PhD thesis

Sensitivity to capsaicin in skin and airways in patients with symptoms elicited by odorous chemicals

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Preface

This thesis is the result of a PhD project in collaboration between the Danish Research Center for Chemical Sensitivities, Gentofte Hospital, University of Copenhagen; the Allergy Unit, National University Hospital, Copenhagen; and the Laboratory for Experimental Pain Research, Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University. The study was conducted between February 2006 and January 2009. The thesis is based on two papers and one submitted manuscript (in the text referred to in roman numerals). The original papers are enclosed.


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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>Aδ-fibres</td>
<td>Fast-velocity vagal myelinated fibres</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>A.U.</td>
<td>Arbitrary unit</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>C2</td>
<td>Capsaicin concentration causing two coughs or more</td>
</tr>
<tr>
<td>C5</td>
<td>Capsaicin concentration causing five coughs or more</td>
</tr>
<tr>
<td>C-fibres</td>
<td>Subset of primary neurons with unmyelated axons</td>
</tr>
<tr>
<td>CFS</td>
<td>Chronic fatigue syndrome</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CP</td>
<td>Chronic pain</td>
</tr>
<tr>
<td>CS</td>
<td>Central sensitization</td>
</tr>
<tr>
<td>CSS</td>
<td>Central sensitivity syndrome</td>
</tr>
<tr>
<td>EC</td>
<td>Eczema patients</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced expiratory volume in the first second</td>
</tr>
<tr>
<td>FM</td>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>MCS</td>
<td>Multiple Chemical Sensitivity</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RARs</td>
<td>Rapidly adapting receptors</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator curve</td>
</tr>
<tr>
<td>SP</td>
<td>Substance P</td>
</tr>
<tr>
<td>SPPH</td>
<td>Secondary pinprick hyperalgesi</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin prick test</td>
</tr>
<tr>
<td>TiVi</td>
<td>Tissue Viability</td>
</tr>
<tr>
<td>TRP</td>
<td>The transient receptor potential</td>
</tr>
<tr>
<td>TRPV-1</td>
<td>Transient Receptor Potential Vanilloid-1</td>
</tr>
<tr>
<td>TS</td>
<td>Temporal Summation</td>
</tr>
<tr>
<td>WDR</td>
<td>Wide dynamic-range neurons</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VAS\text{MAX}</td>
<td>Maximal pain on Visual analogue scale</td>
</tr>
</tbody>
</table>
The primary aim of this thesis was to investigate patho-physiological mechanisms in either patients fulfilling Cullen’s criteria for Multiple Chemical Sensitivity (MCS) or eczema patients (EC) with airway symptoms elicited by odorous chemicals (study 2). The main focus was to investigate the sensitivity to capsaicin by stimulating the Transient Receptor Potential Vanilloid-1 distributed in both skin and airways. This could support the development of tools for more objective diagnosis of such patients. The second aim was to determine the influence of menstrual phase, sex-specific differences, and reproducibility of bronchial challenge with capsaicin in healthy individuals (study 1). This clarification was valuable for the interpretation of our test results and a necessity when establishing reference values. The third aim was to investigate whether sensitivity to capsaicin in skin and airways was correlated.

We measured C5 - the capsaicin concentration causing five coughs or more - using single inhalation test of incremental concentrations. In the first study, participants underwent two intradermal injections of capsaicin with the same concentration in the forehead and forearm. For women the two tests were conducted during their ovulation and menstrual phase. In the second study, two different concentrations of capsaicin were used in the forearm. In both studies, measurements included pain intensity, distribution, visual flare, and secondary pin-prick hyperalgesia (SPPH) (increased response to a normally painful stimulus). Further, in the second study, capsaicin-induced SPPH and temporal summation (TS)(increasing pain sensation generated by repetitive stimulation) were quantified as markers of abnormal central nociceptive processing. Finally, local neurogenic inflammatory response (visual flare) at the site of injection was measured.

In the first study, C5 did not differ between sexes or between menstrual phases, and reproducibility was good. Women had a stronger response to capsaicin in skin than did men, but no difference between menstrual phases was demonstrated. In the second study, lower airway symptoms were significantly correlated to an increased cough reflex sensitivity to capsaicin across groups (MCS and EC). The association between C5 and lower airway symptoms was not affected by being a smoker or by, having asthma, neither was it influenced by applying for pension. Intradermal injections of capsaicin evoked increased SPPH and TS response in patients with subjective symptoms elicited by odorous chemicals (both EC and MCS patients). In addition, patients with co-morbidity of fibromyalgia, chronic pain, and CFS showed significantly enhanced TS compared with patients without co-morbidity. Further, no difference was found in flare area between groups.

This is the first evidence of abnormal central processing of pain in MCS and EC patients. These findings could be a step towards a more mechanism-based diagnose with objective criteria. Future studies need to address whether our finding of an increased SPPH in patients with MCS can be reproduced and whether this phenomenon occurs persistently or intermittently. However, the lack of difference between menstrual phases in women in both skin and airways, when stimulated with capsaicin, simplifies the establishment of future research. In addition, the capsaicin challenge test can be used to verify the presence of symptoms from the lower airways in patients with multiple chemical sensitivity and eczema patients with airway symptoms elicited by odorous chemicals.
Denne afhandling havde til formål at undersøge patofysiologiske mekanismer hos patienter, med symptomer udløst af eksponering af lave koncentrationer af luftbårne kemiske stoffer (studie 2); enten opfyldende Cullens kriterier for duft- og kemikalieoverfølsomhed, eller eksempatienter, henvist til udredning for lidelser i hud og luftveje. Til at belyse disse mekanismer blev capsaicin følsomheden undersøgt i henholdsvis hud og luftveje ved stimulering af den capsaicin følsomme receptor Transient Receptor Potential Vanilloid-1. Formålet var samtidig at vurdere, om det capsaicinudløste respons var kønsrelateret, dels om den hormonale cyklus influerede på følsomheden (studie 1). Endelig, om der var en sammenhæng mellem capsaicinfølsomheden i hud og luftveje.


Vi fandt, at hostetærsklen hverken var forskellig mellem kønnene eller påvirket af hormoncyklus. Derudover var der god reproducerbarhed for den bronkiale provokationstest. I studie 2 fandt vi endvidere at rapportering af nedre luftvejssymptomer var signifikant associeret til lavere hostetærskel hos begge patientgrupper, og at denne association var uafhængig af forekomst af astma, rygning og om man var pensionssøgende.

I studie 1 fandt vi, at kvinder havde et større smerterespnds end mænd, men smerten varierede ikke over cyklus. I studie 2 fandt vi et signifikant større hudområde med sekundær hyperalgesi hos patienter (på tværs af grupperne) i forhold til raske deltagere, samt en øget ophobning af smerte ved gentagen stimulation hos patienter, der opfyldte Cullens kriterier for duft- og kemikalieoverfølsomhed. Denne ophobning af smerteresponenter var særligt udtalt hos patienter, der samtidig rapporterede andre kroniske lidelser som fibromyalgi, kronisk træthedssyndrom og kronisk smerte. Arealet af hudrødmen omkring injektionstraumet, som er tegn på neurogen inflammation, var ens i kontrol- og patientgrupper. Slutteligt fandtes ingen sammenhæng mellem capsaicin følsomheden i hud og luftveje.

