Pain resulting from burn injuries is one of the most excruciating pain sensations that can be experienced. It is estimated that 1 in 3000 people suffers burn injuries annually world-wide. According to WHO statistics, almost 11 million required medical attention due to the severity of their injuries in 2004 (WHO, 2008). Although, Europe has a lower incidence of burn injury than other areas in the world, annually 0.2–2.9/100,000 inhabitants suffer severe burn injury (Brusselaers et al., 2010). Improvements in resuscitation, wound care, critical illness support and infection control have improved the outcomes of burn injuries, and increased survival significantly (Brusselaers et al., 2005). Yet, pain in burn injury patients, both in the acute and chronic setting, is still a major clinical challenge and an unmet medical need (Carrougher et al., 2003; Dauber et al., 2002). Poorly treated pain in burn injury however, can lead to immense suffering, loss of engagement in treatment, development of chronic pain (Browne et al., 2011) and post traumatic stress disorder (Patterson et al., 2006). Further, the lack of appropriate pain control may significantly hamper successful functional recovery, which can lead to reduced integration into society (Esselman et al., 2001). Therefore, providing appropriate pain control following burn injury is of imperative importance.

The lack of appropriate pain control in burn-injured patients reflects our surprisingly limited understanding of the neuronal mechanisms involved in burn injury-associated pain. Understanding the signalling between the burned and subsequently inflamed tissues, and the sensory neurons that innervate those tissues, particularly at a cellular and molecular level therefore, must be
one of the priorities in pain research. In this review, we will give an account on already known and putative signalling events occurring between burned tissues and primary sensory neurons, in the hope that we initiate further studies to elucidate peripheral mechanisms of burn injury-associated pain which will ultimately lead us to better pain management in these patients.

2. Pathology of burn injury

The aetiology of burn injury is diverse and it includes contacts with hot liquids, (scalds), various chemicals (chemical burn), strong electrical currents (electrical burn), flames or hot objects (contact burn). All tissues, which have some contact with the external environment, are susceptible to direct burn damage, including the skin, gastrointestinal and respiratory tracts. However, the majority of burn injuries are due to heat impact (Brusselaers et al., 2010) and affect the skin. Therefore, in this review we will concentrate on mechanisms involved in the development and maintenance of pain associated with heat-induced injury of the skin.

Although burn injury has a diverse aetiology, there is some consistency in the overall pathological process of burns to the skin and its subsequent impact on pain. The skin is a bilayer organ with both protective and immunological functions (DeSanti, 2005; Kao and Garner, 2000). It comprises of the outer epidermal layer, predominantly of keratinocytes, and the inner dermal layer. The dermis further divides into the superficial papillary dermis, and the deeper, reticular dermis. The papillary dermis has greater capacity to regenerate than the reticular dermis. Therefore, the depth of the injury to the skin determines healing and management (DeSanti, 2005; Kinsella and Rae, 1997). The depth of injury has also been used to classify or predict pain following burn injury (Kinsella and Rae, 1997; see Table 1).

The progression of burn injury involves initial tissue damage, inflammation and then healing and remodelling. Primary tissue damage occurs predominantly via thermal denaturing of proteins and loss of the plasma membrane integrity leading to cell death and the leakage of a series of agents (Evers et al., 2010; Fig. 1). Following the initial loss of tissues, there is an enormous inflammatory response (Allgower et al., 1995; Fig. 1). Locally this involves a prolonged influx of inflammatory cells that release various agents which, on the one hand, coordinate the action of immuno-competent cells, while on the other hand, act on sensory neurons inducing either direct activation or sensitisation (Fig. 1). The action of

<table>
<thead>
<tr>
<th>Depth</th>
<th>Layer of skin involved</th>
<th>Pain</th>
<th>Healing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial</td>
<td>Epidermis</td>
<td>– Moderate to severe pain</td>
<td>3–7 days</td>
</tr>
<tr>
<td>Superficial partial</td>
<td>Superficial papillary dermis</td>
<td>– Severe pain</td>
<td>1–3 weeks, long term pigmentation changes may occur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Intact nerve endings in wound</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Pain exacerbated by contact with surfaces</td>
<td></td>
</tr>
<tr>
<td>Deep partial</td>
<td>Deeper reticular dermis</td>
<td>– Minimal pain</td>
<td>3–6 weeks with scars</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Nerve endings damaged but not completely destroyed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Endings have ability to transmit noxious stimuli</td>
<td></td>
</tr>
<tr>
<td>Deep</td>
<td>Full thickness of skin and into the subcutaneous fat or deeper</td>
<td>– Minimal/pain free state</td>
<td>Does not heal by primary intention and requires skin grafting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Complete destruction of nerves</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Schematic drawing of event leading to the initiation, development and maintenance of pain following burn injury. The dashed line and solid line boxes identify events leading to the formation of the early and late burn injury tissue fluid.
immuno-competent cells is accompanied by localised oedema, altered perfusion and systemic inflammatory response (Fig. 1). The healing phase involves re-epithelialisation of the wound by the body itself or by surgical aid. During this period there is nerve healing, re-growth and sprouting. Lastly the rehabilitation stage is characterised by wound closure, scar maturation and optimisation of functional outcome (Summer et al., 2007a).

