Br J Pharmacol 41*, 49–56.

Cui, M., & Nicol, G. D. (1995). Cyclic AMP mediates the prostaglandin E2-induced potentiation of bradykinin excitation in rat sensory neurons. *Neuroscience 66*, 459–466.

Daher, J. B., & Tonussi, C. R. (2003). A spinal mechanism for the peripheral anti-inflammatory action of indomethacin. *Brain Res 962*, 207–212.

Davies, P., Bailey, P. J., Goldenberg, M. M., & Ford-Hutchinson, A. W. (1984). The role of arachidonic acid oxygenation products in pain and inflammation. *Annu Rev Immunol 2*, 335–357.

Davison, C., Guy, J. L., Levitt, M., & Smith, P. K. (1961). The distribution of certain non-narcotic analgetic agents in the CNS of several species. *J Pharmacol Exp Ther 134*, 176–183.

Day, R. O., Graham, G. G., & Williams, K. M. (1988). Pharmacokinetics of non-steroidal anti-inflammatory drugs. *Bailliere's Clin Rheumatol*, 363–393.

Day, R. O., McLachlan, A. J., Graham, G. G., & Williams, K. M. (1999). Pharmacokinetics of nonsteriodal anti-inflammatory drugs in synovial fluid. *Clin Pharmacokinet 36*, 191–210.

Dickenson, A., Stanfa, L., Chapman, V., & Yaksh, T. (1997). Response properties of dorsal horn neurons: pharmacology of the dorsal horn. In T. Yaksh, C. Lynch, W. Zapol, M. Maze, J. Biebuyck, & L.J. Saidman

(Eds.), *Anesthesia: Biologic Foundations* (pp. 611–624). Philadelphia, PA: Lippincott-Raven.

Dinchuk, J. E., Liu, R. Q., & Trzaskos, J. M. (2003). COX-3: in the wrong frame in mind. *Immunol Lett 86*, 121.

Dirig, D. M., Konin, G. P., Isakson, P. C., & Yaksh, T. L. (1997). Effect of spinal cyclooxygenase inhibitors in rat using the formalin test and in vitro prostaglandin E2 release. *Eur J Pharmacol 331*, 155–160.

Dirig, D. M., Isakson, P. C., & Yaksh, T. L. (1998). Effect of COX-1 and COX-2 inhibition on induction and maintenance of carrageenan-evoked thermal hyperalgesia in rats. *J Pharmacol Exp Ther 285*, 1031–1038.

Di Rosa, M., Giroud, J. P., & Willoughby, D. A. (1971). Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J Pathol 104*, 15–29.

Ebersberger, A., Grubb, B. D., Willingale, H. L., Gardiner, N. J., Nebe, J., & Schaible, H. G. (1999). The intraspinal release of prostaglandin E2 in a model of acute arthritis is accompanied by an up-regulation of cyclo-oxygenase-2 in the spinal cord. *Neuroscience 93*, 775–781.

England, S., Bevan, S., & Docherty, R. J. (1996). PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade. *J Physiol 495*(Pt2), 429–440.

Evans, A. R., Vasko, M. R., & Nicol, G. D. (1999). The cAMP transduction cascade mediates the PGE2-induced inhibition of potassium current in rat sensory neurons. *J Physiol 516*, 165–178.

Fabbri, A., Cruccu, G., Sperti, P., Ridolfi, M., Ciampani, T., Leardi, M. G., et al. (1992). Piroxicam-induced analgesia: evidence for a central component which is not opioid mediated. *Experientia 48*, 1139–1142.

Ferracuti, S., Leardi, M. G., Cruccu, G., Fabbri, A., & Itil, T. M. (1994). Analgesic–antiinflammatory drugs inhibit orbicularis oculi reflexes in humans via a central model of action. *Prog Neuro-Psychopharmacol Biol Psychiatry 18*, 101–113.

Ferreira, S. (1972). Prostaglandins, aspirin-like drugs and analgesia. *Nat New Biol 240*, 200–203.

Ferreira, S. H., & Lorenzetti, B. B. (1981). Prostaglandin hyperalgesia: IV. A metabolic process. *Prostaglandins 21*, 789–792.

Ferreira, S. H., Lorenzetti, B. B., & Correa, F. M. (1978). Central and peripheral antialgesic action of aspirin-like drugs. *Eur J Pharmacol 53*, 39–48.

