

Neuropeptides, neurogenic inflammation and complex regional pain syndrome (CRPS)

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ABSTRACT

This review explains symptoms and nature of neuropeptide signaling and its importance for clinical symptoms of CRPS. Neurogenic inflammation regularly accompanies excitation of primary afferent nociceptors. It has two major components—plasma extravasation and vasodilatation. The most important mediators are the calcitonin gene-related peptide (CGRP) and substance P (SP). After peripheral trauma immune reaction (e.g. cytokines) and the attempts of the tissue to regenerate (e.g. growth factors) sensitize nociceptors and amplify neurogenic inflammation. This cascade of events has been demonstrated in rat models of CRPS. Clinical findings in these animals strongly resemble clinical findings in CRPS, and can be prevented by anti-cytokine and anti-neuropeptide treatment. In CRPS patients, there is meanwhile also plenty of evidence that neurogenic inflammation contributes to clinical presentation. Increased cytokine production was demonstrated, as well as facilitated neurogenic inflammation. Very recently even “non-inflammatory” signs of CRPS (hyperhidrosis, cold skin) have been linked to neuropeptide signaling. Surprisingly, there was even moderately increased neurogenic inflammation in unaffected body regions. This favors the possibility that CRPS patients share genetic similarities. The future search for genetic commonalities will help us to further unravel the “mystery” CRPS.

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About 100 years ago Bayliss described antidromic vasodilation following electrical stimulation of centrally cut dorsal roots [4]. Meanwhile it has become clear that action potentials from activated nociceptors invade end branches of primary afferent neurons by retrograde conduction (“axon reflex”) and release neuropeptides from their terminals [62]. The acute effects of primary afferent fiber induced neuropeptide release are vasodilation and protein extravasation, which has been therefore termed “neurogenic inflammation”. Pivotal neuropeptides in the induction of neurogenic inflammation are calcitonin gene-related peptide (CGRP) for vasodilation and substance P (SP) for the induction of protein extravasation—at least in rodents [27]. As a result of previous studies, mechano-insensitive, but heat- and chemo-sensitive C-nociceptors have been found to be responsible for the neurogenic vasodilation in pig [38] and human skin [57] whereas in rodent skin polymodal nociceptors play a crucial role [16].

A few approaches so far have tried to directly measure neuropeptide release in the skin following afferent fiber excitation, e.g. ex vivo analysis by superfusion of excised rat skin [30,34]. In most studies neurogenic vasodilation and protein extravasation have

been used to functionally assess nociceptor activation [15]. Vasodilation has been measured by laser Doppler techniques and infrared thermography. Assessment of protein extravasation requires more invasive techniques. Recent advances in dermal microdialysis have opened new possibilities by allowing in vivo measurement of local mediator concentrations, including protein extravasation [52].

In addition to the classical clinical features of neurogenic inflammation, neuropeptides may also be involved in more complex regulations of tissue perfusion and pain after trauma. Only recently the importance of endothelin-1 (ET-1), a potent vasoconstrictive neuropeptide, has been explored for posttraumatic pain. ET-1, mainly secreted from inflammatory cells and keratinocytes, contributes to primary and secondary hyperalgesia, i.e. it sensitizes primary afferent neurons in the periphery and second order neurons in the spinal cord [43]. Furthermore, neuropeptides directly interact with inflammatory cells. Primary afferent nociceptors release a variety of neuropeptides like neurokinin A, neurokinin B, somatostatin, corticotropin-releasing hormone (CRH), pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive inhibitory peptide (VIP), galanin, somatostatin and endomorphins [60]. As an example, nerve growth factor (NGF) derived from keratinocytes and mast cells sensitizes nociceptors and also increases expression of mast cell tryptase and histamine [20] in mast cells. Histamine and tryptase activate nociceptive terminals via H1 and

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proteinase-activated receptors (PAR2) and release SP from the terminals [61]. SP via activation of NK1 receptors on the mast cells [65] increases expression of histamine and tumor necrosis factor alpha (TNF) [2,13], which in turn sensitizes nociceptive terminals via activation of the TNF receptor.