General characteristics of burn injury-associated pain

While superficial burn injuries are associated with severe pain, the area of deep burn injuries, due to the loss sensory nerve endings, are significantly less painful (Table 1). However burns involve a combination of depths of injury and certainly with deep burns there will be more shallow burn areas around the deep burn area. Therefore, essentially all burn injured patients experience severe pain in smaller or larger areas of the injury.

Pain from burn injury encompasses a number of different modalities, including heat and mechanical hyperalgesia and allodynia (Pedersen and Kehlet, 1998). Furthermore, the hyperalgesia associated with burn injury is both primary, within the damaged tissue area, as well as secondary, in the adjacent uninjured tissue (Moiniche et al., 1993; Raja et al., 1984). Regarding its emergence, burn injury-associated pain is usually classified as background pain, procedural pain and breakthrough pain which all can occur in all the three phases of the burn injury. While this classification is appropriate for describing the emergence of pain, for the elucidation of the cellular and molecular mechanisms involved in the development of pain, it is more useful to look at pain mechanistically at the phase of injury, from the burn to subsequent recovery. This classification encompasses pain arising due to heat impact and subsequent cell death, pain due to the inflammatory response, and finally pain associated with tissue healing and remodeling (Summer et al., 2007a). In the present review we will focus on mechanisms involved in the development of pain during and immediately after the heat impact and during the inflammatory response.

Like the development of pain associated with other peripheral pathologies, the development of burn-injury associated pain also involves nociceptive signaling between the injured tissues and nerve fibres innervating those tissues, as well as nociceptive signaling in the central nervous system. It is believed however, that the great majority of the characteristics of burn injury-associated pain are due to signaling between the burn tissues and primary sensory nerve fibres. Below we will discuss signaling occurring between the injured tissues and primary sensory neurons.

3. Nociceptive signalling in burn tissues

3.1. Mechanism and molecules involved in the development of immediate and early pain

Heat which can cause tissue damage (above ~43 °C) evokes pain immediately. This pain is due to heat-evoked direct activation of a sub-class of primary sensory fibres in the skin, where the heat impact has occurred (Fig. 1). Heat-sensitive primary sensory fibres are nociceptors, which are processes of small diameter, polymodal nociceptive primary sensory neurons. These nociceptive cells are responsive to high intensity mechanical, thermal and chemical stimuli (Nagy, 2004). Previously, two ion channels, the transient receptor potential vanilloid type 1 ion channel (TRPV1; Caterina et al., 1997; Nagy and Rang, 1999a) and the transient receptor potential vanilloid type 2 ion channel (TRPV2; Ahluwalia et al., 2002; Caterina et al., 1999; Nagy and Rang, 1999b) were implicated in the detection of heat above 43 °C and 52 °C, respectively, in nociceptive primary sensory fibres/neurons. Recently, a third ion channel, the transient receptor potential melastatin 3 ion channel (TRPM3; Vriens et al., 2011) was also shown to respond to noxious heat in primary sensory neurons, though the heat threshold of TRPM3, unlike those of TRPV1 and TRPV2, is below the body temperature. Nevertheless, activation of TRPV1, TRPV2, as well as TRPM3, results in the development of nociceptive behaviour in laboratory animals including increased responses to both noxious and innocuous heat (heat hyperalgesia and allodynia; Caterina et al., 1997, 2000; Davis et al., 2000; Vriens et al., 2011). In addition, TRPV1 activation, through spinal cord processes, also results in the development of mechanical allodynia (LaMotte et al., 1991). Increased expression of the activated form of the extra-cellular signal-regulated kinase 1/2 (pERK1/2) in spinal cord neurons is a marker for spinal nociceptive processing (Ji et al., 1999). Hence, our recent finding that there are significantly less pERK1/2-expressing spinal cord neurons in TRPV1−/− than in wild type mice, 5 min following a scalding type injury, shows that TRPV1 indeed contributes to the development of pain during the heat impact (White et al., 2011).