Forster, C., Anton, F., Reeh, P. W., Weber, E., & Handwerker, H. O. (1988). Measurement of the analgesic effects of aspirin with a new experimental algesimetric procedure. *Pain 32*, 215–222.

Forster, C., Magerl, W., Beck, A., Geisslinger, G., Gall, T., Brune, K., et al. (1992). Differential effects of dipyrone, ibuprofen, and paracetamol on experimentally induced pain in man. *Agents Actions 35*, 112–121.

Garavito, R. M., & deWitt, D. L. (1999). The cyclooxygenase isoforms: structural insights into the conversion of arachidonic acid to prostaglandins. *Biochim Biophys Acta 1441*, 278–287.

Garavito, R. M., Malkowski, M. G., & deWitt, D. (2002). The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins Other Lipid Mediat 68–69*, 129–152.

Geisslinger, G., Ferreira, S. H., Menzel, S., Schlott, D., & Brune, K. (1994). Antinociceptive actions of *R*(−)-flurbiprofen—a non-cyclooxygenase inhibiting 2-arylpropionic acid—in rats. *Life Sci 54*, PL173–PL177.

Gilroy, D.W., Colville-Nash, P.R., Willis, D., Chivers, J., Paul-Clark, M.J., & Willoughby, D.A. (1999). Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med 5*, 698–701.

Gold, M. S., Reichling, D. B., Shuster, M. J., & Levine, J. D. (1996a). Hyperalgesic agents increase a tetrodotoxin-resistant Na+ current in nociceptors. *Proc Natl Acad Sci U S A 93*, 1108–1112.

Gold, M. S., Shuster, M. J., & Levine, J. D. (1996b). Role of a Ca^{2+}-dependent slow after hyperpolarization in prostaglandin E2-induced sensitization of cultured rat sensory neurons. *Neurosci Lett 205*, 161–164.

Gold, M. S., Levine, J. D., & Corea, A. M. (1998). Modulation of TTX-r INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. *J Neurosci 18*, 10345–10355.

Goppelt-Struebe, M., & Beiche, F. (1997). Cyclooxygenase-2 in the spinal cord: localization and regulation after a peripheral inflammatory stimulus. *Adv Exp Med Biol 433*, 213–216.

Gordon, S. M., Brahim, J. S., Rowan, J., Kent, A., & Dionne, R. A. (2002). Peipheral prostanoid levels and nonsteriodal anti-inflammatory drug analgesia: replicate clinical trials in a tissue injury model. *Clin Pharmacol Ther 72*, 175–183.

Growcott, J., Stone, A., Beise, R., Stammer, H., Tetzloff, W., & DeMey, C. (2000). Sensitivity of repeated interdigital web pinching to detect antinociceptive effects of ibuprofen. *Br J Clin Pharmacol 49*, 331–336.

Guhring, H., Gorig, M., Ates, M., Coste, O., Zeilhofer, H. U., Pahl, A., et al. (2000). Suppressed injury-induced rise in spinal prostaglandin E2 production and reduced early thermal hyperalgesia in iNOS-deficient mice. *J Neurosci 20*, 6714–6720.

Guieu, R., Blin, O., Pouget, J., & Serratrice, G. (1992). Analgesic effect of indomethacin shown using the nociceptive flexion reflex in humans. *Ann Rheum Dis 51*, 391–393.

Hamilton, J. A., Vadas, P., & Hay, J. B. (1981). Measurement of blood flow and vascular permeability changes in response to 12-*O*-tetradecanoyl-phorbol-13-acetate and to asbestos fibers. *J Toxicol Environ Health 8*, 205–214.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., & Joris, J. (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain 32*, 77–88.

Harvey, R., Depner, U., Wassle, H., Ahmadi, S., Heindl, C., Reinold, H., et al. (2004). GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science 304*, 884–887.

Hawkey, C. J. (1999). COX-2 inhibitors. *Lancet 353*, 307–314.

Hay, C., & de Belleroche, J. (1997). Carrageenan-induced hyperalgesia is associated with increased cyclo-oxygenase-2 expression in spinal cord. *NeuroReport 8*, 1249–1251.

Hay, C. H., & de Belleroche, J. S. (1998). Dexamethasone prevents the induction of COX-2 mRNA and prostaglandins in the lumbar spinal cord following intraplantar FCA in parallel with inhibition of oedema. *Neuropharmacology 37*, 739–744.