In the past animal models have been used to study the nature of neuropathic pain. Most studies focused on mechanisms and symptoms of pain but did not assess symptoms which characterize neurogenic inflammation. Nevertheless, these pain models indicate that cytokines (TNF-alpha, and NGF) were upregulated in the injured nerve [26,53] and that neutralizing TNF-alpha reduces pain behavior [59]. In the meanwhile these shortcomings were eliminated and preclinical models for CRPS have been established.

Most cases of CRPS I develop after bone fractures, e.g. to the wrist or ankle. Therefore, the most interesting animal model may be a controlled fracture of a large extremity bone. This model of course cannot account for all CRPS symptoms, in particular the CNS-derived signs [42], but it helps to explain the peculiar peripheral changes. In that way skin temperature increase, edema, plasma protein extravasation, osteoporosis and hyperalgesia-like behavior for weeks has been observed [21]. As indicated above, these are signs of neurogenic inflammation, which can also be found in acute CRPS after bone fracture. These signs were even pronounced if the limbs of the rats were immobilized—as it is common treatment in many human fractures. Supporting the neurogenic inflammation hypothesis of these symptoms, skin temperature difference, edema and pain behavior can be reversed by NK1-antagonism [31] and glucocorticoid administration [22]. Very recently the contribution of posttraumatically released NGF [51] and TNF-alpha [50] for pain and hyperalgesia in this CRPS I model have been established. As indicated above, both NGF and TNF amplify neurogenic inflammation by amplifying neuronal and non-neuronal neuropeptide production.

The sciatic nerve transection model was introduced as a surrogate for CRPS II. Soon after transection of the sciatic nerve, similar to the fracture model, rats develop increased skin temperature, paw edema, regional osteoporosis and hyperalgesia-like behavior. Thus, symptoms after nerve transection also resemble acute CRPS. In this model, different treatments were tested. Steroids were also able to reverse inflammatory symptoms [32], and as described in the bone fracture model, NK1 antagonism was also effective [31].

Without a detailed neurological investigation CRPS I and CRPS II cannot be distinguished. CRPS II can be diagnosed when there is obvious evidence of peripheral nerve lesion—clinically (sensory loss, muscle weakness, reflex loss) or neurophysiologically (nerve conduction, electromyography) [24]. However, these peripheral nerve lesions must involve either major nerves or skin nerves to be clinically recognized. Nerve lacerations (from broken bones) or stretches (from joint distraction) are usually unrecognizable. Injuries to small distal nerve branches may thus go undetected in most clinical settings [46,47]. Thus, we will not continue the traditional but often virtual differentiation between CRPS I and II in this review.

Pain and hyperalgesia are the most distressing symptoms. Since the initial trauma for CRPS does usually not include a complete nerve lesion, there are no serious reasons to doubt that pain and hyperalgesia result, at least partially, from activation or sensitization of peripheral nociceptors. If these nociceptors are sensitized, they might be spontaneously active causing pain at rest [49], or they respond more vigorously to physiological stimuli causing pain, e.g. while moving a joint [54]. Nociceptor sensitization furthermore drives spinal sensitization, which reinforces mechanical hyperalgesia. Since neurogenic inflammation, e.g. the release of neuropeptides from nociceptors, is inevitably linked to nociceptor activation (even subthreshold for pain might be enough [39]),

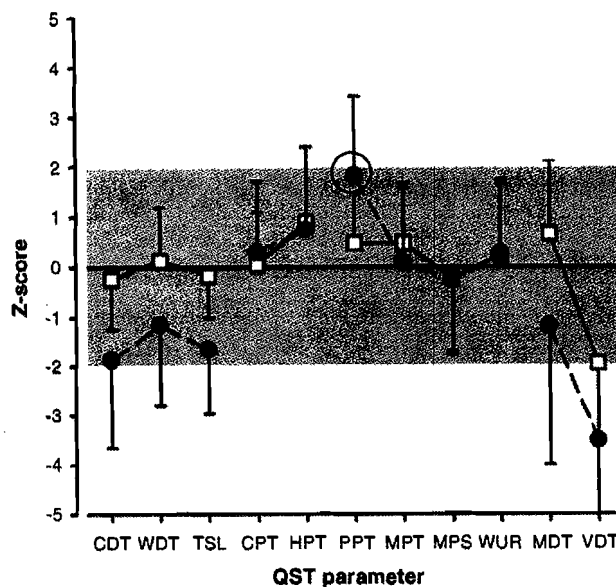


Fig. 1. QST profile of 40 cold type CRPS patients is shown. The affected side is displayed as blue circles, the unaffected side as open squares. Sensory profile points to loss of sensation on the affected side (negative scores). However, gain of function (hyperalgesia) was found for blunt pressure (red circle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