Denne afhandling er den første af sin art, der har fundet abnormt, centratl udløst neurogent respons hos patienter med duft- og kemikalieoverfølsomhed. Disse fund kan være første skridt på vejen mod en mere mekanisme-orienteret diagnose baseret på objektive kriterier. Der er dog lang vej, inden en sådan test kan finde klinisk anvendelse. I første omgang skal resultaterne reproduceres, og det skal kortlægges om den øgede sensibilisering varierer over tid hos det samme individ. Resultaterne fra studie 1 har gjort det lettere at opstille efterfølgende forsøg, idet der ikke nødvendigvis skal tages hensyn til menstruations cyklus. Derimod er det vigtigt, at der tages højde for den kønsbestemte forskel i sekundær hyperalgesi i fremtidige studier.
Introduction and background

Exposure to low concentrations of odorous chemicals in the environment such as perfumes, car exhaust, cleaning agents, freshly printed-paper, and flower scents are normally regarded as non-toxic. However, certain groups of individuals exhibit symptoms from the central nervous system (CNS), skin, gastrointestinal system and airways when exposed to odorous chemicals. These symptoms have been proposed to be diagnosed as Multiple Chemical Sensitivity (MCS). Many theories and hypothesis have been advanced for the causes of MCS, ranging from disorders such as allergy or other immunological disturbances, through cardiovascular and neurological diseases to predominately psychiatric disorders. The underlying cause of MCS remains disputed, and the debate regarding both the existence and definition of MCS is ongoing. Although a contradictory picture is presented, there is growing evidence that MCS is not a single clinical entity.

1.1 Definition of Multiple Chemical Sensitivity (MCS)

The condition was first defined and the term coined by Cullen in 1987 containing the following criteria for MCS:

1) The disorder is acquired in relation to some documentable environmental exposure(s), insult(s), or illness(es).
2) Symptoms involve more than one organ system
3) Symptoms recur and abate in response to predictable stimuli
4) Symptoms are elicited by exposures to chemicals of diverse structural classes and toxicological modes of action
5) Symptoms are elicited by exposures that are demonstrable (albeit of low level)
6) Exposures that elicit symptoms must be very low (below those known to cause adverse effects in the general population)
7) No single, widely available test of organ system function that can explain the symptoms.

At present, at least 9 different case definitions for MCS are existing. The most profound and well-known case definition was elaborated on a MCS consensus conference in the United States in 1999, “The 1999 consensus report”, based on the study by Nethercoott. et al. These criteria resemble those outlined by Cullen a decade before. However, Cullen proposed the disorder to be acquired in relation to documented environmental exposure, insult or illness, whereas the combined criteria of the consensus-conference definition take into account self-reported, exposure-related circumstances that patients experience as MCS. Lacour et al. validated the US MCS-case definition on the basis of a systematic literature-review in order to derive standards for differential diagnostic procedures recently. The review revealed that for MCS, exposure-related unspecific symptoms of the CNS are a predominant feature. The author therefore made a proposal for a further extension of the US MCS-case definition from 1999, in order to specify this criterion: exposure-related symptoms associated with self-reported multiple chemical sensitivities are to be divided into non-specific complaints from the central nervous system-CNS (mandatory diagnostic criterion) and functional disturbance in other organ systems (optional complaints). Further, that the symptoms has been causing a significant lifestyle or functional impairment for the last 6 month before being diagnosed as MCS.

As stated above there are several other case definitions, which might not have been used as extensively as the ones by Cullen and the consensus report in 1999. The MCS criteria used in this thesis is based on the broad definition adopted by Graveling in 1999, in order not to miss, any exciting material. Here, MCS was defined as symptoms from more than one organ system elicited by various unrelated chemicals at very low levels of exposure. This definition encompasses the original criteria outlined by Cullen and the US MCS-case definition, although in the lack of good exposure data in currently published studies, the definition by Graveling did not include exposure-response relations. For the experiments, we chose Cullen’s criteria, originally developed for research purposes, in order to obtain a more homogeneous selection of MCS patients.
Chapter 1

1.2 Prevalence

There is a wide range of estimates for the prevalence of MCS within the general population, reflecting both the type of information elicited and the definitions applied. This raises questions regarding the accuracy of questionnaire-based diagnosis of chemical sensitivity. Furthermore, most questionnaires are directed towards general chemical sensitivity rather than MCS specifically. A recent postal survey conducted in Copenhagen, Denmark showed, that 27% of the respondents reported symptoms related to inhalation of airborne chemicals. Three point three percentage of the respondents adjusted their social life or occupational conditions due to such symptoms and 0.5% were making adjustments to both social life and occupational conditions. These results are in accordance with similar studies in the United States where approximately 4% of the adult population had experienced discomfort due to symptoms when shopping in stores or eating in restaurants. Further, approximately 1.5% had lost or had to give up a job or occupation as a consequence of their symptoms. In general, women reported a greater number of annoying exposures than men and women had more symptoms related to inhalation of airborne chemicals. The gender difference could reflect a biological difference (e.g. genetic and hormonal), psychological, environmental, and socio-cultural mechanisms (where men under-report symptoms), respectively. Rates of self-reported sensitivity among patients with chronic fatigue syndrome (CFS), fibromyalgia (FM) and temporomandibular disorders have been reported to be higher than healthy controls. In high prevalence of chemical sensitivity among asthmatic and eczema patients (EC) have been observed in several studies. The overlap of symptoms among asthmatic, eczema and FM patients adjusted their social life or occupational conditions due to such symptoms and 0.5% were making adjustments to both social life and occupational conditions. These results are in accordance with similar studies in the United States where approximately 4% of the adult population had experienced discomfort due to symptoms when shopping in stores or eating in restaurants. Further, approximately 1.5% had lost or had to give up a job or occupation as a consequence of their symptoms. In general, women reported a greater number of annoying exposures than men and women had more symptoms related to inhalation of airborne chemicals. The gender difference could reflect a biological difference (e.g. genetic and hormonal), psychological, environmental, and socio-cultural mechanisms (where men under-report symptoms), respectively. Rates of self-reported sensitivity among patients with chronic fatigue syndrome (CFS), fibromyalgia (FM) and temporomandibular disorders have been reported to be higher than healthy controls. In high prevalence of chemical sensitivity among asthmatic and eczema patients (EC) have been observed in several studies.

1.3. Main theories of possible causative mechanisms

In sections 1.4-1.7 some of the recent findings and proposals relevant for investigations of pathophysiological mechanisms in MCS will be addressed. Although, there are no symptom constellations that constitute MCS, non-specific complaints of CNS, especially headaches, fatigue and cognitive deficits are the most frequently described symptoms in studies and reviews. Further, complains from the respiratory system with symptoms from both upper and lower airways (e.g. eye irritation, sinusitis, hoarseness, cough, chest pain and dyspnoea) are commonly reported. In view of the latter, there have been many proponents for immunological disorders or deficiencies; however convincing evidence is still lacking. The overlap of symptoms between MCS, CFS and FM provide substantial diagnostic challenges. It has also lead to advances, as common pathophysiological mechanisms have recently been proposed to be involved, primarily activation of nociceptors and central sensitization (CS) (for details see section 1.7). The airway reactions to chemical stimuli are conducted by afferent sensory receptors located in and essential protective physiological mechanisms. However, the general reactivity to airborne chemicals in MCS has been related to altered function of the respiratory mucosa suggesting that chemicals stimulate a non-specific pathway involving sensory fibre (for details see section 1.6).

1.4 Allergy as a possible causative mechanism for MCS

Allergic reactions have underlying immune mechanisms, triggered by allergens. Unlike proteins, chemicals must associate with larger carrier molecules in order to induce immune response. Further, it is difficult to explain how structurally different chemicals give rise to diverse symptoms and organ involvement due to an adverse effect on the immune system. In addition, correlates are rarely found altered (or only mildly altered) in MCS patients, suggesting that MCS is not mediated through allergic mechanisms. On top, bronchial obstruction was not provoked when methacholine tested in MCS patients in a previous study. Nevertheless, the higher prevalence of chemical sensitivity among asthmatic and EC patients estimated from questionnaires remains unexplained, and results cannot exclude that MCS could be more prevalent in asthmatics. However, since both asthma and eczema are common diseases, it is more likely that there are patients with these diseases and coexistent MCS. This would partly explain, why only EC who suffer from airway symptoms elicited by odours chemicals have increased sensitivity to inhaled capsaicin, compared
to EC without airway symptoms\textsuperscript{16,17}. Further, that no correlation was found between cough threshold and methacholine provocations; suggesting that cough sensitivity and bronchial responsiveness may be independently potentiated by different mechanisms in chronic airway inflammation\textsuperscript{25,26}. However, MCS and EC patients with airway symptom elicited by odours chemicals has not yet been provoked by capsaicin in the same experimental set up, in order to explore if these patients may share some overlapping mechanisms with MCS as suggested in a case study\textsuperscript{23}.