Hemler, M., & Lands, W. E. (1976). Purification of the cyclooxygenase that forms prostaglandins: demonstration of two forms of iron in the holoenzyme. *J Biol Chem 251*, 5575–5579.

Hingtgen, C. M., & Vasko, M. R. (1994). Prostacyclin enhances the evoked-release of substance P and calcitonin gene-related peptide from rat sensory neurons. *Brain Res 655*, 51–60.

Hla, T., & Neilson, K. (1992). Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A 89*, 7384–7388.

Horton, E. W. (1963). Action of prostaglandin E1 on tissues which respond to bradykinin. *Nature 200*, 892–893.

Hua, X. Y., Chen, P., & Yaksh, T. L. (1999). Inhibition of spinal protein kinase C reduces nerve injury-induced tactile allodynia in neuropathic rats. *Neurosci Lett 276*, 99–102.

Hunskaar, S., & Hole, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain 30*, 103–114.

Ichitani, Y., Shi, T., Haeggstrom, J., Sammuelssn, B., & Hökfelt, T. (1997). Increased levels of cycloxygenase-2 mRNA in the rat spinal cord after peripheral inflammation: an in situ hybridization study. *NeuroReport 8*, 2949–2952.

Inoue, A., Ikoma, K., Morioka, N., Kumagai, K., Hashimoto, T., Hide, I., et al. (1999). Interleukin-1beta induces substance P release from primary afferent neurons through the cyclooxygenase-2 system. *J Neurochem 73*, 2206–2213.

Jolliet, P., Simon, N., Bree, F., Urien, S., Pagliara, A., Carrupt, P. A., et al. (1997). Blood-to-brain transfer of various oxicams: effects of plasma binding on their brain delivery. *Pharm Res 14*, 650–656.

Juhlin, L., & Michaelsson, G. (1969). Cutaneous vascular reactions to prostaglandins in healthy subjects and in patients with urticaria and atopic dermatitis. *Acta Derm Venereol 49*, 251–261.

Jurna, I. (1997). Opioid and nonopioid analgesic agents: some aspects of central (spinal) and peripheral actions. *Pain Rev 4*, 205–229.

Jurna, I., Spohrer, B., & Bock, R. (1992). Intrathecal injection of acetylsalicylic acid, salicylic acid and indomethacin depresses C fibre-evoked activity in the rat thalamus and spinal cord. *Pain 49*, 249–256.

Karim, S. (1971). Action of prostaglandin in the pregnant woman. *Ann N Y Acad Sci 180*, 483–498.

Kaufmann, W. E., Andreasson, K. I., Isakson, P. C., & Worley, P. F. (1997). Cyclooxygenases and the central nervous system. *Prostaglandins 54*, 601–624.

Kawamura, T., Yamauchi, T., Koyama, M., Maruyama, T., Akira, T., & Nakamura, N. (1997). Expression of prostaglandin EP2 receptor mRNA in the rat spinal cord. *Life Sci 61*, 2111–2116.

Kilo, S., Forster, C., Geisslinger, G., Brune, K., & Handwerker, H. O. (1995). Inflammatory models of cutaneous hyperalgesia are sensitive to effects of ibuprofen in man. *Pain 62*, 187–193.

Kirtikara, K., Swangkul, S., & Ballou, L. R. (2001). The analysis of nonsteriodal antiinflammatory drug selectivity in prostaglandin G/H synthase (PGHS)-null cells. *Inflamm Res 50*, 327–332.

Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., & Herschman, H. R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem 15*, 12866–12872.

Kumazawa, T., Mizumura, K., Koda, H., & Fukusako, H. (1996). EP receptor subtypes implicated in the PGE2-induced sensitization of polymodal receptors in response to bradykinin and heat. *J Neurophysiol 75*, 2361–2368.

Langenbach, R., Morham, S. G., Tiano, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., et al. (1995). Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell 83*, 483–492.

Langenbach, R., Loftin, C. D., Lee, C., & Tiano, H. (1999). Cyclooxygenase-deficient mice: a summary of their characteristics and susceptibilities to inflammation and carcinogenesis. *Ann N Y Acad Sci 889*, 52–61.

Lapicque, F., Vergne, P., Jouzeau, J. Y., Loeuille, D., Gillet, P., Vignon, E., et al. (2000). Articular diffusion of meloxicam after a single oral dose: relationship to cyclo-oxygenase inhibition in synovial cells. *Clin Pharmacokinet 39*, 369–382.