we regard pain and hyperalgesia as the first clinical symptom of neurogenic inflammation in CRPS. Quantitative sensory testing furthermore revealed that on CRPS affected skin there is a moderate decrease (about 4 °C) of heat pain thresholds [63]. Heat pain sensitization of skin nociceptors is regarded as a clinical sign for peripheral sensitization [3]. Even more obvious was the decrease of mechanical pain thresholds on the affected side. We investigated CRPS patients and found presence of hyperalgesia to brief mechanical impact stimuli (e.g. pinprick hyperalgesia) in patients characterized by inflammatory signs [58] and hyperalgesia to blunt pressure, even when inflammatory signs were less obvious (see Fig. 1).

In about 80% of posttraumatic CRPS cases, skin temperature is increased during the first 6 months of the disease. The maximum skin temperature difference might be up to 10 °C [7]. Accordingly, skin color in this stage is reddish and the skin appears hyperemic. With disease chronification, skin temperature decreases on the affected side, the skin becomes bluish and appears thin and shiny (Fig. 2) [5]. In about 20% of posttraumatic CRPS cases, skin temperature is cold and color bluish right from the beginning [66]. Since cold skin might also result from neuropeptide release (e.g. endothelin, see above and below) both warm and cold CRPS extremities might be the result of exaggerated posttraumatic inflammation.

In 50% of the patients with CRPS increased sweating can be clinically observed [10]. In acute CRPS, hyperhidrosis has been located to the peripheral nervous system [10]. From recent experiments we now know, that CGRP, which seems responsible for increased skin temperature and perfusion in CRPS, enhances sweat gland activity in physiological concentrations [56]. The mechanism might be a leftward shift of the dose–response function of nicotinic acetylcholine receptors by CGRP and in particular its fragments [14,17,45].

Edema is the most striking clinical sign in CRPS. During acute stages almost all patients have visible edema on the affected limb [8]. The edema is pronounced at backs of hands or feet. After exercise or during the course of a day, when repeated nociceptor activation has occurred, edema increases. Only if CRPS persists longer and becomes chronic, edema dwindles [6].

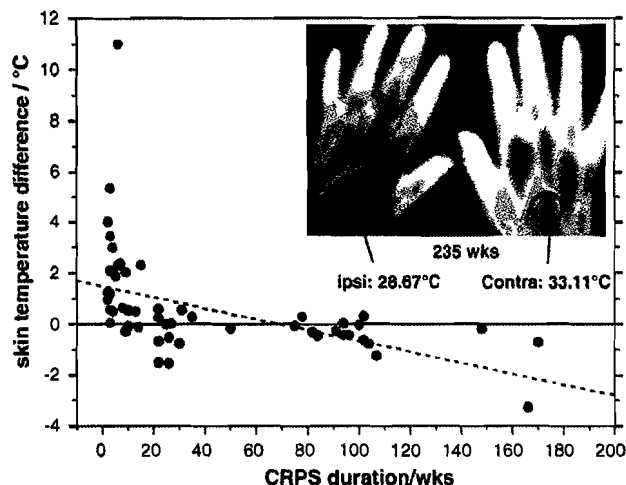


Fig. 2. Thermographic recordings of skin temperature differences between affected and unaffected sides in CRPS patients. The longer CRPS duration is the more prevalent are colder extremities.

Trophic changes can be found in more than 50% of patients. Increased hair- and nail growth appears several days after the onset of symptoms [8]. The well-known periarticular patchy osteoporosis, which can be seen on X-ray pictures, belongs to the same group of symptoms [41]. This osteoporosis is a so-called high-turnover osteoporosis with increased osteoclastic activity [37]. Interestingly, TNF- α and SP [18] activate osteoclasts, and CGRP promotes hair growth [23,23].