In addition to inducing depolarising currents which can induce the generation of action potentials, noxious heat, through activating TRPV1 and TRPV2, and possibly also TRPM3, induces the release of various agents including the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) from the peripheral terminals of nociceptive primary sensory fibres (Holme et al., 1986; Kessler et al., 1999; Zimmermann et al., 2005; Fig. 1). These neuropeptides, together with other molecules released from either “heat-shocked” cells or degenerating tissues form an “early burn injury tissue fluid”, a mixture of agents found in the intercellular space at the area of the burn injury (Fig. 1). At present very little is known about the composition of this fluid however, many of its molecules may significantly contribute to the development of pain during and immediately after the heat impact. Both SP and CGRP are likely to be part of the early burn injury tissue fluid as their levels are elevated in burn injury patients (Onuoha and Alpar, 2001) and have been identified in animal models of acute burn injury (Jonsson et al., 1986; Saria, 1984; Papp and Valtonen, 2006). Both SP and CGRP are important mediators of neurogenic inflammation (Holzer, 1998) during which CGRP induces vasodilatation, whereas SP induces plasma extravasation and the release of agents from immuno-competent cells such as lymphocytes, monocytes/macrophages, eosinophils, neutrophils and mast cells (Scholzen et al., 1998; Fig. 1). Through this action, SP and CGRP initiate an inflammatory response for the protection and restoration of tissue integrity and function. However, many of the mediators released by SP and CGRP during the neurogenic inflammation also activate or sensitise nociceptive primary sensory neurons, and that activation and/or sensitisation result in further release of SP and CGRP (Fig. 1). In addition to acting on vascular smooth muscle and endothelial cells, and immuno-competent cells, both SP and CGRP also act directly on nociceptive primary sensory neurons inducing excitation (Szucs et al., 1999; Natura et al., 2005; Segond von Banchet et al., 2002; Fig. 1). This SP- and CGRP-induced activation may recruit additional primary afferents. Further this activation, again, may result in further release of these neuropeptides (Fig. 1). Thus, SP and CGRP, both through direct and indirect mechanisms, initiate and maintain reverberating signalling between burn tissues and nociceptive primary sensory fibres which result in increased nociceptive input into the central nervous system, hence pain (Fig. 1). Through these mechanisms therefore SP and CGRP, in areas where sensory fibres survive, seem to contribute to the development of pain arising immediately as well as later stages after the heat impact.

Noxious temperatures denature proteins and destroy cell membranes. Hence, the content of degenerating cells is released into the intercellular space where they also become part of the
early burn injury tissue fluid (Fig. 1). It is believed that ATP should be a principal algogenic component of the early burn injury tissue fluid because ATP is released from degenerating cells, and its extracellular concentration during tissue injury is sufficient enough to activate purinergic P2X3 and P2X2/3 ion channels expressed by nociceptors (North, 2003). In addition to the initial pain however, ATP should also be involved in maintaining pain in later stages of burn injuries because P2X2 receptor expression is up-regulated following burn injury (Gao et al., 2010, Xu et al., 2009). This up-regulation should be use-dependent, hence driven by the presence of ATP.

Mice lacking P2X3 and/or P2X2 exhibit reduced mechanical and thermal stimulation-evoked responses as well as reduced inflammatory pain-related behaviour (Cockayne et al., 2000, 2005; Souslova et al., 2000). Hence, P2X2 and P2X3 channels might be involved in the development of both heat and mechanical hyperalgesia and allodynia in burn injury. Notably, the ATP metabolite adenosine has been found in burn-induced blister within 12 h of the injury (Shaked et al., 2007). At present, it is not clear whether this adenosine is formed from ATP leaked from degenerated cells or released de novo for example by immuno-competent cells. Nevertheless, adenosine has been shown to exert antinociceptive rather then nociceptive effects (Sjolund et al., 1999) and its role in burn pain is unclear.

A recent work has revealed that exposure of skin to noxious heat (48 °C for 10 min) in vitro results in the production of the lipid metabolites 9- and 13-hydroxyoctadecadienoic acids (9- and 13-HODE) which activate TRPV1 directly (Patwardhan et al., 2010; Fig. 1). Both of these compounds, when injected into the hind paw of laboratory animals, produce nocifensive behavioural and heat hyperalgesia (Patwardhan et al., 2010). At present it is not known whether, in addition to cells in the skin such as keratinocytes, other cells such as invading immuno-competent cells or fibroblasts in deep connective tissue layers also have enzymatic capacity to synthesise 9- and 13-HODE. Nevertheless, Patwardhan et al. (2010) findings indicate that 9- and 13-HODE could be part of the early burn injury tissue fluid, at least in areas where some keratinocytes survive the impact. In addition to 9- and 13-HODE, other lipid metabolites, which are produced either in surviving cells or produced from disrupted cell membranes, are also expected to be present in the early burn injury tissue fluid.