\section*{1.5 Capsaicin and the Transient Receptor Potential Vanilloid-1 (TRPV-1)}

The key to, how different sensory fibres translate various kinds of stimuli (e.g. the perception of chemicals and touch) into an electrical signal, interpreted in the nervous system, was revealed by the cloning of the super family of transient receptor potential (TRP) ion channels. These channels subserve a whole host of cellular roles including many features of the sensory transduction\textsuperscript{27}. They are important means for multiple organ systems to interact with their environment\textsuperscript{27,28} and enable humans to sense conditions such as temperature, noxious stimuli, stretch, osmolarity, and pain. They are divided into the sub modalities mechanosensation, temperature sensation, chemosensation, and nociception\textsuperscript{27}. The receptor TRPV-1, one of eight subfamilies of TRP, is a six-trans membrane-spanning protein with a pore region\textsuperscript{29}. The TRPV-1 channel is activated by diverse range of chemical ligands such as capsaicin and other vanilloids (e.g. reiñifaratoxin and anandamide), as well as low extra cellular pH, noxious heat, and diverse lipid derivatives\textsuperscript{30}. Capsaicin (the piquancy of hot chilli -peppers) is a versatile odourless natural compound, acting as a ligand solely to the TRPV-1 channel\textsuperscript{31}. It has achieved widespread use in clinical research because it induces cough in the airways and pain in a dose-dependent and reproducible manner\textsuperscript{32-35} with almost no adverse events reported\textsuperscript{33,35,36}.

The strong indications of the involvement of the TRPV-1 channel in several diseases, comes from the close correlations between levels of TRPV-1 channel expression when immunostained in biopsies, and disease symptoms\textsuperscript{27,37,38}, and/or enhanced responsiveness from the central nervous system, gut, and airways when experimentally provoked by capsaicin\textsuperscript{37,39-41}. This close link was also found for MCS patients with lower airway symptoms\textsuperscript{34}, but has only been suggested theoretically in regards to CNS symptoms and pain\textsuperscript{18,42}.

\section*{1.6 Increased expression of TRPV-1 in the airways as possible causative mechanisms}

Increased cough sensitivity to bronchial challenge test with capsaicin was not only found in patients with lower airway symptoms, but also in a group of MCS patients with no history of coughing\textsuperscript{43}. In general lower, but not upper respiratory symptoms were also significantly associated with increased capsaicin sensitivity in eczema patients with respiratory symptoms elicited by perfume\textsuperscript{44}. A cough reflex can be triggered by several inflammatory or mechanical changes in the airways and by inhalation of chemical and mechanical irritants\textsuperscript{35}. Sensory nerve receptors responding to these stimuli are defined by their conductive properties as rapidly adapting receptors (RARs), slowly adapting receptors, or C-fibre receptors\textsuperscript{39}. RARs are discharged when stimulated by, for example cigarette smoke, acid, mechanical stimulation and alkaline solutions and are conducted in fast-velocity vagal myelinated (A\textdelta) fibres\textsuperscript{19}. C-fibre receptors (TRPV-1) are highly sensitive to chemicals and thus often referred to as chemosensors. The TRPV-1 channel seen both on RARs and C-fibres has been localised to epithelial nerves in the upper and lower airways\textsuperscript{19,45,46}.

Capsaicin activation of the TRPV-1 in peripheral nerve endings in mucous membranes promotes orthodromic propagation (afferent function) of the depolarising stimulus, whereby it contributes to reflex responses, including cough (lower airways)\textsuperscript{30}. In the upper airways, inhalation of capsaicin causes a hot, burning sensation. It is unknown whether increased cough sensitivity is caused by peripheral or central sensitization\textsuperscript{19}. However, the expression of TRPV-1 was found increased in patients with chronic cough irrespective of the causes\textsuperscript{38}. The author suggested the up-regulation of TRPV-1 to be of major importance for peripheral sensitization\textsuperscript{38}. Little is known about the neuronal circuit in the brain\textsuperscript{47}. The cough reflex is believed to involve multiple primary and secondary pathways and processes in the brainstem and being modulated by cortical control to shape the response\textsuperscript{47} (Figure 1).
A significant overlap between the brain regions activated in both peripheral somatosensory (by nociceptors) and airway sensory stimulation has recently been proposed. Although it is likely that the respiratory mucosa can act as both a target and an effector organ for MCS, this hypothesis alone does not account for the wider multisystem symptoms reported in MCS.

### 1.7 Plastic change in the central nervous system as possible causative mechanisms

Pain such as headache, chest pain, muscle -, and joint pain is frequently reported symptoms among MCS patients. Pain is a multidimensional experience that involves not only the transduction of noxious environmental stimuli, but also congestive and emotional processing in the brain. Pain is signalled through nociceptors. They are divided into thinly myelinated, intermediate conducting Aδ-fibres and unmyelinated slowly conducting C-fibres. The Aδ-fibres mediate a rapid, acute, sharp pain whereas C-fibres mediate a more diffuse, dull pain (Figure 2 A).

TRPV-1 is located on the free sensory nerve endings of the C-fibres in skin. The C-fibres transmit information through the dorsal root/trigeminal ganglion and enter the dorsal horn of the spinal cord where the fibres bifurcate in Lissauer’s tract. After bifurcation, the nerve fibres travel 2–3 spinal segments in both rostral and caudal directions, sending collateral projections into the dorsal horn to transmit pain signals across multiple segments of the spinal cord. In the dorsal horn the primary afferent fibres synapses either directly or indirectly (via interneurons) in lamina I, II and IV–VI. There are two major types of pain-related neurons, the lamina I neurons with a high activation threshold which respond only to noxious mechanical stimuli, and the wide dynamic range neurons (WDR) in lamina V which respond over both the innocuous and noxious range. These neurons are subjected to powerful control by supraspinal sites and function as a relay for nociceptive signals. Axons of nociceptive dorsal neurons in these laminae cross to the contra lateral side to form the ascending tract, which terminates in the brainstem and several distinct areas of the

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**Figure 1**

**Cough processing pathways**

Laryngeal and pulmonary receptors, such as rapidly adapting receptors (RAR) and C-fiber, are stimulated by peripheral noxious stimulations. The activation of sensory nerve fibers then leads to orthodromic activation of the brainstem. Here, the signal is modulated via interneurons in the nucleus tractus solitarius and transmitted to the central cough generator, which establishes and coordinates cough. Moreover, neuropeptides are transported antidromically via the sensory fibers back to the peripheral endings in the airway. Here, they are locally released and propagate neurogenic inflammation. Further, the central cortex can control the output of cough volitionally, or influence the urge to cough sensation.