Inflammation in the classical sense with positive blood markers has not been proven, but any inflammation has a “neurogenic component” [12]. As indicated above, peripheral trauma, in particular if it is accompanied by partial peripheral nerve lesion, causes a rapid release of NGF and cytokines. Both are able to activate and sensitize primary afferents, locally at the injury site or proximally in the respective nerve trunk. Accordingly, increased levels of proinflammatory cytokines have been shown in CRPS skin by analysis of suction blister fluids [25,29,44] and in CRPS spinal fluid [1]. Our own group concentrated on cytokines in blood samples. We have been able to demonstrate increased TNF- α in plasma samples of two different independent CRPS patients groups [40]. Increase of TNF- α in that study was correlated to mechanical hyperalgesia. In a very recent more extensive study we confirmed these findings—not only on protein but also RNA level in CRPS blood samples [64]. Different proinflammatory cytokines were upregulated while anti-inflammatory cytokines were downregulated in the patients.

Cytokines also increase the neuropeptide content of primary afferent neurons [48]. Activation of sensitized primary afferents then causes an increased release of neuropeptides into the affected body region. Chronic release of neuropeptides might be responsible for the above-mentioned CRPS symptoms. In serum samples from patients with acute CRPS, CGRP [9], SP [55] and bradykinin [11] were found to be significantly increased, in particular when clinical inflammatory signs were present. Subsequent to these exploratory investigations, neurogenic inflammation was elicited directly in the skin by transcutaneous electrical stimulation via intradermal microdialysis capillaries by our group. We first investigated the flare by Laser-Doppler scanning on the affected and on the unaffected side in our CRPS patients. Neurogenic flare was significantly more intense in patients—on both the affected and the clinically unaffected side [36]. Another characteristic of neurogenic inflammation in rodents is SP-mediated plasma protein extravasation (PPE). In healthy humans, C-fibers usually contain too little SP to induce PPE. In CRPS, however, significant PPE could be shown in

almost all patients investigated. In contrast to the flare response, this increased PPE was limited to the affected side [68]. These results suggested two possible pathomechanisms leading to facilitated neurogenic inflammation in CRPS—either increased release or hampered inactivation of neuropeptides, or both. In order to further unravel these mechanisms, we perfused SP in ascending concentrations through dermal microdialysis fibers in CRPS patients and controls. We found SP significantly more effective at inducing PPE in CRPS patients. Similar to the increased flare response, this increased responsiveness to SP was present on both the affected and unaffected limbs [35].

As already mentioned above not only classical signs of inflammation but also “cold” CRPS might be related to increased neuropeptide signaling. In suction blister fluids from cold regulation type CRPS patients [67] the vasoconstrictive neuropeptide endothelin-1 was found to be significantly increased as compared to control subjects [19].

To summarize, all these experiments indicate a trauma-related up-regulation of neuropeptides on the affected side and in addition an impaired inactivation of neuropeptides on both sides, possibly as a predisposing factor. Neuropeptide action in human tissue is terminated by degradation by peptidases. The most familiar peptidase, which cleaves SP and CGRP is the angiotensin converting enzyme (ACE). However, in a linkage study in CRPS families we failed to show co-segregation of an insertion/deletion (I/D) polymorphism in intron 16 and CRPS phenotype [28]. Therefore we will have to focus on other enzymes, which are involved in neuropeptide degradation. One example might be the neutral endopeptidase (NEP). Blocking the NEP, facilitates neurogenic inflammation in human skin a way, which resembles the findings in CRPS patients on the unaffected side [33].