High temperature-induced effects on the integrity of vessels (Jackson, 1953) and subsequent formation of molecules are also highly likely to contribute to the early burn injury tissue fluid and the development of pain immediately after the heat impact (Fig. 1). Damaged vasculature leads to the activation of the coagulation cascade, which results in the formation of the "zone of coagulation" (Jackson, 1953). Thrombin formed during the coagulation cascade, on the one hand, catalyses the formation of fibrin, while on the other hand, could activate protease-activated receptor (PAR) 1, 3 and 4, which have been shown to be expressed by sub-populations of nociceptive primary sensory neurons (Russell et al., 2010; Zhu et al., 2005). Activation of PAR-1 and PAR-4 results in TRPV1 sensitisation (Vellani et al., 2010), hence thrombin may contribute to the development of the burning pain immediately after the burn injury.

The coagulation zone of the burn-injured site is surrounded by the "zone of stasis" (Jackson, 1953). The resulting hypoxia in these two zones, through subsequent acidosis, could also be a major contributor to pain immediately after, as well as later stages of, burn injury (Fig. 1). Protons may act on at least four types of ion channels in nociceptive primary sensory fibres (White et al., 2010) TRPV1 (Caterina et al., 1997), the transient receptor vanilloid type 4 (TRPV4; Suzuki et al., 2003), P2X2/P2X2/3 (King et al., 1997) and acid-sensing ion channels (ASIC; White et al., 2010). Among the ASICs, ASIC3 seems to be the most important, responding to protons in primary sensory neurons (Deval et al., 2008). While protons activate TRPV1, TRPV4 and ASICs directly, they only modulate the activity of P2X2/P2X3 channels evoked by ATP (Caterina et al., 1997; King et al., 1997). However, protons also sensitize the effect of other activators on TRPV1 (Tominaga et al., 1998). Through these ion channels, protons can produce both heat hyperalgesia and allodynia (TRPV1, TRPV4: P2X2/P2X3,) and mechanical allodynia (TRPV4, P2X2/P2X2/3; ASICs; Caterina et al., 2000; Cockayne et al., 2000, 2005; Davis et al., 2000; Grant et al., 2007; Sluka et al., 2003; Souslova et al., 2000; Todaka et al., 2004).

3.2. Mechanism and molecules involved in the development of pain during the inflammatory phase

At the inflammatory stage, the composition of the extracellular fluid should be significantly changed when compared to the early burn injury tissue fluid because invading immunocompetent cells produce and release a large number of various agents (Fig. 1). In addition, molecules released from the degenerating cells come into contact with cells brought in by the circulation, and it is expected that many of them are metabolised. Therefore, it is useful to differentiate this burn injury tissue fluid from that emerging in the early phase, and refer to it as the “late burn injury tissue fluid”. Similarly to the composition of the early burn injury tissue fluid, we know little about the composition of the late burn injury tissue fluid. Establishing the molecular components of the late burn injury tissue fluid is however important, as among its components there must be mediators which maintain pain in the inflammatory phase of the injury.

Often, when investigators try to establish the components of the late burn injury tissue fluid, they assess serum levels and extrapolate those data to the injury site. However, as pointed out by Summer et al. (2007b), mediators are released locally and may undergo metabolism prior to reaching the systemic circulation. Conversely, agents which are detected in serum may never reach the site of the burn injury (Summer et al., 2007b). Therefore, it is more useful to assess the composition of the burn injury tissue fluid locally. Such analysis of late burn injury tissue fluid, so far, has revealed the presence of many agents, including prostaglandin (PG) F1α, PGE2, thromboxane B2 (Heggers et al., 1980), histamine (Horakova and Beavan, 1974), adenosine (Shaked et al., 2007), interleukin (IL) 1α, IL-1β, epithelial growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-β2 (TGFβ2), TGFeα, TGFp1, IL-6, platelet-derived growth factor (PDGF) and IL-8 (Ono et al., 1995). In addition to these agents, others, though not yet shown, are also highly likely to be present. Nevertheless, looking at the list of so far known agents in the late burn injury tissue fluid, it is clear that the inflammatory stage of burn injury is characterised by a complex interplay between the component of this tissue fluid, the immuno-competent cells infiltrating the wound, and primary sensory fibres. Below we will discuss briefly how known and some of the putative agents could contribute to pain developing in the inflammatory phase of burn injury.

