



Murdoch
UNIVERSITY

MURDOCH RESEARCH REPOSITORY

This is the author's final version of the work, as accepted for publication following peer review but without the publisher's layout or pagination.

The definitive version is available at

<http://dx.doi.org/10.1016/j.expneurol.2010.01.012>

Drummond, P.D. (2010) Inflammatory consequences of cutaneous stimulation. *Experimental Neurology*, 222 (2). pp. 181-183.

<http://researchrepository.murdoch.edu.au/1937/>

Copyright: © 2010 Elsevier Inc.

It is posted here for your personal use. No further distribution is permitted.

Inflammatory consequences of cutaneous stimulation

Peter D. Drummond

School of Psychology, Murdoch University, Perth, 6150, Western Australia

Keywords: Inflammation; Tumor necrosis factor- α ; Pain; Skin; Axon-reflex

Among other functions, resident skin cells monitor their local environment for potential sources of threat and mount defenses against invasion by foreign micro-organisms. Most prominent in this innate defense system are keratinocytes, mast cells and dendritic cells (Metz and Maurer, 2009). Each of these cell types participate in cutaneous immune and allergic responses and, together, provide an integrated defense against infection and injury. Activation of epidermal keratinocytes by ultraviolet light or stimulation of toll-like receptors on their cell membrane evokes the release of antimicrobial peptides and secretion of pro-inflammatory cytokines. Mast cells are acutely sensitive to their local environment and, when activated, secrete a wide array of biological mediators that play a pivotal role in the response to infection and tissue injury. Immune cells are drawn to the site of injury by chemical signals (chemokines) released by injured cells and by the actions of the innate defense system. Dermal and epidermal dendritic cells (Langerhans cells) then initiate immune responses by presenting antigens to cells of the adaptive immune system.

These defensive processes are monitored and coordinated by sensory nerves. Noxious mechanical, chemical, thermal and electrical stimuli initiate axon-reflex vasodilatation (a flare) around the site of stimulation, thereby helping to disperse harmful substances and to recruit protective blood-borne cells and agents that quarantine and destroy invading microbes. This local response is triggered by the sensory afferents that detect noxious stimuli, and involves release of vasoactive neuropeptides from the axonal network around the site of stimulation. In humans, the axon-reflex flare is mediated primarily by calcitonin gene-related peptide (CGRP) with an additional contribution from substance P (Sauerstein et al., 2000). Besides their effect on vascular smooth muscle and endothelial cells, these neuropeptides communicate with resident skin cells. For example, stimulation of neurokinin-1 receptors by substance P induces proliferation of keratinocytes, release of cytokines from mast cells and functional activation of dendritic cells (Peters et al., 2006), responses that are important for wound healing and protection against infection. Conversely, pro- and anti-inflammatory mediators released from resident skin and infiltrating immune cells influence the excitability of cutaneous sensory neurons. The two-way communication between these cells and sensory nerves helps to fine-tune local inflammatory responses while also informing the central nervous system about developments that might require systemic intervention.

One of the key regulators of inflammation is tumor necrosis factor- α (TNF- α) (Bradley, 2008). Although activated macrophages and T lymphocytes provide the main source of TNF- α in the skin, a wide range of resident skin cells can also produce this cytokine (Metz and Maurer, 2009). TNF- α is a modulator of the host response to infection and of programmed cell death, and acts upon the vascular endothelium to increase adhesion and transendothelial migration of leukocytes to sites of inflammation (Bradley, 2008). TNF- α also generates a cytokine cascade that can sensitize cutaneous sensory afferents via at least two pathways. The

first involves cyclo-oxygenase with production of hyperalgesic prostaglandins, whereas the second involves chemokines and the subsequent release of sympathetic amines (Cunha et al., 1992 and Cunha et al., 2008). As noted below, the second pathway could implicate the sympathetic nervous system in neuro-inflammatory pain syndromes.

Given these properties, it is not surprising that TNF- α plays an important role in various chronic inflammatory diseases, including rheumatoid arthritis, neuropathic pain, inflammatory bowel disease and psoriasis (Bradley, 2008). TNF- α is elevated in the affected tissue of patients with complex regional pain syndrome (CRPS) (Huygen et al., 2002 and Bernateck et al., 2010), a chronic pain condition that generally starts after peripheral nerve or tissue injury (Drummond, 2001 and Gibbs et al., 2008). The consequences of this elevation were explored recently in an animal model of CRPS involving distal tibia fracture (Sabsovich et al., 2008). TNF- α was found to be over-expressed in affected skin and sciatic nerve four weeks after the fracture. This was associated with behavioural signs of pain and spinal Fos activation, a marker of nociceptive signaling. These responses were blocked by pre-emptive treatment with a TNF- α antagonist, suggesting that TNF- α is an important mediator of pain following tibial fracture. Further characterization of the pain model indicated that substance P signaling through enhanced neurokinin-1 receptor expression in keratinocytes activated pro-inflammatory cytokines (Wei et al., 2009). This raises the possibility that release of substance P from nociceptive neurons evokes a cytokine cascade from resident skin cells that ultimately results in sensitization of the nociceptors that began the process. If left unchecked, this vicious circle could result in chronic inflammation and pain.