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References

- [1] G.M. Alexander, M.A. van Rijn, J.J. van Hilten, M.J. Perreault, R.J. Schwartzman, Changes in cerebrospinal fluid levels of pro-inflammatory cytokines in CRPS, *Pain* 116 (2005) 213–219.
- [2] J.C. Ansel, J.R. Brown, D.G. Payan, M.A. Brown, Substance P selectively activates TNF- α gene expression in murine mast cells, *J. Immunol.* 150 (1993) 4478–4485.
- [3] R. Baron, Peripheral neuropathic pain: from mechanisms to symptoms, *Clin. J. Pain* 16 (2000) 12–20.
- [4] W.M. Bayliss, On the origin from the spinal cord of the vaso-dilator fibres of the hindlimb, and on the nature of these fibers, *J. Physiol. (Lond.)* 32 (1901) 1025–1043.
- [5] F. Birklein, B. Riedl, D. Claus, B. Neundörfer, Pattern of autonomic dysfunction in time course of complex regional pain syndrome, *Clin. Aut. Res.* 8 (1998) 79–85.
- [6] F. Birklein, B. Riedl, N. Griessinger, B. Neundörfer, Komplexes regionales Schmerzsyndrom. Klinik und autonome Störungen während akuter und chronischer Krankheitsstadien, *Nervenarzt* 70 (1999) 335–341.
- [7] F. Birklein, B. Riedl, H.O. Handwerker, B. Neundörfer, Störung der thermoregulatorischen Hautdurchblutung bei sympathischer Reflexdystrophie, *Akt. Neurol.* 24 (1997) 61–66.
- [8] F. Birklein, B. Riedl, N. Sieweke, M. Weber, B. Neundörfer, Neurological findings in complex regional pain syndromes—analysis of 145 cases, *Acta Neurol. Scand.* 101 (2000) 262–269.
- [9] F. Birklein, M. Schmelz, S. Schiffer, M. Weber, The important role of neuropeptides in complex regional pain syndrome, *Neurology* 57 (2001) 2179–2184.
- [10] F. Birklein, R. Sittl, A. Spitzer, D. Claus, B. Neundörfer, H.O. Handwerker, Sudomotor function in sympathetic reflex dystrophy, *Pain* 69 (1997) 49–54.
- [11] S.J. Blair, M. Chinthagada, D. Hoppenstehdt, R. Kijowski, J. Fareed, Role of neuropeptides in pathogenesis of reflex sympathetic dystrophy, *Acta Orthop. Belg.* 64 (1998) 448–451.
- [12] S.D. Brain, T.J. Williams, Substance P regulates the vasodilator activity of calcitonin gene-related peptide, *Nature* 335 (1988) 73–75.
- [13] R. Cocchiara, N. Lampiasi, G. Alibegiani, A. Bongiovanni, A. Azzolina, D. Geraci, Mast cell production of TNF- α induced by substance P evidence for a modulatory role of substance P-antagonists, *J. Neuroimmunol.* 101 (1999) 128–136.