Though bradykinin has not been shown directly in burn injury tissue fluid, previously it has been noted that blister fluid taken from severely burned patients has a bradykinin like effect (Saint-Blancard et al., 1966). Further, bradykinin is formed in injured tissues locally, and by injection it induces a burning pain sensation (Marceau et al., 1998). Hence, bradykinin should be a major component of the late burn injury tissue fluid. Bradykinin is a potent activator of primary nociceptive primary sensory fibres/neurons (Oh and Weinreich, 2004) through the G protein-coupled B1 and B2 receptors (Moreau et al., 2005). Both B1 and B2 receptors are expressed by nociceptors though B1 expression occurs only in pathological conditions, for example in inflammation (Fox et al.,
Recently, histamine levels were shown to be increased in an injury likely to be released from activated mast cells and platelets in burn (Armstrong et al., 1953; Dallob et al., 1987; McGivern and Basran, 1984). Bradykinin exerts its excitatory effects mainly via other channels including TRPV1 and the transient receptor potential ankyrin type 1 ion channel (TRPA1; Amaya et al., 2006; Bautista et al., 2006; Cesare et al., 1999; Kollarik and Undem, 2004; Katanosaka et al., 2008). Regarding TRPV1 activation, bradykinin can achieve that activation through at least two indirect mechanisms; through lowering the heat threshold of TRPV1 so that body temperature activates this ion channel (Cesare and McNaughton, 1996; Sugiuura et al., 2002), and through inducing the production of the lipid metabolite 12-hydroperoxyeicosatetraenoic acid, which directly activates TRPV1 (Shin et al., 2002). However, our recent finding that the number of pERK1/2-expressing neurons in the spinal dorsal horn in wild type and TRPV1−/− mice is not different 30 min, 60 min and 3 h after the burn injury indicates that TRPV1 might not be involved the maintenance of pain during the inflammatory phase of the injury (White et al., 2011).

While TRPA1 has been identified as a molecule responsive to noxious cold stimuli, its activation for example by cinnamaldehyde results in burning pain (Namer et al., 2005). Further, there is compelling evidence that TRPA1 plays a pivotal role in the development of inflammatory thermal hyperalgesia and mechanical allodynia (Petrus et al., 2007; Tsagareli et al., 2010). Therefore, bradykinin-evoked TRPA1 activation/sensitisation could contribute to both thermal and mechanical pain in burn injury. However, at present, there is no direct evidence whether or not TRPA1 activation contributes to pain in burn injury.

In 1953 Armstrong demonstrated that serotonin (5-HT) when applied topically to a blister base caused pain similar to that caused by the blister fluid itself (Armstrong et al., 1953). Indeed, 5-HT has been found in microdyalisates taken from burn injury sites as well as uninjured sites of burn injured patients (Samuelson et al., 2008). Mast cells and platelets, which are accumulated and activated at the site of the burn injury, release 5-HT (Kushnir-Sukhov et al., 2007; White et al., 1994). Hence, it is reasonable to assume that these cells constitute the source of 5-HT found by Samuelson et al. (2008). 5-HT induces nociceptor sensitisation (Dray, 1995; Steen et al., 1996) and mechanical hyperalgesia (Oliveira et al., 2007), which might be mediated both directly and indirectly through multiple 5-HT receptors expressed by primary sensory neurons (Loyd et al., 2011) as well as some immuno-compotent cells (Tambeli et al., 2006). 5-HT directly activates sensory fibres through 5-HT1 receptors (Hicks et al., 2002). However, Oliveira et al. (2007) have shown that the 5-HT-induced hyperalgesia depends on local release of prostaglandins and norepinephrine, and that the effect of norepinephrine depends on β2 adrenergic receptors. In addition, 5-HT2 receptor activation by serotonin sensitises ASICs (Qui et al., 2012). However, it should also be noted that activation of 5-HT1A and 5-HT1D receptors exerts inhibitory actions on primary sensory neurons (Edvinsson and Petersen, 2007).

Similarly to 5-HT, histamine, when applied to blister base also induces pain (Armstrong et al., 1953). Further, histamine is also present in burn tissues (Horakora and Beavan, 1974). More recently, histamine levels were shown to be increased in an injury depth-dependent manner (Papp et al., 2005). Histamine is also likely to be released from activated mast cells and platelets in burn injured tissues (Mannion et al., 1997; Riley, 1953). Histamine induces Ca2+ influx from extracellular fluid as well as Ca2+ outflux from intracellular stores in primary sensory neurons (Nicolsson et al., 2002). Hence, histamine induces neuronal excitation through activating the H3 receptors (Nicolsson et al., 2002; Parada et al., 2001; Ting et al., 2007). Recently, Kajihara et al. (2010) have reported that H3 receptor-mediated excitation in primary sensory neurons, at least in part, depends on TRPV1. These authors have also shown that histamine potentiates the excitatory action of protons of on TRPV1. In addition to H1, H4 receptors have also been shown to contribute to the development of thermal hyperalgesia following acute inflammation (Coruzzi et al., 2007). Hence, histamine may contribute to the development of burning pain in the inflammatory phase of burn injury.