It is becoming increasingly clear that pro-inflammatory processes involving keratinocytes are fundamental in certain forms of neuropathic pain. To illustrate this point, Radtke et al. (2010) transplanted human keratinocytes at the site of a severed sciatic nerve in a rat model of neuropathic pain. The keratinocytes assembled into a cellular structure that resembled the stratum spinosum of the epidermis. Levels of the prototypic neurotrophin, nerve growth factor (NGF), rose substantially at the transplant site. In addition, the cell bodies of primary afferent neurons whose cut axons were present near the transplanted keratinocytes became highly excitable and animals displayed behavioural signs of pain (autotomy of the nerve-injured limb). These effects were greater than those induced by ligation alone; moreover, keratinocytes injected into an *intact* sciatic nerve neither assembled into a cellular structure nor induced behavioural or electrophysiological signs of pain. NGF is crucial for the survival of sensory and sympathetic neurons, and also regulates axonal growth, synaptic plasticity, the expression of ion channels and neurotransmission via tyrosine kinase A receptors (Reichardt, 2006). Some of these effects may be driven by TNF- α during inflammation, as TNF- α enhances the synthesis of NGF from cultured human keratinocytes (Takaoka et al., 2009). Together, the findings presented by Radtke et al. show that the context in which sensory afferents and resident skin cells interact is critically important—and shifts dramatically after nerve injury.

In a study published recently in *Experimental Neurology*, Eberle et al. (2010) measured intradermal TNF- α of healthy volunteers in response to two forms of sensory stimulation. The first involved shooting small plastic cylinders (0.5 g at 11 m/s) 120 times each at three adjacent skin sites in the forearm over a 30-min period. This produced a mildly painful sensation by the end of stimulation (rated 0.9 on a 0–10 pain rating scale) but no tenderness in the surrounding skin. The second form of stimulation involved intradermal electrical

impulses (1 Hz, 0.5 ms duration, increasing to 20 mA within the first 5 min of a 30-min period of stimulation). This produced a moderately painful sensation by the end of stimulation (rated 6.3 on the pain scale) and half of the participants reported tenderness in surrounding skin, reflecting sensitization of central nociceptive neurons. Laser Doppler imaging indicated that electrical stimulation produced a flare that persisted for at least 90 min, whereas impact stimuli produced a flare during the first 10 min that subsided thereafter. Surprisingly, there was no apparent association between the neuropeptide-mediated flare and intradermal TNF- α , as TNF- α increased approximately 5-fold during the hour following impact stimuli but remained unchanged both during and after painful electrical stimulation.

These findings are interesting for at least two reasons. First, they illustrate that activity in cutaneous nociceptors (which generates pain, tenderness and flares) does not necessarily result in release of TNF- α . *In vitro*, substance P and CGRP trigger the release of TNF- α and other cytokines in a concentration-dependent manner from fibroblasts prepared from human dental pulp tissue (Yamaguchi et al., 2004), and increase mRNA for a range of cytokines including TNF- α in cultured human keratinocytes (Dallos et al., 2006). Thus, the machinery exists for cells (including human skin cells) to manufacture cytokines in response to stimulation by neuropeptides. However, outcomes may be more complex *in vivo* as CGRP impedes immune cell activity (Gomes et al., 2005) and inhibits production of TNF- α in Langerhans cells following exposure to lipopolysaccharide (Ding et al., 2007). Thus, the net result following nociceptor activation might depend on the relative concentrations of pro-inflammatory neuropeptides, such as substance P, and anti-inflammatory neuropeptides like CGRP. In human skin, a predominance of CGRP secreted from the peripheral terminals of nociceptive neurons during axon-reflex flares (Sauerstein et al., 2000) may explain why TNF-

α did not increase following intradermal electrical stimulation in the study by Eberle et al. (2010). It would be interesting to determine whether electrical stimulation is more effective in inflammatory and neuropathic pain syndromes characterized by heightened TNF- α signaling, because confirmation of a vicious circle between substance P and cytokine release could lead to insights about the pathogenesis of these syndromes.