Lee, H. M., Kim, H. I., Shin, Y. K., Lee, C. S., Park, M., & Song, J. H. (2003). Diclofenac inhibition of sodium currents in rat dorsal root ganglion neurons. *Brain Res 992*, 120–127.

Lin, J., Zhang, W., Jones, A., & Doherty, M. (2004). Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials. *BMJ 329*, 324.

Lopshire, J. C., & Nicol, G. D. (1998). The cAMP transduction cascade mediates the prostaglandin E2 enhancement of the capsaicin-elicited current in rat sensory neurons: whole-cell and single-channel studies. *J Neurosci 18*, 6081–6092.

Maihofner, C., Tegeder, I., Euchenhofer, C., deWitt, D., Brune, K., Bang, R., et al. (2000). Localization and regulation of cyclo-oxygenase-1 and -2 and neuronal nitric oxide synthase in mouse spinal cord. *Neuroscience 101*, 1093–1108.

Malmberg, A. B., & Yaksh, T. L. (1992). Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. *J Pharmacol Exp Ther 263*, 136–146.

Malmberg, A. B., & Yaksh, T. L. (1993). Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. *Anesthesiology 79*, 270–281.

Malmberg, A. B., & Yaksh, T. L. (1994). Antinociception produced by spinal delivery of the *S* and *R* enantiomers of flurbiprofen in the formalin test. *Eur J Pharmacol 256*, 205–209.

Malmberg, A. B., & Yaksh, T. L. (1995). Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. *J Neurosci 15*, 2768–2776.

Matoga, M., Pehourcq, F., Lagrange, F., Tramu, G., & Bannwarth, B. (1999). Influence of molecular lipophilicity on the diffusion of arylpropionate non-steroidal anti-inflammatory drugs into the cerebrospinal fluid. *Arzneim Forsch 49*, 477–482.

Matoga, M., Pehourcq, F., Lagrange, F., Fawaz, F., & Bannwarth, B. (2002). Influence of a polymeric formulation of ketoprofen on its diffusion into cerebrospinal fluid in rats. *J Pharm Biomed Anal 27*, 881–888.

McAdam, B. F., Mardini, I. A., Habib, A., Burke, A., Lawson, J. A., Kapoor, S., et al. (2000). Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. *J Clin Invest 105*, 1473–1482.

McCormack, K., & Brune, K. (1991). Dissociation between the antinociceptive and anti-inflammatory effects of the nonsteroidal anti-inflammatory drugs: a survey of their analgesic efficacy. *Drugs 41*, 533–547.

McCormack, K., & Urquhart, E. (1995). Correlation between nonsteroidal anti-inflammatory drug efficacy in a clinical pain model and the dissociation of their anti-inflammatory and analgesic properties in animal models. *Clin Drug Investig 9*, 88–97.

Meller, S. T., & Gebhart, G. F. (1997). Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. *Eur J Pain 1*, 43–52.

Meyer, R., Campbell, J., & Raja, S. (1994). Peripheral neural mechanisms of nociception. In P.D.W.R. Melzack (Ed.), *Textbook of pain* (pp. 13–44). Edinburgh: Lingvistone Churchill.

Minami, T., Uda, R., Horiguchi, S., Ito, S., Hyodo, M., & Hayaishi, O. (1992). Allodynia evoked by intrathecal administration of prostaglandin F2 alpha to conscious mice. *Pain 50*, 223–229.

Minami, T., Uda, R., Horiguchi, S., Ito, S., Hyodo, M., & Hayaishi, O. (1994). Allodynia evoked by intrathecal administration of prostaglandin E2 to conscious mice. *Pain 57*, 217–223.

Minami, T., Nakano, H., Kobayashi, T., Sugimoto, Y., Ushikubi, F., Ichikawa, A., et al. (2001). Characterization of EP receptor subtypes responsible for prostaglandin E2-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br J Pharmacol 133*, 438–444.

Mizumura, K., Sato, J., & Kumazawa, T. (1987). Effects of prostaglandins and other putative chemical intermediaries on the activity of canine testicular polymodal receptors studied in vitro. *Pflugers Arch 408*, 565–572.

Mnich, S. J., Veenhuizen, A. W., Monahan, J. B., Sheehan, K. C., Lynch, K. R., Isakson, P. C., et al. (1995). Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E2. *J Immunol 155*, 4437–4444.