A number of eicosanoids have been found in burn tissue and blister fluid. Burn tissue and inflammatory cells, including neutrophils and macrophages, both release arachidonic acid which forms the basis for the syntheses of prostanooids and thromboxanes via the cyclooxygenase (COX) enzyme system or leukotrienes via the 5 lipooxygenase enzyme system.

Among prostaglandins, PGE2 might be one of the most important in the development of pain in burn injury. PGE2 injection into rat paws leads to hyperalgesia (Kuhn and Willis, 1973). This effect is produced through multiple mechanisms in primary sensory neurons; PGE2 sensitises the effect of bradykinin (Kumazawa et al., 1996), sensitises TTX-resistant sodium channels (England et al., 1996), inhibits voltage-gated potassium currents (England et al., 1996), sensitises TRPV1 by reducing its heat threshold below the body temperature (Moriyama et al., 2005) and sensitises the effect of histamine (Nicolsson et al., 2007). Further, PGE2 increases the expression of the pro-inflammatory cytokine IL-6, of the neuropeptides SP and CGRP, and of the SP-responding neurokinin 1 receptor in primary sensory neurons (Ma, 2010; Segond von Banchet et al., 2003; St Jacques and Ma, 2011). In addition to PGE2, other prostaglandins at least through their metabolites, such as the PGD2 metabolite 15-deoxy-D12,14-prostaglandin J2 (Taylor-Clark et al., 2008). Notably, exposure of primary sensory neurons to bradykinin induces the synthesis and release of PGE2 from these neurons (Inoue et al., 2006).

Leukotrienes (LT) have been known to be present in the late burn injury tissue fluid for a long time (Denzlinger et al., 1985). They are released by macrophages and neutrophils once these immuno-compotent cells have infiltrated wounds. In addition to having various affects on non-neuronal cells, LT also act on primary sensory neurons. Among LT, LTB4 seems to be the most important agent in contributing to the development of pain in burn injury. Injection of this agent induces both heat and mechanical hyperalgesia (Bisgaard and Kristensen, 1985; Martin et al., 1988). The cognate receptor for LTB4, BLT1 is expressed by a major sub-population of TRPV1 expressing primary sensory neurons (Andoh and Kuraishi, 2005, Okubo et al., 2010). However, LTB4 can directly activate TRPV1 (Hwang et al., 2000). Hence it is not surprising that LTB4 induces Ca2+ influx into primary sensory neurons (Andoh and Kuraishi, 2005), hence induces excitation. In addition to BLT1 and TRPV1, LTB4 may also act on primary sensory neurons through the peroxisome proliferator-activated receptor α (LoVerme et al., 2006; Narala et al., 2010). However, through this latter action LTB4 should produce an antinoceptive effect (LoVerme et al., 2006).

Platelet activating factor (PAF) production is increased by polymorphonuclear cells isolated from burn patients (Lavaud et al., 1988). PAF decreases nociceptive thresholds in rats as well as humans (Dallob et al., 1987; McGivern and Basran, 1984). Further, intrathecal administration of PAF induces both burn and mechanical pain which depends on the expression of the PAF receptor (Tsuda et al., 2007). PAF has a direct excitatory effect on primary sensory neurons (Tsuda et al., 2007). However, that effect could be mediated not through its cognate receptor, as the PAF receptor mRNA has been found only in satellite cells which surround primary sensory neurons (Hasegawa et al., 2010). Through the PAF receptor expressed in satellite cells, PAF also seems to up-regulate the production of tumour necrosis factor alpha (TNFα) and IL-1β in satellite cells (Hasegawa et al., 2010). Hence, PAF seems to indirectly contribute to the development of
burn injury-associated pain in the inflammatory stage. This indirect algesic effect consists of multiple mechanisms, including activation of TRPV1 (Marotta et al., 2009).

Interleukin 1β is released by macrophages and neutrophils and has been demonstrated at low levels in blister fluid (Ono et al., 1995). IL-1β is also produced by primary sensory neurons (Coprøy et al., 2001; Hasegawa et al., 2010) and the IL-1β receptor 1 is also expressed by a sub-population of these neurons (von Banchet et al., 2011). IL-1β induces heat hyperalgesia and mechanical allodynia when injected subcutaneously (Safieh-Garabedian et al., 1995; Sach et al., 2002). This effect is underlain by IL-1β-evoked increase in responses of primary afferent fibres to mechanical as well as thermal stimuli (Fukuda et al., 1994). The excitatory effects of IL-1β on primary sensory neurons are mediated by multiple mechanisms involving up-regulation of TTX-resistant Na+ currents (Binshtok et al., 2008), suppression of voltage-gated K+ currents (Takeda et al., 2008) and sensitisation of TRPV1 (Obreja et al., 2002). Recently von Banchet et al. (2011) have shown that IL-1β application prevents B2 receptor internalisation, hence IL-1β increases the effect of bradykinin on primary sensory neurons. In addition to the short term effects on primary sensory neurons, IL-1β also have long term effects on other cells; it up-regulates the synthesis and release of the pivotal inflammatory mediator, nerve growth factor (NGF) (Safieh-Garabedian et al., 1995).