- [14] S. Di Angelantonio, R. Giniatullin, V. Costa, E. Sokolova, A. Nistri, Modulation of neuronal nicotinic receptor function by the neuropeptides CGRP and substance P on autonomic nerve cells, *Br. J. Pharmacol.* 139 (2003) 1061–1073.
- [15] C. Geber, R. Fondel, H.H. Kramer, R. Rolke, R.D. Treede, C. Sommer, F. Bircklein, Psychophysics, flare, and neurosecretory function in human pain models: capsaicin versus electrically evoked pain, *J. Pain* 8 (2007) 503–514.
- [16] M.D. Gee, B. Lynn, B. Cotsell, The relationship between cutaneous C fibre type and antidromic vasodilatation in the rabbit and the rat, *J. Physiol. (Lond.)* 503 (1997) 31–44.
- [17] R. Giniatullin, S. Di Angelantonio, C. Marchetti, E. Sokolova, L. Khiroug, A. Nistri, Calcitonin gene-related peptide rapidly downregulates nicotinic receptor function and slowly raises intracellular Ca^{2+} in rat chromaffin cells in vitro, *J. Neurosci.* 19 (1999) 2945–2953.
- [18] T. Goto, T. Yamaza, M.A. Kido, T. Tanaka, Light- and electron-microscopic study of the distribution of axons containing substance P and the localization of neurokinin-1 receptor in bone, *Cell Tissue Res.* 293 (1998) 87–93.
- [19] J.G. Groeneweg, F.J. Huygen, C. Heijmans-Antonissen, S. Niehof, F.J. Zijlstra, Increased endothelin-1 and diminished nitric oxide levels in blister fluids of patients with intermediate cold type complex regional pain syndrome type 1, *BMC. Musculoskelet. Disord.* 7 (2006) 91.
- [20] D.A. Groneberg, F. Serowka, N. Peckenschneider, M. Artuc, A. Grutzkau, A. Fischer, B.M. Henz, P. Welker, Gene expression and regulation of nerve growth factor in atopic dermatitis mast cells and the human mast cell line-1, *J. Neuroimmunol.* 161 (2005) 87–92.
- [21] T.Z. Guo, S.C. Offley, E.A. Boyd, C.R. Jacobs, W.S. Kingery, Substance P signaling contributes to the vascular and nociceptive abnormalities observed in a tibial fracture rat model of complex regional pain syndrome type I, *Pain* 108 (2004) 95–107.
- [22] T.Z. Guo, T. Wei, W.S. Kingery, Glucocorticoid inhibition of vascular abnormalities in a tibia fracture rat model of complex regional pain syndrome type I, *Pain* 121 (2006) 158–167.
- [23] S. Hagner, R.V. Haberberger, D. Overkamp, R. Hoffmann, K.H. Voigt, G.P. McGregor, Expression and distribution of calcitonin receptor-like receptor in human hairy skin, *Peptides* 23 (2002) 109–116.
- [24] N. Harden, S. Bruehl, Diagnostic criteria: the statistical derivation of four criterion factors, in: P. Wilson, M. Stanton-Hicks, N. Harden (Eds.), *CRPS: Current Diagnosis and Therapy*, IASP Press, Seattle, 2005.
- [25] C. Heijmans-Antonissen, F. Wesseldijk, R.J. Munnikes, F.J. Huygen, M.P. van der, W.C. Hop, H. Hooijkaas, F.J. Zijlstra, Multiplex bead array assay for detection of 25 soluble cytokines in blister fluid of patients with complex regional pain syndrome type 1, *Mediators Inflamm.* 2006 (2006) 28398.
- [26] U. Herzberg, E. Eliav, J.M. Dorsey, R.H. Gracely, I.J. Kopin, NGF involvement in pain induced by chronic constriction injury of the rat sciatic nerve, *Neuroreport* 8 (1997) 1613–1618.
- [27] P. Holzer, Neurogenic vasodilatation and plasma leakage in the skin, *Gen. Pharmacol.* 30 (1998) 5–11.
- [28] K. Hühne, S. Leis, M. Schmelz, B. Rautenstrauss, F. Bircklein, A polymorphic locus in the intron 16 of the human angiotensin-converting enzyme (ACE) gene is not correlated with complex regional pain syndrome I (CRPS I), *Eur. J. Pain* 8 (2004) 221–225.
- [29] F.J. Huygen, A.G. De Bruijn, M.T. De Bruin, J.G. Groeneweg, J. Klein, F.J. Zijlstra, Evidence for local inflammation in complex regional pain syndrome type 1, *Mediators Inflamm.* 11 (2002) 47–51.
- [30] S. Kilo, C. Harding-Rose, K.M. Hargreaves, C.M. Flores, Peripheral CGRP release as a marker for neurogenic inflammation: a model system for the study of neuropeptide secretion in rat paw skin, *Pain* 73 (1997) 201–207.