Modified from Groneberg et al, 2004
Third order neurons further project to various cortical and limbic structures, where pain signals are received and interpreted in the somatosensory areas of cortex (localization, duration, and intensity of pain) as well as limbic structures and anterior cingulate cortex ACC (emotional responses to pain) (Figure 2B). The ACC is the most commonly activated structure in functional imaging studies of pain and is suggested to play a pivotal role in nociceptive processing. This also partly explains the significantly increased activation of the ACC that was found in a group of MCS patients stimulated with the pure olfactory odorant vanillin recorded by PET scan which in parallel showed a reduced activation of the olfactory region. The nociceptors are not only unique in the sense that they can detect a wide range of stimulus modalities, including those of physical and chemical nature, they are also unique since their receptive properties can be modulated. Thus, nociceptors not only signal acute pain, but also contribute to persistent and pathological pain conditions that occur in the setting of injury (peripheral sensitization), whereby injury-induced discharge of nociceptive afferents trigger central sensitization (CS). This modulation of nociceptive synaptic transmission either outlasts the initiating input or requires a low-level peripheral drive to maintain it. The WDR neurons, in particular, change structure, function and biochemistry in longstanding CS. Secondary hyperalgesia (SH) refers to increased pain sensitivity in undamaged skin that surrounds an injury site. Electrophysiological studies provide strong evidence against any sensitization of primary nociceptive afferents in undamaged skin. Therefore, SH can only be conceived as CS, presumably heterosynaptic facilitation of second order neurons in the spinal cord. Like SH, “wind-up” is also related to CS. This phenomenon involves a slowly increasing number of action potentials generated by the postsynaptic neurons induced by repetitive stimulation. The perceptual correlate to wind-up is temporal summation (TS) of second pain, but unlike SH, it is explained by homosynaptic facilitations primarily mediated by the WDR neurons in lamina V.
Central sensitization, SH and wind-up have been induced experimentally by various methods in order to study pain mechanisms in both animal and human models. Capsaicin-induced SH has been found increased in patients compared with controls in clinical studies on rheumatoid arthritis, vulvodynia and FM\textsuperscript{39-41}. These conditions including MCS have recently been suggested classified as central sensitivity syndromes (CSS) with the underlying patho-physiological mechanism suggested being CS\textsuperscript{18} (Figure 3). However, CS as a pathological mechanism is unexplored in patients with symptoms induced by odorous chemicals. This includes MCS patients. In addition, recent investigations into FM have focused on wind-up and central sensitization. In comparison, the perceived pain was increased, as were the level of TS within a series of stimuli when induced experimentally in FM patients and controls\textsuperscript{58}.

1.8 Neurogenic inflammation as a possible causative mechanism

Neurogenic inflammation in the airways may participate in the development and progression of chronic inflammatory diseases such as asthma and chronic obstructive pulmonary disease \textsuperscript{58}. Alterations in sensory nerves and neurogen inflammation has also been suggested in patients with chronic non-productive cough\textsuperscript{59} and patients with airway symptoms from scents and chemicals\textsuperscript{60}. In these conditions increased levels of substance P and nerve growth factor (NGF) was found in the upper airways\textsuperscript{59,60}, respectively. Further, the levels of neuropeptides were significantly correlated to cough sensitivity to capsaicin in both conditions\textsuperscript{59,60}. The imbalance of NGF found in patients with sensory hyperreactivity has been suggested to be the same for MCS patients\textsuperscript{60}. However, in comparison with patients suffering from asthma and allergy, the level of NGF was discrete, and the author emphasizes an inconsistency between the patho-physiological mechanisms in allergy and MCS / sensory hyperreactivity\textsuperscript{60}. Another study included both patients with MCS and atopic eczema/dermatitis syndrome, and found initial plasma levels of substance P, vasoactive peptide and NGF to be increased in both groups, whereas histamine was only elevated in eczema patients\textsuperscript{61}. Exposure to volatile organic compounds did not increase plasma levels in eczema patients\textsuperscript{61}. In contrast, all parameters were increased in MCS patients, indicating that volatile organic compounds may enhance neurogenic response\textsuperscript{61}. Thus, results are in line with Millqvist et al. suggesting the patho-physiological mechanisms in eczema and MCS to be different.

Tissue injury, irritation and infection can similarly induce inflammation in the skin. The classical observation of redness (rubber/flare), heat (calor/flush) and swelling (tumour/wheal), are invariably accompanied by pain (dolor)\textsuperscript{62}. Experimentally, pain evoked by intradermal injection of capsaicin, is well known to be inflammatory\textsuperscript{63}. Several studies have showed that CS is induced by neurogenic inflammation\textsuperscript{57,64,65}. However, in clinical studies there has been a lack of consistency as to whether CS co-exists with neurogenic inflammation both within the same disorder\textsuperscript{66-68} and between conditions suggested to be part of CSS\textsuperscript{59,67,68}. Lewis was first to describe the erythema arising in human skin in the surroundings

![Central sensitisation and CSS](image-url)

**Figure 3**

The central sensitivity syndromes (CSS)
The proposed link of pathophysiological mechanism among them is central sensitisation. Irritable bowel syndrome (IBS), tension-type headache (T-T headache), temporomandibular disorders (TMD), myofascial pain syndrome (MPS), fibromyalgia (FM), multiple chemical syndrome (MCS), female urethral syndrome (FUS), interstitial cystitis (IC), posttraumatic stress syndrome (PTSD).

Modified from Yunus, 2008
Figure 4
The process of neurogenic inflammation
Activation of the nociceptor transmits afferent messages to the spinal cord dorsal horn and initiates antidromic release of neuropeptides (substance P (SP) and calcitonin gene related peptide (CGRP)) from the peripheral terminals. The release induces vasodilatation and plasma extravasation, as well as activation of many non-neuronal cells, including mast cells and neutrophils. These cells in turn contribute additional elements to the inflammatory soup by sensitize (lower the threshold) of the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons, creating an inflammatory loop.

of trauma as part of the triple response to noxious stimuli. From these findings and earlier work, Lewis developed the concept of axon reflex flare, i.e. the notion that nociceptive nerve fibres excited by a trauma send impulses not only into the CNS but also via axon branches into the surrounding skin (antidromic conduction), where they trigger the release of a vasodilating substance from the nerve endings, primarily calcitonin gene-related peptide (CGRP) and substance P. The antidromic release of neuropeptides is followed by redness and warmth (secondary to vasodilatation), swelling (secondary to plasma extravasation) and hypersensitivity concurrently referred to as neurogenic inflammation (Figure 4).

According to this model the area of flare is affected by the anatomical relationship of two elements: the size of the collateral nerve network innervating the skin and the distribution of vasculature. Neurogenic inflammation induced by for example capsaicin has mainly been measured by the investigator’s subjective visual inspection. This area was found to be smaller than the area of SH is some studies, and was taken as evidence that different mechanisms generate SH and neurogenic inflammation. The development of new and versatile technologies for investigating neurogenic inflammation including laser Doppler flowmetry and thermography have facilitated objective measurements and have demonstrated that the area of neurogenic inflammation to be larger than found when visually inspected. Further, the area of SH was found to be surrounded by the area of neurogenic inflammation, and a possible contribution of neurogenic inflammation to SH has recently been proposed.

It remains to be investigated to what extent neurogenic inflammation contributes to the pathological mechanism induced by odorous chemicals in MCS patients, and if so, whether this inflammation can be correlated to enhanced responsiveness of neurons within the dorsal horn followed by enhanced SH.

1.9 Sex Hormones
One of the prominent aspects in MCS is the disproportionate representation of women in line with many chronic pain syndromes. There is growing evidence that estrogens act directly on the nervous system thus affecting the severity of pain. Estrogen receptors are expressed by sensory neurons, and in trigeminal ganglia, 17 ß-estradiol can indirectly enhance nociception by stimulating...
expression and release of prolactin, which increases phosphorylation of the TRPV-1 channel\(^5\). However, in human experimental pain studies there is disagreement as to the influence of sex\(^3\). One possible factor contributing to this inconsistency could be that women in general have been provoked during various phases of their menstrual cycle and this subject needs to be further addressed by e.g. hormone samples during experiments in women\(^3\). Similarly, a clarification of the menstrual cycles influence on airway functions is highly relevant, as chronic persistent nonproductive cough has been found more frequently in women\(^76,77\). 