Interleukin 6 has been demonstrated in high levels in blister fluid (Ono et al., 1995). Recently, IL-6 has been shown in increased levels at burn injury sites in rats, which correlated with ipsilateral mechanical hyperalgesia (Summer et al. 2007b). Summer et al. (2007b) also found that the burn injury-induced mechanical hyperalgesia is attenuated by anti-IL-6 antibodies. These findings strongly argue for a pivotal role of IL-6 in the development of burn injury-associated pain. This view is supported by a recent finding that PGE2 (Heggers et al., 1980) which itself is present in the late burn injury tissue fluid up-regulates IL-6 expression in primary sensory neurons (St Jacques and Ma, 2011).

Intraplantar injection of IL-6 causes mechanical pain in rats (Cunha et al., 1992). Primary sensory neurons may express both IL-6 and its receptor as well as its signal transducer glycoprotein, gp130 (Cadient and Otten, 1996; Obreja et al., 2005). Recently, Obreja et al. (2002) showed that IL-6 when bound to its soluble receptor sensitizes primary afferents which is underlain by increased TRPV1-mediated currents and reduced heat threshold of this ion channel (Obreja et al., 2005). However, as mentioned, TRPV1 might not be involved in maintaining pain in the inflammatory phase of burn injury (White et al., 2011). At present it is not known what other molecules might be targeted by IL-6 signalling in primary sensory neurons.

Tumour necrosis factor alpha is another proinflammatory cytokine which has been found in blister fluid (Onuoha and Alpar, 2001). Serum levels of TNFα are also increased following burn injury (Onuoha and Alpar, 2001). In naive conditions, TNFα is predominantly synthesised by mast cells (Ackermann and Harvima, 1998). However in injury, TNFα is synthesised by a variety of cells including keratinocytes (Corsi and Gelli, 1998), fibroblasts (Fujisawa et al., 1997), macrophages and neutrophils (Khanolkar-Young et al., 1995). Schwann cells also synthesise TNFα particularly after peripheral nerve injury, which occurs in burn injury (Wagner and Myers, 1996). Further, TNFα induces the production of other cytokines, including IL-1β, IL-6, and IL-8, and triggers COX which synthesise prostaglandins (Cunha et al., 1992). TNFα should have a direct action on primary sensory neurons because one of the TNFα cognate receptors, TNFR1, is expressed in a large proportion of neurons in the dorsal root ganglion. In addition to the direct effect however, circulating TNFα may also have an indirect effect on primary sensory neurons as another TNFα receptor, TNFR2, is expressed by the satellite cells, and those cells produce various other inflammatory mediators including TNFα itself (Hasegawa et al., 2010) which can act directly on primary sensory neurons.

Local injection of TNFα induces thermal hyperalgesia and/or mechanical allodynia and/or hyperalgesia (Fernandes et al., 2011; Oppre and Kress, 2000; Sommer et al., 1998). The algesic effect of local TNFα injection could be underlain by multiple effects. Exposure of primary sensory neurons to TNFα results in increased TRPV1-mediated responses (Nicol et al., 1997). This effect can be blocked by indomethacin, as well as by a COX-2 inhibitor (Nicol et al., 1997). Hence the sensitising effect of TNFα on TRPV1 seems to be indirect. In addition to TRPV1, TRPA1 also seems to be a target for TNFα-induced signalling because blocking the activity of TRPA1 at peripheral tissues results in reduced mechanical hyperalgesia (Fernandes et al., 2011). However, P2X3 seems to be unaffected by TNFα (de Oliveira Fusaro et al., 2010).