- [31] W.S. Kingery, M.F. Davies, J.D. Clark, A substance P receptor (NK1) antagonist can reverse vascular and nociceptive abnormalities in a rat model of complex regional pain syndrome type II, *Pain* 104 (2003) 75–84.
- [32] W.S. Kingery, T. Guo, G.S. Agashe, M.F. Davies, J.D. Clark, M. Maze, Glucocorticoid inhibition of neuropathic limb edema and cutaneous neurogenic extravasation, *Brain Res.* 913 (2001) 140–148.
- [33] H.H. Krämer, K. Schmidt, S. Leis, M. Schmelz, C. Sommer, F. Bircklein, Inhibition of neutral endopeptidase (NEP) facilitates neurogenic inflammation, *Exp. Neurol.* 195 (2005) 179–184.
- [34] M. Kress, C. Guthmann, B. Averbek, P.W. Reeh, Calcitonin gene-related peptide and prostaglandin E2 but not substance P release induced by antidromic nerve stimulation from rat skin, in vitro, *Neuroscience* 89 (1999) 303–310.
- [35] S. Leis, M. Weber, A. Isselmann, M. Schmelz, F. Bircklein, Substance-P-induced protein extravasation is bilaterally increased in complex regional pain syndrome, *Exp. Neurol.* 183 (2003) 197–204.
- [36] S. Leis, M. Weber, M. Schmelz, F. Bircklein, Facilitated neurogenic inflammation in unaffected limbs of patients with complex regional pain syndrome, *Neurosci. Lett.* 359 (2004) 163–166.
- [37] T. Leitha, A. Staudenherz, M. Korpan, V. Fialka, Pattern recognition in five-phase bone scintigraphy: diagnostic patterns of reflex sympathetic dystrophy in adults, *Eur. J. Nucl. Med.* 23 (1996) 256–262.
- [38] B. Lynn, S. Schutterle, F.K. Pierau, The vasodilator component of neurogenic inflammation is caused by a special subclass of heat-sensitive nociceptors in the skin of the pig, *J. Physiol. (Lond.)* 494 (1996) 587–593.
- [39] W. Magerl, G. Geldner, H.O. Handwerker, Pain and vascular reflexes elicited by prolonged noxious mechano-stimulation, *Pain* 43 (1990) 219–225.
- [40] C. Maihofner, H.O. Handwerker, B. Neundorfer, F. Bircklein, Mechanical hyperalgesia in complex-regional pain syndrome: a role for TNF-alpha, *Neurology* 65 (2005) 311–313.
- [41] V. Maresca, Human calcitonin in the management of osteoporosis: a multicentre study, *J. Int. Med. Res.* 13 (1985) 311–316.
- [42] G.L. Moseley, S.C. Gandevia, Sensory-motor incongruence and reports of 'pain', *Rheumatology (Oxford)* 44 (2005) 1083–1085.
- [43] F.H. Mujenda, A.M. Duarte, E.K. Reilly, G.R. Strichartz, Cutaneous endothelin-A receptors elevate post-incisional pain, *Pain* 133 (2007) 161–173.
- [44] R.J. Munnikes, C. Muis, M. Boersma, C. Heijmans-Antonissen, F.J. Zijlstra, F.J. Huygen, Intermediate stage complex regional pain syndrome type 1 is unrelated to proinflammatory cytokines, *Mediators Inflamm.* 2005 (2005) 366–372.
- [45] A. Nistri, S. Di Angelantonio, Enhancement of neuronal nicotinic receptor activity of rat chromaffin cells by a novel class of peptides, *Ann. NY Acad. Sci.* 971 (2002) 100–107.
- [46] A.L. Oaklander, F. Bircklein, Peripheral somatosensory abnormalities - pathology and pathophysiology, in: M. Stanton Hicks, N. Harden, P.R. Wilson (Eds.), *CRPS: Current Diagnosis and Therapy*, IASP Press, Seattle, 2005.
- [47] A.L. Oaklander, J.G. Rissmiller, L.B. Gelman, L. Zheng, Y. Chang, R. Gott, Evidence of focal small-fiber axonal degeneration in complex regional pain syndrome-I (reflex sympathetic dystrophy), *Pain* 120 (2006) 235–243.
- [48] A. Oprea, M. Kress, Involvement of the proinflammatory cytokines tumor necrosis factor-alpha, IL-1 beta, and IL-6 but not IL-8 in the development of heat hyperalgesia: effects on heat-evoked calcitonin gene-related peptide release from rat skin, *J. Neurosci.* 20 (2000) 6289–6293.
- [49] K. Orstavik, C. Weidner, R. Schmidt, M. Schmelz, M. Hilliges, E. Jorum, H. Handwerker, E. Torebjörk, Pathological C-fibres in patients with a chronic painful condition, *Brain* 126 (2003) 567–578.