Further, the cough reflex has been found more sensitive in women in general, as compared to men, when experimentally induced by capsaicin\(^77,79\).

1.10 Symptoms elicited by odorous chemicals –relationship to other diseases

In the previous sections overlapping conditions to MCS has been mentioned. They mainly fall into two categories 1) CNS symptoms, recently proposed to be mandatory diagnostic criterion\(^5\) (e.g. headache, fatigue, dizziness inability to concentrate) overlapping with conditions such as fibromyalgia (FM), chronic pain (CP) and chronic fatigue syndrome (CFS) 2) Airway symptoms, optional criterion\(^5\) (e.g. cough, bronchial phlegm, chest tightness sore throat ear and eye irritation) overlapping with asthma and eczema patients with airway symptoms elicited by odorous. Since the co-occurrence/interaction of symptoms between MCS and asthma and/or eczema contributes to diagnostic difficulties, we chose to enrol a group of EC patients with upper and/or lower airways symptoms induced by odorous chemicals, as this patient group could in some aspects be regarded as a sub-condition of MCS. Further, as there has not been any symptom constellation that constitutes MCS and no fulfilling diagnostic criteria, we chose not to exclude patients that have developed FM, CP or CFS in the course of MCS as suggested by Lacour et al.\(^5\).
Aims of the PhD project

The overall aim of this PhD project was to investigate patho-physiological mechanisms in Multiple Chemical Sensitivity (MCS) by applying experimental models of intradermal and bronchial provocations of capsaicin in patients either fulfilling Cullen’s criteria for MCS or eczema patients with airway symptoms elicited by odorous chemicals (EC) as compared with controls. This could lead to the development of diagnostic tools for future diagnosis of patients with MCS. Furthermore, the models were also applied in healthy men and women, in order to gain information about reproducibility, sex-related difference, and influence of menstrual cycle of cough threshold and pain reactivity.

Capsaicin-induced cough reflex

The study aims were to investigate:
- To what extent menstrual cycle influences the cough threshold in healthy women and reproducibility of test (men)
- Whether the threshold of the capsaicin-induced cough reflex was enhanced in patients (paper I)
- To what extent the reporting of lower airway symptoms related to odorous chemicals influenced the patients’ cough thresholds to capsaicin (paper I)

Capsaicin-induced pain

The study aims were:
- To assess to what extent menstrual cycle influences pain reactivity in healthy women and reproducibility of test (men)
- To assess pain reactivity, hyperalgesia and temporal summation in patients (paper II)
- To assess pain reactivity in relation to hormone level in patients (paper II)
- To investigate to what extent capsaicin-induced cough is associated with secondary hyperalgesia and temporal summation

Capsaicin-induced neurogenic inflammation

The study aims were to investigate:
- The capsaicin-induced neurogenic vasodilatations (peripheral inflammation) in the skin by measuring erythema intensity and visual flare in patients (paper III)
- The interrelationship between the erythema intensity and flare in patients (paper III)
- To what extent capsaicin-induced cough is associated with erythema intensity and visual flare
Figure 5. Outline of the PhD project
Capsaicin-induced cough reflex

3.1 Measurements of cough receptor sensitivity to capsaicin

The experimental induction of cough has become an important tool in clinical cough research, because it allows quantification of coughing sensitivity. In essence, there are two main methods used for cough challenges; namely single dose inhalation and the dose-response (Figure 6). Single dose inhalation involves inhalation of one concentration of the tussive agent, and is mainly applied for screening large populations.80 The dose-response challenges, involves inhalation of incremental concentrations of tussive agent.80 This method can be further subdivided into single breath and fixed time inhalation, depending on the length of inhalation.80 While the tidal method measures the capability to habituate to a response induced over a protracted period, the single breath inhalation test measures the first response that provokes the cough reflex.

The most commonly used tussive agents include capsaicin, citric acid and ultrasonically nebulised distilled water (fog).81 Capsaicin induces cough by stimulating the TRPV-1 receptor in a safe, dose-dependent, and reproducible manner. It has been used for cough challenge in more than 20 years.32,34,80-82 Short-term tachyphylaxis has been demonstrated when fixed time inhalations are applied for one minute, although much less pronounced for capsaicin compared with citric acid, and distilled water.83 However, significant long-term tachyphylaxis for both capsaicin and citric acid has been reported when applying repeated challenges 82,83, using four inhalations of each dose with a 10 sec interval between each of the four inhalations, repeated at 10 min intervals for the first 40 min and then at 240 and 360 min.82,83 Further, the order of inhaled capsaicin concentration was found to influence the number of cough significantly.84 This difference increased with increasing sensitivity to capsaicin.84 The tussive response to a single breath of capsaicin is immediate and brief. Therefore, only coughs that occur within the first 10-15 sec. of dose delivery of capsaicin, should be counted.82,83 Thresholds of inhalation cough challenge testing, especially with a dose–response method, are C2 and C5, i.e. the administered doses of capsaicin inducing at least two or at least five coughs, respectively.82 C5 has been shown to have better reproducibility than C2, especially in the short-term assessments (14-day interval).82

Several factors are known to influence the cough reflex: capsaicin cough sensitivity increases during upper respiratory infections due to transient hyperresponsiveness of cough receptors and it has been recommended that subjects who have had infection within the previous 4 weeks should be excluded from clinical studies.85 Likewise, cough reflex sensitivity was found enhanced as soon as 2 weeks after smoking cessation.86 In contrast, healthy cigarette smokers have a diminished cough reflex sensitivity relative to non-smokers of the same sex, most likely due to chronic, cigarette smoke-induced desensitization of airway cough receptors.86 Therefore, clinical trials should control for smoking and non-smoking status.81 Some contradictory elements exist regarding whether sex influences cough sensitivity. The majority of studies showed both healthy women and female patients with chronic cough to have enhanced cough reflex to capsaicin as compared with men.76,78,87

Figure 6 An overview of methodology of inhalation cough challenge

Modified from Morice et al, 2001)
Chapter 3

The capsaicin challenge test previously applied in patients with symptoms triggered by exposure to chemicals has mainly been the tidal breathing challenge test (fixed time inhalation)\textsuperscript{24,43,44,88}. Whereas, the single breath inhalation test has primarily been used when measuring cough reflex in patients with various causes of chronic cough\textsuperscript{38,85,87,89}. However, as results are not easily comparable with those obtained from the single breath inhalation test (dosimeter method)\textsuperscript{24,80}, we chose to use the single breath method, in this thesis. In the upper airway mucosal symptoms such as nasal irritation, rhinorrhea, and eye irritation are frequently reported during capsaicin challenge test\textsuperscript{44,60}. These symptoms resembles the ones reported by patients when exposure to odours chemicals\textsuperscript{10,16,17}. However, the mechanisms behind these reactions are not fully explained\textsuperscript{91,60}. They could be due to capsaicin reaching the nose from pharynx, or that some of the nebulizer solution are dispersed and inhaled through the nose, as participants did not use a nose clip\textsuperscript{60}. In this thesis these symptoms are assembled in the term “upper airway symptoms” and breathing problems and cough as lower airways symptoms.