Murata, T., Ushikubi, F., Matsuoka, T., Hirata, M., Yamasaki, A., Sugimoto, Y., et al. (1997). Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature 388*, 678–682.

Nakamura, F., & Strittmatter, S. M. (1996). P2Y1 purinergic receptors in sensory neurons: contribution to touch-induced impulse generation. *Proc Natl Acad Sci U S A 93*, 10465–10470.

Netter, P., Lapicque, F., Bannwarth, B., Tamisier, J. N., Thomas, P., & Royer, R. J. (1985). Diffusion of intramuscular ketoprofen into the cerebrospinal fluid. *Eur J Clin Pharmacol 29*, 319–321.

Netter, P., Bannwarth, B., & Royer-Morrot, M. J. (1989). Recent findings on the pharmacokinetics of non-steroidal anti-inflammatory drugs in synovial fluid. *Clin Pharmacokinet 17*, 145–162.

Nicol, G. D., Klingberg, D. K., & Vasko, M. R. (1992). Prostaglandin E2 increases calcium conductance and stimulates release of substance P in avian sensory neurons. *J Neurosci 12*, 1917–1927.

Nicol, G. D., Vasko, M. R., & Evans, A. R. (1997). Prostaglandins suppress an outward potassium current in embryonic rat sensory neurons. *J Neurophysiol 77*, 167–176.

Nielsen, J. C., Bjerring, P., Arendt-Nielsen, L., & Petterson, K. J. (1990). A double-blind, placebo controlled, cross-over comparison of the analgesic effect of ibuprofen 400 mg and 800 mg on laser-induced pain. *Br J Clin Pharmacol 30*, 711–715.

Nishihara, I., Minami, T., Watanabe, Y., Ito, S., & Hayaishi, O. (1995). Prostaglandin E2 stimulates glutamate release from synaptosomes of rat spinal cord. *Neurosci Lett 196*, 57–60.

Ochs, H. R., Greenblatt, D. J., Abernethy, D. R., Arendt, R. M., Gerloff, J., Eichelkraut, W., et al. (1985). Cerebrospinal fluid uptake and peripheral distribution of centrally acting drugs: relation to lipid solubility. *J Pharm Pharmacol 37*, 428–431.

Oida, H., Namba, T., Sugimoto, Y., Ushikubi, F., Ohishi, H., Ichikawa, A., et al. (1995). In situ hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. *Br J Pharmacol 116*, 2828–2837.

Pehourcq, F., Matoga, M., & Bannwarth, B. (2004). Diffusion of arylpropionate non-steroidal anti-inflammatory drugs into the cerebrospinal fluid: a quantitative structure–activity relationship approach. *Fundam Clin Pharmacol 18*, 65–70.

Pellerin, M., Hardy, F., Abergel, A., Boule, D., Palacci, J. H., Babinet, P., et al. (1987). Chronic refractory pain in cancer patients. Value of the spinal injection of lysine acetylsalicylate. 60 cases. *Presse Med 16*, 1465–1468.

Petersen, K. L., Brennum, J., & Dahl, J. B. (1997). Experimental evaluation of the analgesic effect of ibuprofen on primary and secondary hyperalgesia. *Pain 70*, 167–174.

Reddy, S. T., & Herschman, H. R. (1997). Prostaglandin synthase-1 and prostaglandin synthase-2 are coupled to distinct phospholipases for the generation of prostaglandin D2 in activated mast cells. *J Biol Chem 272*, 3231–3237.

Resnick, D. K., Graham, S. H., Dixon, C. E., & Marion, D. W. (1998). Role of cyclooxygenase 2 in acute spinal cord injury. *J Neurotrauma 15*, 1005–1013.

Romsing, J., Moiniche, S., Ostergaard, D., & Dahl, J. B. (2000). Local infiltration with NSAIDs for postoperative analgesia: evidence for a peripheral analgesic action. *Acta Anaesthesiol Scand 44*, 672–683.

Samad, T. A., Moore, K. A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., et al. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature 410*, 471–475.

Sandrini, G., Ruiz, L., Capararo, M., Garofoli, F., Beretta, A., & Nappi, G. (1992). Central analgesic activity of ibuprofen: a neurophysiological study in humans. *Int J Clin Pharmacol Res 12*, 197–204.