- [50] I. Sabsovich, T.Z. Guo, T. Wei, R. Zhao, X. Li, D.J. Clark, C. Geis, C. Sommer, W.S. Kingery, TNF signaling contributes to the development of nociceptive sensitization in a tibia fracture model of complex regional pain syndrome type I, *Pain*; 2007 Dec 14 [epub ahead of print].
- [51] I. Sabsovich, T. Wei, T.Z. Guo, R. Zhao, X. Shi, X. Li, D.C. Yeomans, M. Klyukin, W.S. Kingery, J.D. Clark, Effect of anti-NGF antibodies in a rat tibia fracture model of complex regional pain syndrome type I, *Pain*; 2007 Nov 20 [epub ahead of print].
- [52] K. Sauerstein, M. Klede, M. Hilliges, M. Schmelz, Electrically evoked neuropeptide release and neurogenic inflammation differ between rat and human skin, *J. Physiol.* 529 (2000) 803–810.
- [53] M. Schafers, C. Geis, D. Brors, T.L. Yaksh, C. Sommer, Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve, *J. Neurosci.* 22 (2002) 536–545.
- [54] H.G. Schaible, A. Ebersberger, G.S. von Banchet, Mechanisms of pain in arthritis, *Ann. NY Acad. Sci.* 966 (2002) 343–354.
- [55] C. Schinkel, A. Gaertner, J. Zaspel, S. Zedler, E. Faist, M. Schuermann, Inflammatory mediators are altered in the acute phase of posttraumatic complex regional pain syndrome, *Clin. J. Pain* 22 (2006) 235–239.
- [56] T. Schlereth, J.O. Dittmar, B. Seewald, F. Bircklein, Peripheral amplification of sweating - a role for calcitonin gene-related peptide, *J. Physiol.* 576 (2006) 823–832.
- [57] M. Schmelz, K. Michael, C. Weidner, R. Schmidt, H.E. Torebjörk, H.O. Handwerker, Which nerve fibers mediate the axon reflex flare in human skin? *Neuroreport* 11 (2000) 645–648.
- [58] N. Sieweke, F. Bircklein, B. Riedl, B. Neundorfer, H.O. Handwerker, Patterns of hyperalgesia in complex regional pain syndrome, *Pain* 80 (1999) 171–177.
- [59] C. Sommer, T. Lindenlaub, P. Teuteberg, M. Schafers, T. Hartung, K.V. Toyka, Anti-TNF-neutralizing antibodies reduce pain-related behavior in two different mouse models of painful mononeuropathy, *Brain Res.* 913 (2001) 86–89.
- [60] M. Steinhoff, S. Stander, S. Seeliger, J.C. Ansel, M. Schmelz, T. Luger, Modern aspects of cutaneous neurogenic inflammation, *Arch. Dermatol.* 139 (2003) 1479–1488.
- [61] M. Steinhoff, N. Vergnolle, S.H. Young, M. Tognetto, S. Amadesi, H.S. Ennes, M. Trevisani, M.D. Hollenberg, J.L. Wallace, G.H. Caughey, S.E. Mitchell, L.M. Williams, P. Geppetti, E.A. Mayer, N.W. Bunnett, Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism, *Nat. Med.* 6 (2000) 151–158.
- [62] J. Szolcsanyi, Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroregulatory mechanism, *Prog. Brain Res.* 113 (1996) 343–359.
- [63] A.J. Tahmouh, R.J. Schwartzman, J.L. Hopp, J.R. Grothausen, Quantitative sensory studies in complex regional pain syndrome type 1/RSD, *Clin. J. Pain* 16 (2000) 340–344.
- [64] N. Uceyler, T. Eberle, R. Rolke, F. Bircklein, C. Sommer, Differential expression patterns of cytokines in complex regional pain syndrome, *Pain* 132 (2007) 195–205.
- [65] H.P. van der Kleij, D. Ma, F.A. Redegeld, A.D. Kraneveld, F.P. Nijkamp, J. Biemont, Functional expression of neurokinin 1 receptors on mast cells induced by IL-4 and stem cell factor, *J. Immunol.* 171 (2003) 2074–2079.
- [66] P.H.J.M. Veldman, H.M. Reynen, I.E. Arntz, R.J.A. Goris, Signs and symptoms of reflex sympathetic dystrophy: prospective study of 829 patients, *Lancet* 342 (1993) 1012–1016.
- [67] G. Wasner, J. Schattschneider, K. Heckmann, C. Maier, R. Baron, Vascular abnormalities in reflex sympathetic dystrophy (CRPS I): mechanisms and diagnostic value, *Brain* 124 (2001) 587–599.
- [68] M. Weber, F. Bircklein, B. Neundorfer, M. Schmelz, Facilitated neurogenic inflammation in complex regional pain syndrome, *Pain* 91 (2001) 251–257.