Administration of capsaicin

The cough challenge with capsaicin relies on the delivery of the tussive agents as aerosols from a nebuliser. The nebuliser should produce a high ratio of respirable aerosol particles at low volume and output\textsuperscript{80}, which can be obtained by compressed gas as the driving force\textsuperscript{60}. In this PhD project, a sidestream\textsuperscript{®} nebuliser (MediAid, Sussex, UK), primed with 3.00 ml solution and a nebulizer-output of 0.24 ml-min\textsuperscript{-1} was used. The cough response to capsaicin depends on the diameter of the aerosol particles produced\textsuperscript{91}. Whilst large (5-10 μm) and small particles (less than 5 μm) of capsaicin have a similar deposition in the larynx, the small particles have better peripheral penetration and are more potent in inducing cough, suggesting more peripherally distributed capsaicin-sensitive nerves\textsuperscript{91}. In addition, lower inspiratory flow rate is associated with a greater cough stimulus, possible due to increased deposition in the intrapulmonary Airways\textsuperscript{81}. For these reasons, the dosimeter controlled jet nebulisers have become the preferred delivery systems for the inhalation delivery\textsuperscript{40}. In this PhD project such an aerosol provocation system Masterscope\textsuperscript{®} + software, APS version 5.02, Jaeger (Würzburg, Germany) was used. The inhalation from the dosimeter elicits a burst of compressed air that gives a nebulisation of a fixed duration. This facilitates accurate calibration of the output although some variation in the velocity of inhalation might still occur\textsuperscript{80}.

As stated in the introduction, the dose–response method can employ either single, vital-capacity breaths of incremental concentrations of capsaicin or tidal-breathing inhalations over a fixed period, usually 15–60 sec, but in former tidal-breathing studies regarding symptoms elicited by odorous chemicals, 6 minutes have usually been applied\textsuperscript{24,44,88}. In this PhD project the single-breath, dose–response method was used with capsaicin in doubling concentrations until, C5, the concentration that provoked at least five coughs was obtained.

3.2 Sex difference and influence of menstrual cycle on capsaicin cough reflex in healthy controls

Background

Multiple chemical sensitivity (MCS) occurs with a higher prevalence in women than in men\textsuperscript{10,12,13}. Many studies have demonstrated a significant sex difference in cough reflex sensitivity when applying the capsaicin single breath inhalation test (dosimeter method)\textsuperscript{77–79}. Further, a recent study found the cough sensitivity was enhanced in menopausal women\textsuperscript{77}. Before the MCS patients were challenged we wanted to investigate whether the threshold of the capsaicin-induced cough reflex was influenced by menstrual phase in healthy women and, further, to what extent sex influenced cough threshold and finally to evaluated reproducibility of bronchial challenge with capsaicin when applying the single breath inhalation test.

Material and Methods

Subjects

Study participants were 26 healthy Caucasians, 13 women and 13 men (Table 1), recruited by advertisement. Exclusion criteria were previous diagnoses of chronic respiratory diseases or current respiratory symptoms, eczema, smoking, stroke, use of medication; and for women use of oral contraception for at least four months before the study, pregnancy and breastfeeding. The women all had normal 28-31 day menstrual cycles. The study
was approved by a local ethics committee on human research (VN20060037), and all subjects gave full informed consent to participate in the study in accordance with the Helsinki II declaration.

**Design**

Two experimental sessions were conducted for every volunteer, in men on two separate days > 48 hours apart to avoid a possible effect of long-term tachyphylaxis, and in women, bronchial provocations were performed during menstrual phase (day 1 or 2 of menstrual bleeding) and luteal phase (day 1 or 2 from confirmed ovulation). We enrolled 7 of the 13 women during their menstrual phase (first visit) and continued the experimental procedure during their luteal phase (second visit). The remaining 6 women were enrolled reversely, minimizing any order effect. During the periovulatory period, women were requested to perform a daily urine luteal test to confirm ovulation. Each session was conducted at the same time of day. The volunteers completed a standardized questionnaire regarding diagnoses of respiratory and allergy diseases and underwent a lung function test. Before provocations with capsaicin, venous blood samples were collected for later analyses.

**Measurements of hormones**

An enzyme-linked immunoassay technique involving monoclonal antibody technology, with a minimum detection sensitivity level of 50mIU/L, was used to detect the luteinizing hormone surge in the urine at luteal (Uniplan® luteal test, Birkerød, Denmark)\(^9\). Luteal was later confirmed by blood samples analysed for estradiol and progesterone on E170 module for modular analytics (Roche, Germany).

**Lung function**

Spirometric parameters were recorded Masterscope® + software, APS version 5.02, Jaeger (Würzburg, Germany), and the best forced expiratory volume in first second (FEV\(_1\)) and forced vital capacity (FVC) from three technically satisfactory attempts were used. Data are expressed as percentage of predicted values\(^9\).

**Capsaicin cough challenge**

ERS Guidelines on the assessment of cough were followed as closely as possible\(^8\). Capsaicin (15.64 mg) was dissolved in Polysorbat 80 (2.05 mg) and then dissolved in 0.9% saline (to 100 ml) to provide a stock solution of 512x10\(^{-6}\) M.\(^9\). Fresh serial dilutions were prepared from stock solution each day of testing using saline diluent to produce doubling concentrations from 0.50 to 512 µM. Doses were administered from a compressed air driven sidestream® nebuliser (MedicAid, Sussex, UK) controlled by a Masterscope® + software, APS version 5.02, Jaeger (Würzburg, Germany). The nebulizer output was 0.24 ml·min\(^{-1}\) . The duration of aerosol delivery was programmed to 0.4 sec, thereby providing 0.002 ml per breath. The aerodynamic mass diameter of the particle was 3.20 ± 1.8 µm\(^9\). The subjects inhaled three single vital capacity breaths before capsaicin solution was ejected in the fourth inhalation with increasing doses every minute\(^9\). The cough response in the 10 sec. following each inhalation of capsaicin was recorded\(^8\). The concentration of capsaicin that cause five or more coughs (C5) was registered. A value of 1024 µM was assigned if five cough had not been obtained at 512 µM. Subjects were not informed that the end point of the study was the number of coughs induced.

**Statistics**

Analysis of variance and paired comparisons for continuous data when variance homogeneity and normal distributions could not be rejected (Age and FEV1) or Mann–Whitney rank sum test (BMI, estrogen and progesterone) due to variance in homogeneity. For discrete binary data Fisher’s exact test was used C5 data were shown to be normally distributed after a logarithmic transformation (logC5), Bland–Altman plot. Repeatability data were analyzed using students paired t-test. Odds ratios with 95% confidence limits were calculated. Significance levels throughout this study were P < 0.05. Statistical tests were performed using SPSS software, version 15.0 (SPSS, Chicago, USA).

**Results**

Basic and clinical characteristics are shown in Table 1. All 26 subjects completed the challenge test twice.
### Table 1. Characteristics of the subjects studied (mean ± SD or counts)

<table>
<thead>
<tr>
<th></th>
<th>Females (n=13)</th>
<th>Males (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>28.7 ± 7.5</td>
<td>24.8 ± 4.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 5</td>
<td>181 ± 7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.8 ± 4.5</td>
<td>75.1 ± 9.5</td>
<td>0.002*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 ± 2.0</td>
<td>22.8 ± 2.9</td>
<td>0.88</td>
</tr>
<tr>
<td>FEV1 before expected (% predicted)</td>
<td>110 ± 10</td>
<td>116 ± 13</td>
<td>0.20</td>
</tr>
<tr>
<td>FEV1 after expected (% predicted)</td>
<td>109 ± 10</td>
<td>114 ± 12</td>
<td>0.30</td>
</tr>
<tr>
<td>Serum estrogen luteal or males visit 1 (nmol/L)</td>
<td>0.80 ± 0.43</td>
<td>0.11 ± 0.02</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum estrogen menstrual or males visit 2 (nmol/L)</td>
<td>0.19 ± 0.16</td>
<td>0.12 ± 0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Serum progesterone luteal or males visit 1 (nmol/L)</td>
<td>3.65 ± 4.41</td>
<td>2.41 ± 0.82</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum progesterone menstrual or males visit 1 (nmol/L)</td>
<td>4.17 ± 5.27</td>
<td>2.46 ± 0.75</td>
<td>0.46</td>
</tr>
<tr>
<td>C5 luteal or males visit 1 (µmol/L)</td>
<td>620 ± 354</td>
<td>544 ± 421</td>
<td>0.28</td>
</tr>
<tr>
<td>C5 menstrual or males visit 2 (µmol/L)</td>
<td>600 ± 367</td>
<td>537 ± 427</td>
<td>0.33</td>
</tr>
<tr>
<td>Asthma (%)</td>
<td>0</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>Allergic rhinitis (%)</td>
<td>8</td>
<td>23</td>
<td>0.59</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>15</td>
<td>15</td>
<td>1.00</td>
</tr>
<tr>
<td>Food allergy (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Psoriasis (%)</td>
<td>0</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Depression (%)</td>
<td>15</td>
<td>0</td>
<td>0.48</td>
</tr>
<tr>
<td>Anxiety (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Epilepsy (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Age to FEV1 incl. was compared by two-sided t-test.
Estrogen and progesterone were compared by Mann-Whitney test due to variance in homogeneity.
C5 data were compared by two-sided t-test after log-transformation.
The binary data from asthma to epilepsy were compared by Fisher’s exact test.