Saunders, M. A., Belvisi, M. G., Cirino, G., Barnes, P. J., Warner, T. D., & Mitchell, J. A. (1999). Mechanisms of prostaglandin E2 release by intact cells expressing cyclooxygenase-2: evidence for a 'two-component' model. *J Pharmacol Exp Ther 288*, 1101–1106.

Schaible, H. -G. (2004). Spinal mechanisms contributing to joint pain. *Novartis Found Symp 260*, 4–22.

Schaible, H. -G., Ebersberger, A., & Von Banchet, G. S. (2002). Mechanisms of pain in arthritis. *Ann N Y Acad Sci*, 343–354.

Scheuren, N., Neupert, W., Ionac, M., Neuhuber, W., Brune, K., & Geisslinger, G. (1997). Peripheral noxious stimulation releases spinal PGE2 during the first phase in the formalin assay of the rat. *Life Sci 60*, PL295–PL300.

Schou, S., Nielsen, H., Nattestad, A., Hillerup, S., Ritzau, M., Branebjerg, P. E., et al. (1998). Analgesic dose–response relationship of ibuprofen 50, 100, 200 and 400 mg after surgical removal of third molars: a single-dose, randomized, placebo-controlled, and double-blind study in 304 patients. *J Clin Pharmacol*, 447–454.

Schwab, J. M., Beiter, T., Linder, J. U., Laufer, S., Schulz, J. E., Meyermann, R., et al. (2003). COX-3—a virtual pain target in humans? *FASEB J 17*, 2174–2175.

Seybold, V. S., Jia, Y. P., & Abrahams, L. G. (2003). Cyclo-oxygenase-2 contributes to central sensitization in rats with peripheral inflammation. *Pain 105*, 47–55.

Smith, W. L., Meade, E. A., & deWitt, D. L. (1994). Interactions of PGH synthase isozymes-1 and -2 with NSAIDs. *Ann N Y Acad Sci 744*, 50–57.

Stein, C., Millan, M. J., & Herz, A. (1988). Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. *Pharmacol Biochem Behav 31*, 451–455.

Stock, J. L., Shinjo, K., Burkhardt, J., Roach, M., Taniguchi, K., Ishikawa, T., et al. (2001). The prostaglandin E2 EP1 receptor mediates pain perception and regulates blood pressure. *J Clin Invest 107*, 325–331.

Sycha, T., Gustorff, B., Lehr, S., Tanew, A., Eichler, H. G., & Schmetterer, L. (2003). A simple pain model for the evaluation of analgesic effects of NSAIDs in healthy subjects. *Br J Clin Pharmacol 56*, 165–172.

Tanaka, H., & Mizojiri, K. (1999). Drug-protein binding and blood–brain barrier permeability. *J Pharmacol Exp Ther 288*, 912–918.

Tegeder, I., Niederberger, E., Israr, E., Guhring, H., Brune, K., Euchenhofer, C., et al. (2001a). Inhibition of NF-kappaB and AP-1 activation by *R*- and *S*-flurbiprofen. *FASEB J 15*, 2–4.

Tegeder, I., Pfeilschifter, J., & Geisslinger, G. (2001b). Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J 15*, 2057–2072.

Tilley, S., Coffman, T., & Koller, B. (2001). Mixed messages: modulation of inflammtion and immune responses by prostaglandins and thromboxanes. *J Clin Invest 108*, 15–23.

Tjolsen, A., Berge, O. G., Hunskaar, S., Rosland, J. H., & Hole, K. (1992). The formalin test: an evaluation of the method. *Pain 51*, 5–17.

Tomlinson, A., Appleton, I., Moore, A. R., Gilroy, D. W., Willis, D., Mitchell, J. A., et al. (1994). Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenan-induced pleurisy. *Br J Pharmacol 113*, 693–698.

Tonai, T., Taketani, Y., Ueda, N., Nishisho, T., Ohmoto, Y., Sakata, Y., et al. (1999). Possible involvement of interleukin-1 in cyclooxygenase-2 induction after spinal cord injury in rats. *J Neurochem 72*, 302–309.

Torebjork, H. E., Lundberg, L. E., & LaMotte, R. H. (1992). Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *J Physiol 448*, 765–780.

Uda, R., Horiguchi, S., Ito, S., Hyodo, M., & Hayaishi, O. (1990). Nociceptive effects induced by intrathecal administration of prostaglandin D2, E2, or F2 alpha to conscious mice. *Brain Res 510*, 26–32.