The second intriguing observation by Eberle et al. (2010) was the increase in levels of TNF- α in the dermal interstitial fluid 1 h after the train of impact stimuli. Inflammatory responses generally evolve over a period of hours or days rather than minutes because it takes time to manufacture cytokines. For instance, exposure to ultraviolet B light induces a biphasic increase in pro- and anti-inflammatory cytokines with an initial peak at 3–6 h and a second peak at 48 h (Saade et al., 2008). Curiously, both peaks depend to some extent upon the viability of capsaicin-sensitive cutaneous nociceptors and sympathetic efferents. Specifically, sympathetic efferents are required for TNF- α production both after ultraviolet B stimulation (Saade et al., 2008) and after intraplantar injection of bacterial endotoxin (Safieh-Garabedian et al., 2002); this inflammatory link might account, at least in part, for involvement of the sympathetic nervous system in CRPS (Gibbs et al., 2008). To explain the time course of TNF- α release after mechanical stimulation, Eberle et al. postulated that impact stimuli dislodged preformed stores of TNF- α into the interstitial fluid, possibly from mast cells (Gibbs et al., 2001). Alternatively, rapid *de novo* synthesis might account for the 90-min delay between stimulus onset and detection of TNF- α in the dermal interstitial fluid.

At present, the relevance of these findings for neuropathic pain remains unclear. Both in terms of neural discharge and pain behaviour, sensitivity to TNF- α increased in uninjured

primary afferent neurons after spinal nerve ligation (an animal model of neuropathic pain that resembles CRPS), possibly due to the products of infiltrating immune cells or release of neurotrophins such as NGF (Schafers et al., 2003). Moreover, preemptive treatment with a TNF- α antagonist inhibited allodynia both in this model (Schafers et al., 2003) and after distal tibia fracture (Sabsovich et al., 2008), pointing to the importance of TNF- α in the genesis of neuro-inflammatory pain. Whether preformed stores of TNF- α contribute significantly to this process requires further investigation.

Benefits of TNF- α treatment for CRPS have been identified in uncontrolled case studies (Huygen et al., 2004 and Bernateck et al., 2007) but await confirmation in randomized controlled trials. In the meantime, the findings of Eberle et al. (2010) indicate that innocuous mechanical stimulation of healthy skin is sufficient to raise intradermal levels of TNF- α , independent of activity in cutaneous nociceptive neurons. Cutaneous nerves might be more influential in the context of inflammation or nerve injury, due to sensitization of primary nociceptive afferents and up-regulation of TNF- α and other inflammatory mediators produced by resident skin cells (Sommer and Kress, 2004 and Sabsovich et al., 2008). In any event, the findings of Eberle et al. (2010) suggest that even minor mechanical stimulation of affected regions may be sufficient to maintain inflammation and pain in patients with chronic neuro-inflammatory disease. If so, blocking the inflammatory response ought to be given priority over physical treatments that might inadvertently aggravate symptoms and cause the syndrome to persist.

Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia.

References

- Bernateck, M., Karst, M., Gratz, K.F., Meyer, G.J., Fischer, M.J., Knapp, W.H., Koppert, W., Brunkhorst, T., 2010. The first scintigraphic detection of tumor necrosis factor-alpha in patients with complex regional pain syndrome type 1. *Anesth. Analg.* 110, 211–215.
- Bernateck, M., Rolke, R., Birklein, F., Treede, R.D., Fink, M., Karst, M., 2007. Successful intravenous regional block with low-dose tumor necrosis factor-alpha antibody infliximab for treatment of complex regional pain syndrome 1. *Anesth. Analg.* 105, 1148–1151 table of contents.
- Bradley, J.R., 2008. TNF-mediated inflammatory disease. *J. Pathol.* 214, 149–160.
- Cunha, F.Q., Poole, S., Lorenzetti, B.B., Ferreira, S.H., 1992. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br. J. Pharmacol.* 107, 660–664.
- Cunha, T.M., Verri Jr., W.A., Valerio, D.A., Guerrero, A.T., Nogueira, L.G., Vieira, S.M., Souza, D.G., Teixeira, M.M., Poole, S., Ferreira, S.H., Cunha, F.Q., 2008. Role of cytokines in mediating mechanical hypernociception in a model of delayed-type hypersensitivity in mice. *Eur. J. Pain* 12, 1059–1068.
- Dallos, A., Kiss, M., Polyanka, H., Dobozy, A., Kemeny, L., Husz, S., 2006. Effects of the neuropeptides substance P, calcitonin gene-related peptide, vasoactive intestinal

polypeptide and galanin on the production of nerve growth factor and in inflammatory cytokines in cultured human keratinocytes. *Neuropeptides* 40, 251–263.

Ding, W., Wagner, J.A., Granstein, R.D., 2007. CGRP, PACAP, and VIP modulate Langerhans cell function by inhibiting NF-kappaB activation. *J. Invest. Dermatol.* 27, 2357–2367.

Drummond, P.D., 2001. Mechanism of complex regional pain syndrome: no longer excessive sympathetic outflow. *Lancet* 358, 168–170.