Nerve growth factor is thought to be a pivotal inflammatory mediator in the development of inflammatory heat hyperalgesia (Huang et al., 2006). Indeed, increased NGF levels were found following tissue injury including that evoked by burn (Ueda et al., 2002). Further, anti-NGF treatment alleviates burn-induced hyperalgesia (Summer et al., 2006) indicating that this mediator indeed plays a key role in the development of pain following burn injury. NGF is produced by monocytes, eosinophils and mast cells (Leon et al., 1994). A series of other inflammatory mediators, some of which have been identified in the late burn injury microfluid, up-regulate NGF synthesis (Abe et al., 2007; Hattori et al., 1993; Lipnik-Stangelj, 2006; Toyomoto et al., 2004; Woolf et al., 1997). The great majority of SP- and CGRP-containing primary sensory neurons responds to NGF because they express the high affinity NGF cognate receptor tyrosine kinase A (trkA; McMahon et al., 1994). Local injection of NGF induces heat hyperalgesia and mechanical allodynia (Lewin et al., 1993; Thompson et al., 1995). Data, which have emerged in the last decade, have shown that one of the main targets through which NGF induces heat hyperalgesia is TRPV1 because NGF significantly increases TRPV1 responsiveness. This effect is achieved by at least two mechanisms. First, NGF induces TRPV1 phosphorylation which results in sensitised responses (Bonnington and McNaughton, 2003; Chuang et al., 2001). Second, NGF also induces translocation of TRPV1 from the cytoplasm to cell membrane (Zhang et al., 2005). In addition to these effects, NGF also up-regulates TRPV1 transcription (Xue et al., 2007). Recent findings however indicate that in addition to TRPV1, other ion channels also constitute targets for NGF-trkA signalling in primary sensory neurons. TRPA1, ASIC3 and P2X3 expression is controlled and up-regulated by NGF (D’Arco et al., 2007; Mamet et al., 2003; Obata et al., 2005; Ramer et al., 2001). Further, NGF also sensitises TRPA1, ASIC3- and P2X3-mediated responses (D’Arco et al., 2007; Malin et al., 2001; Mamet et al., 2002). Taken together, NGF indeed seems to be a key player in maintaining burn injury-associated heat and mechanical pain.

Stimulation of peripheral beta adrenergic receptors on primary nociceptive afferents has been linked to inflammatory hyperalgesia. This view is supported by the finding that epinephrine injection, in a dose-dependent manner, reduces the mechanical threshold which effect is attenuated by the selective β1 antagonist atenolol (Dina et al., 2001, Khasar et al., 1999). Variation in catechol-o-methyltransferase (COMT) genotype had been linked to prediction of acute pain (Diatchenko et al., 2005). The question whether or not COMT genotype is related to the severity of burn injury pain has recently been addressed in burn patients (Orrey et al., 2012). Using a haplotype based approach to COMT variables, one of three haplotypes located in the central COMT locus has previously been associated with low pain sensitivity, increased COMT activity, and has been deemed to be “pain protective”. If no copies of this gene were present in burn injured patients they were presumed to have a COMT pain vulnerable genotype.
Comparing the vulnerable, with the non-vulnerable genotype, a statistically significant increase in overall pain, “least pain” and awakening pain scores were demonstrated. Expression of the vulnerable genotype was also a stronger predictor of overall pain severity than size, type or depth of burn. Based on these data, we cannot conclude at present that exhibiting the vulnerable genotype is sign for a genetic predisposition for burn injury-associated pain. However, these data do call for further studies on the role of pathways involving catecholamines in the development of pain with peripheral origin including pain associated with burn injuries.

4. Conclusions

Burn injury is associated with enormous suffering due the severe pain it induces. Whilst burn injury-associated pain can be regarded as part of a tissue-protecting/tissue regeneration-promoting mechanism, due to its severity, pain in burn injury becomes counteractive in every sense rather than beneficial. At present, the most common analgesia used in burn injury pain in burn units are opioids. However, in the majority of the cases, opioids do not provide sufficient pain control and may even induce severe side effects in already highly vulnerable patients. Therefore, the discovery of novel targets for the development of new analgesics to improve pain control in burn-injured patients is important.

Cognate receptors in nociceptors for agents found in burn injury tissue fluid may represent ideal novel targets for drug development and intervention. However as outlined above, the composition of these tissue fluids is incompletely established at present. Further, at present it seems unlikely that there is any “leading” compound(s) within burn tissue fluids, the inactivation of which alone, would result in a significant reduction of pain. Therefore, we should devote more resources and use high throughput approaches to establish the composition of burn injury tissue fluid both in animal models of burn injury and in burn injured patients. In addition, investigating the cellular and molecular factors of the components of these burn injury tissue fluids on sensory neurons as well as tissue regeneration is also needed, as downstream molecules in nociceptors might provide better targets than those of individual cognate receptors for agents alone.

References


Eddvinsson, L., Petersen, K.A., 2007. CGRP-receptor antagonism in migraine treat-


Englund, S., Bevan, S., Docherty, R.J., 1996. PGE2 mediates the tetrodotoxin-
resistant sodium current in neonatal rat dorsal root ganglion neurons via the cAMP-protein kinase A cascade. J. Physiol. 495, 429–440.