Vane, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br J Pharmacol 23*, 360–373.

Vane, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol 231*, 232–235.

Vane, J. R., Bakhle, Y. S., & Botting, R. M. (1998). Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol 38*, 97–120.

Vanegas, H. (2002). Bases for a spinal analgesic action of cyclooxygenase inhibitors. *Proc West Pharmacol Soc 45*, 225–227.

Vanegas, H. (2004). To the descending pain-control system in rats, inflammation-induced primary and secondary hyperalgesia are two different things. *Neurosci Lett 361*, 225–228.

Vanegas, H., & Schaible, H. -G. (2001). Prostaglandins and cyclo-oxygenases in the spinal cord. *Prog Neurobiol*, 327–363.

Vasquez, E., Bar, K. J., Ebersberger, A., Klein, B., Vanegas, H., & Schaible, H. -G. (2001). Spinal prostaglandins are involved in the development but not maintenance of inflammation-induced spinal hyperexcitability. *J Neurosci 21*, 9001–9008.

Willer, J., & Bathien, N. (1977). Pharmacological modulations on the nociceptive flexion reflex in man. *Pain 3*, 111–119.

Willer, J., & Harrewyn, J. (1987). Central inhibitory effect of intravenous ketoprofen on the nociceptive flexion reflex in man. *Presse Med 16*, 63–67.

Willer, J., De Broucker, T., Bussel, B., Roby-Brami, A., & Harrewyn, J. (1989). Central analgesic effect of ketoprofen in humans: electrophysiological evidence for a supraspinal mechansim in a double-blind and cross-over study. *Pain 38*, 1–7.

Willingale, H. L., Gardiner, N. J., McLymont, N., Giblett, S., & Grubb, B. D. (1997). Prostanoids synthesized by cyclo-oxygenase isoforms in rat spinal cord and their contribution to the development of neuronal hyperexcitability. *Br J Pharmacol 122*, 1593–1604.

Willis, A. L. (1969). Parallel assay of prostaglandin-like activity in rat inflammatory exudate by means of cascade superfusion. *J Pharm Pharmacol 21*, 126–128.

Woolf, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature 306*, 686–688.

Woolf, C. J., & Max, M. B. (2001). Mechanism-based pain diagnosis: issues for analgesic drug development. *Anesthesiology 95*, 241–249.

Woolf, C. J., & Salter, M. W. (2000). Neuronal plasticity: increasing the gain in pain. *Science 288*, 1765–1769.

Xie, W., Roberston, D., & Simmons, D. (1992). Mitogen-inducible prostaglandin S/H synthase: a new target for nonsteriodal. *Drug Dev Res 25*, 249–265.

Yamamoto, T., & Nozaki-Taguchi, N. (1996). Analysis of the effects of cyclooxygenase (COX)-1 and COX-2 in spinal nociceptive transmission using indomethacin, a non-selective COX inhibitor, and NS-398, a COX-2 selective inhibitor. *Brain Res 739*, 104–110.

Yang, L. C., Marsala, M., & Yaksh, T. L. (1996). Effect of spinal kainic acid receptor activation on spinal amino acid and prostaglandin E2 release in rat. *Neuroscience 75*, 453–461.

Yashpal, K., & Coderre, T. J. (1998). Influence of formalin concentration on the antinociceptive effects of anti-inflammatory drugs in the formalin test in rats: separate mechanisms underlying the nociceptive effects of low- and high-concentration formalin. *Eur J Pain 2*, 63–68.

Yashpal, K., Mason, P., McKenna, J. E., Sharma, S. K., Henry, J. L., & Coderre, T. J. (1998). Comparison of the effects of treatment with intrathecal lidocaine given before and after formalin on both nociception and Fos expression in the spinal cord dorsal horn. *Anesthesiology 88*, 157–164.

Zecca, L., Broggini, M., Pirola, R., Campi, R., Ferrario, P., Bichisao, E., et al. (1988). The diffusion of pirprofen into the cerebrospinal fluid in man. *Eur J Clin Pharmacol 35*, 81–83.

Zhu, X., & Eisenach, J. C. (2003). Cyclooxygenase-1 in the spinal cord is altered after peripheral nerve injury. *Anesthesiology 99*, 1175–1179.

Zhu, X., Conklin, D., & Eisenach, J. C. (2003). Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. *Pain 104*, 15–23.