The FEV₁ did not change (Table 1), nor was there any difference in FEV₁ between visit 1 and 2 for men or between menstrual and luteal phases for women (data not shown). There were no significant differences between the two groups with regard to anxiety, depression or atopy/allergic rhinitis (Table 1).

### Assessment of cough threshold

We found a wide variation in cough sensitivity in men (Figure 7) with median and 95% percentiles for C5, 512 µmol/L (160-1024 µmol/L) and 512 µmol/L (96-1024 µmol/L) for menstrual- and luteal phase, respectively. Threshold C5 did not differ significantly between sexes (Figure 7 and Table 1). Interclass correlation coefficients for C5 were 0.78 in men and 0.68 in women, between tests (Figure 8).

### Hormones

There was a significant difference between women (luteal phase) as compared with both women (menstrual phase) and men for estradiol level (Table 1). No differences were found between men and women at menstrual phase in estradiol level, and progesterone did not vary significantly at any time between sexes (Table 1).
Chapter 3

Discussion

To our knowledge this study is the first to investigate the influence of menstrual cycle, confirmed by blood sample, on capsaicin-induced cough. Our results support the findings of Fujimura et al.\textsuperscript{77}, who found no difference in bronchial sensitivity between luteal and menstrual phase, in young women (methods of provocation not described). The same investigators previously reported lower capsaicin threshold in both young and middle-aged women compared with age matched men, when applying the tidal method for 15 sec with nebulisation out put similar to the one used in this study but with a delivery time almost forty times longer than that of the present study.\textsuperscript{77} Other studies have likewise found the threshold to be lower in women using the single breath inhalation test, but with a nebuliser output four times higher than that of the present study and/or a longer period of aerosol delivery.\textsuperscript{76,78} Only one study did not find any significant difference between the sexes using almost the same amount of inhaled capsaicin in ml per breath, as in the present study.\textsuperscript{87}

With the lack of a “Gold standard test” for determining cough threshold to capsaicin, the methods chosen vary both in exposure time and amount delivered. In general, studies using longer exposures and higher amounts report sex. One proposed explanation is that women have a smaller airway surface area leading to a greater deposition of capsaicin per area in each inhalation.\textsuperscript{78}

Conclusion

Cough threshold did not vary between sexes using single breath inhalation test. Further, the menstrual cycle did not influence cough sensitivity in this group of women. According to the method of Bland and Altman, good agreement was found when comparing subsequent challenge tests in both sexes.

3.3 The capsaicin cough reflex in patients with symptoms elicited by odorous chemicals

Background

Rhinitis and airway reactivity are common symptoms reported by MCS patients when exposed to odorous chemicals and scents.\textsuperscript{1,97} and similar symptoms have been described in asthmatic and eczema patients.\textsuperscript{9,10,16,17} These symptoms have been related to an altered function of the respiratory mucosa, suggesting that chemicals stimulate a non-specific pathway involving C-fibre neurons.\textsuperscript{21}
Table 2. Demographic and clinical characteristics of controls, eczema patients with airway symptoms elicited by odorous chemicals (EC) and patients with multiple Chemical Sensitivity (MCS)

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 29) (%)*</th>
<th>EC cases (N = 15) (%)*</th>
<th>p-value</th>
<th>MCS cases (N = 16) (%)*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>49 ± 13</td>
<td>47 ± 11</td>
<td>0.68</td>
<td>51 ± 9</td>
<td>0.47</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24 ± 2</td>
<td>25 ± 5</td>
<td>0.77</td>
<td>23 ± 4</td>
<td>0.23</td>
</tr>
<tr>
<td>FEV1(% of predicted)</td>
<td>115 ± 11</td>
<td>101 ± 16</td>
<td>0.001</td>
<td>113 ± 19</td>
<td>0.43</td>
</tr>
<tr>
<td>Cigarette smoking (current)</td>
<td>7</td>
<td>27</td>
<td>0.16</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Upper airway symptoms§</td>
<td>0**</td>
<td>93</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower airway symptoms</td>
<td>0**</td>
<td>67</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS symptoms†</td>
<td>0**</td>
<td>80</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>14</td>
<td>20</td>
<td>0.68</td>
<td>25</td>
<td>0.43</td>
</tr>
<tr>
<td>Asthma</td>
<td>3</td>
<td>27</td>
<td>0.04</td>
<td>38</td>
<td>0.005</td>
</tr>
<tr>
<td>Skin prick test (at least one positive)</td>
<td>45</td>
<td>27</td>
<td>0.33</td>
<td>50</td>
<td>0.74#</td>
</tr>
<tr>
<td>Hand eczema</td>
<td>10</td>
<td>40</td>
<td>0.04</td>
<td>31</td>
<td>0.11</td>
</tr>
<tr>
<td>Perfume contact allergy (patch test)</td>
<td>0</td>
<td>33</td>
<td>0.003</td>
<td>19</td>
<td>0.04</td>
</tr>
<tr>
<td>Food allergy</td>
<td>10</td>
<td>13</td>
<td>1.0</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>Food intolerance</td>
<td>7</td>
<td>7</td>
<td>1.0</td>
<td>38</td>
<td>0.02</td>
</tr>
<tr>
<td>Drug treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2 agonists</td>
<td>0</td>
<td>20</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
<td>0</td>
<td>27</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucotriene antagonists</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-morbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>0</td>
<td>27</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibromyalgia, chronic pain, chronic fatigue</td>
<td>0</td>
<td>7</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum estradiol (nmol/L)</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum progesterone (nmol/L)</td>
<td>3.1 ± 5.2</td>
<td>2.3 ± 3.6</td>
<td>4.5 ± 9.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance and paired comparisons for continuous data when variance homogeneity and normal distributions could not be rejected (Age and FEV1) or Mann-Whitney rank sum test (BMI).

For discrete binary data Fisher’s exact test (2-sided) unless otherwise stated.

P-values for controls versus EC and controls versus MCS
* Prevalence (% of total) unless otherwise stated.
* Pearson’s Chi square
** Selection criteria for controls
§ Rhinorrhea, eye irritation, sinusitis or irritation in mouth or throat elicited by odorous chemicals
¶ Cough and/or breathing problems elicited by odorous chemicals
† Dizziness, exhaustion, concentration problems or fatigue elicited by odorous chemicals
Tidal challenge tests performed in recent years confirm that patients with lower airway symptoms elicited by odorous chemicals have an increased sensitivity to capsaicin as compared with healthy controls. The aim of this part of the study was to test whether the capsaicin-induced cough reflex was enhanced when applying the single breath inhalation method in two groups of patients with symptoms related to odorous chemicals and to investigate to what extent the reporting of lower airway symptoms related to odorous chemicals influenced the cough reflex.