Eberle, T., Doganci, B., Kramer, H., Fechir, M., Wagner, I., Sommer, C., Birklein, F., 2010. Mechanical but not painful electrical stimuli trigger TNF alpha release in human skin.

Gibbs, B.F., Wierecky, J., Welker, P., Henz, B.M., Wolff, H.H., Grabbe, J., 2001. Human skin mast cells rapidly release preformed and newly generated TNF-alpha and IL-8 following stimulation with anti-IgE and other secretagogues. *Exp. Dermatol.* 10, 312–320.

Gibbs, G.F., Drummond, P.D., Finch, P.M., Phillips, J.K., 2008. Unravelling the pathophysiology of complex regional pain syndrome: focus on sympathetically maintained pain. *Clin. Exp. Pharmacol. Physiol.* 35, 717–724.

Gomes, R.N., Castro-Faria-Neto, H.C., Bozza, P.T., Soares, M.B., Shoemaker, C.B., David, J.R., Bozza, M.T., 2005. Calcitonin gene-related peptide inhibits local acute inflammation and protects mice against lethal endotoxemia. *Shock* 24, 590–594.

Huygen, F.J., De Bruijn, A.G., De Bruin, M.T., Groeneweg, J.G., Klein, J., Zijlstra, F.J., 2002. Evidence for local inflammation in complex regional pain syndrome type 1. *Mediators Inflamm.* 11, 47–51.

- Huygen, F.J., Niehof, S., Zijlstra, F.J., van Hagen, P.M., van Daele, P.L., 2004. Successful treatment of CRPS 1 with anti-TNF. *J. Pain Symptom. Manage* 27, 101–103.
- Metz, M., Maurer, M., 2009. Innate immunity and allergy in the skin. *Curr. Opin. Immunol.* 21, 687–693.
- Peters, E.M., Ericson, M.E., Hosoi, J., Seiffert, K., Hordinsky, M.K., Ansel, J.C., Paus, R., Scholzen, T.E., 2006. Neuropeptide control mechanisms in cutaneous biology: physiological and clinical significance. *J. Invest. Dermatol.* 126, 1937–1947.
- Radtke, C., Vogt, P.M., Devor, M., Kocsis, J.D., 2010. Keratinocytes acting on injured afferents induce extreme neuronal hyperexcitability and chronic pain. *Pain* 148, 94–102.
- Reichardt, L.F., 2006. Neurotrophin-regulated signalling pathways. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 361, 1545–1564.
- Saade, N.E., Farhat, O., Rahal, O., Safieh-Garabedian, B., Le Bars, D., Jabbur, S.J., 2008. Ultra violet-induced localized inflammatory hyperalgesia in awake rats and the role of sensory and sympathetic innervation of the skin. *Brain Behav. Immun.* 22, 245–256.
- Sabsovich, I., Guo, T.Z., Wei, T., Zhao, R., Li, X., Clark, D.J., Geis, C., Sommer, C., Kingery, W.S., 2008. TNF signaling contributes to the development of nociceptive sensitization in a tibia fracture model of complex regional pain syndrome type I. *Pain* 137, 507–519.
- Safieh-Garabedian, B., Poole, S., Haddad, J.J., Massaad, C.A., Jabbur, S.J., Saade, N.E., 2002. The role of the sympathetic efferents in endotoxin-induced localized

inflammatory hyperalgesia and cytokine upregulation. *Neuropharmacology* 42, 864–872.

Sauerstein, K., Klede, M., Hilliges, M., Schmelz, M., 2000. Electrically evoked neuropeptide release and neurogenic inflammation differ between rat and human skin. *J. Physiol.* 529 (Pt 3), 803–810.

Schafers, M., Svensson, C.I., Sommer, C., Sorkin, L.S., 2003. Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. *J. Neurosci.* 23, 2517–2521.

Sommer, C., Kress, M., 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci. Lett.* 361, 184–187.

Takaoka, K., Shirai, Y., Saito, N., 2009. Inflammatory cytokine tumor necrosis factor-alpha enhances nerve growth factor production in human keratinocytes, HaCaT cells. *J. Pharmacol. Sci.* 111, 381–391.

Wei, T., Li, W.W., Guo, T.Z., Zhao, R., Wang, L., Clark, D.J., Oaklander, A.L., Schmelz, M., Kingery, W.S., 2009. Post-junctional facilitation of Substance P signaling in a tibia fracture rat model of complex regional pain syndrome type I. *Pain* 144, 278–286.

Yamaguchi, M., Kojima, T., Kanekawa, M., Aihara, N., Nogimura, A., Kasai, K., 2004. Neuropeptides stimulate production of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha in human dental pulp cells. *Inflamm. Res.* 53, 199–204.