**Material and Methods**

Before and after capsaicin cough challenge test 18 different symptoms were rated. The challenge test followed the procedure described in section 3.2.

**Statistics**

Statistics was performed as describe in section 3.2. Further, a step-wise logistic regression analysis starting with a set of candidate explanatory variables and successive elimination of non-significant explanatory variables, was performed between patients with upper and lower airway symptoms to odorous chemicals, respectively.

**Table 3.** Relationship between sensitivity to capsaicin (logC5) and other parameters, examined in stepwise multiple linear regression analysis (healthy volunteers excluded), N= 31.

<table>
<thead>
<tr>
<th>Parameters in models</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
<th>Step 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower airway symptoms</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.006</td>
<td>0.007</td>
<td>0.009</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>0.13</td>
<td>0.15</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>* Applied for pension</td>
<td>0.36</td>
<td>0.35</td>
<td>0.31</td>
<td>0.28</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Retired</td>
<td>0.66</td>
<td>0.65</td>
<td>0.69</td>
<td>0.24</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>0.56</td>
<td>0.55</td>
<td>0.53</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Groups</td>
<td>0.72</td>
<td>0.71</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.81</td>
<td>0.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For “Step 1”, all candidate explanatory variables are included, and the numbers in the column represent the P-values for omission of the variable in question from the multiple regression model. Thus at “Step 1”, “current smoker” is the least significant (highest P-value) and is consequently excluded at “Step 2”. This process is repeated until all remaining variables have P-values > 0.05.

* Variable with 3 categories: working (reference), applying for pension and retired.

# Groups (eczema patients with airway symptoms elicited by odorous chemicals (EC) and patients with Multiple Chemical Sensitivity (MCS))

**Results**

Basic and clinical characteristics are shown in Table 2. The co-existence of asthma and perfume contact allergy was significantly increased in both groups of patients compared with controls. However, 45% of controls were SPT-positive or suffered from allergy/allergy-like conditions (10%, 14%, and 10% had hand eczema, allergic rhinitis, and food allergy, respectively).

**Assessment of cough threshold**

Increased cough sensitivity was found in patients in general, although this difference was significant only for EC patients. Low C5 was associated with presence of lower airway symptoms, independent of patient group, being pre or postmenopausal and co-existence asthma (Table 3). The vast majority of MCS patients did not recover from the provocation as quickly as EC patients or controls.

**Discussion**

The results showed that cough reflex was enhanced in both groups of patients compared with controls – despite the fact that a proportion of controls were sensitised to allergens, which would tend to diminish difference in cough threshold between patients and controls. Of importance, none of the controls had...
any airway or CNS symptoms elicited by odorous chemicals (Table 2).
The tidal breathing and the dosimeter methods have been found equally valuable in assessing cough receptor sensitivity to capsaicin, but the amount of the tussigenic agent delivered with each inhalation can be affected to a larger extent by factors such as an individual's breathing effort and tidal volume when applying the tidal breathing method. It has been argued that the tidal breathing method should be favorable, because the concentration causing five coughs or more is significantly lower than that of the dosimeter method. This is probably true, but an important point is that the dose inhaled in total is much less in the single inhalation compared with the tidal breathing method.
Patients reported more symptoms in general when challenge tested in addition to cough as compared with controls. This is in accordance with provocation studies using the tidal breathing methods.
Further, symptoms ceased more slowly in the MCS group as compared with the EC group, days versus hours, respectively. Although, a subpopulation of patients across groups share some common underlying mechanisms regarding enhanced cough reflex with presence of lower airway symptoms, other mechanisms should be considered as well. Especially, the prolonged CNS symptom in MCS patients when provoked in the airways and the involvement of more than one organ system needs further investigations. It is possible that the differences between groups would have been even more significant if more severely ill patients with MCS had participated.
The MCS patients were recruited by letter, in which they were informed about the study. About half of the group accepted to participate. The part that chose not to participate was very heterogeneous, some were taking medicine mentioned as exclusion criteria, two had experienced a stroke recently, one was epileptic, and one was pregnant. Further, some did not wish to participate because they felt too ill. The participants in the MCS group were considered to be mildly to moderately affected.

Conclusion
Cough reflex was enhanced in both groups of patients compared with controls but the difference was significant only in EC patients. Significantly lower CS was seen in patients with lower airway symptoms.
4.1 Pain assessments

“An ideal pain stimulus evokes a distinct pain sensation with minimal tissue damage, represents a relationship between stimulus intensity and pain intensity, gives reliable inter- and intra-session reproducibility, and excites nociceptors exclusively”, Gracely 200699. Thus, in experimental pain research two major areas are: standardised activation of the nociceptive system and measurement100. As pain is a multidimensional experience, for measurement, combined assessments are generally needed to encompass the sensation101. The induction methods can be phasic (short, lasting for milliseconds) or tonic (long, lasting for minutes)100. Furthermore, the phasic stimuli can be delivered as a single stimulus or as a train of stimuli. If pain pulses are delivered at a rate of 1-3/sec, the pain will integrate, become more painful and summate - known as temporal summation (TS)57,100. Experimental models of pain are important aids in the study of pain mechanisms, one such model is the intradermal injection of capsaicin53,64,101,102. Physiologically, this pain sensation is manifested as a set of measurable changes that fall into four categories29,64,73,103: 1) spontaneous pain, the burning/aching sensation at the site of administration/injury, that is short-lived (less than 30 min) and maximal at the time of injection. 2) allodynia, increased response to non-painful stimuli, e.g. a brush, is divided in to a primary zone within the site of injury and a secondary zone in the undamaged skin that surrounds the injury, lasts for less than 20 minutes. 3) hyperalgesia, increased response to a stimulus, that is normally painful, e.g., punctuate mechanical stimuli, is likewise divided in to a primary and secondary zone lasting 6 to 24 hours. 4) an area of flare induced by neurogenic inflammation, that extends beyond the site of injection and lasts up to 90 minutes. These assessments are dose-dependent49,102,104 similar to the capsaicin challenge test used for bronchial provocations, described in section 3.1. Sex differences in pain perception have been observed in several studies99,105, but the influence of menstrual cycle and ethnicity are disputed35,92,105. Further, a recent study showed capsaicin-evoked sensory and vasomotor manifestations to be different between site innervated by the spinal cord and those innervated by the trigeminal nerve92. Of importance, it was found that the area of secondary hyperalgesia as an indicator of central sensitization were significantly greater in the forearm than the forehead92.

There are no “gold standards” regarding the test dose of capsaicin used to induce pain. In studies involving patients39,41 there is a tendency to use lower doses of capsaicin than in studies of physiological pain response in healthy persons64,102,104. Further, intradermal rather than topical applications are preferential102,106, and the importance of the configuration of the nylon filament (von Frey) is emphasised107. Finally, age differences in the nociceptive pathways are seemingly gaining importance, as some studies have found impairment of the Aδ fibres in the older age group108.

4.2 Sex difference and influence of menstrual cycle on capsaicin-induced pain in healthy controls

Background

In several studies extensive two-way overlaps among CFS, FMS and MCS has been reported109. Further, the majority of women meet criteria for all three disorders109. This pattern is reversed in men; where only one third meet the criteria for all three disorders109. In FMS there is growing evidence that the biological part of the syndrome is a longstanding or permanent change in the function of the nociceptive system causing central sensitization53. Further, there is evidence suggesting that women experience greater pain intensity, lower thresholds, and lower tolerance to experimentally induced pain in healthy controls as well as in patients studies35,92,109. However, the findings on sex differences in human experimental pain research are inconsistent and are reported in only two-thirds of the studies in a recent review110. The lack of consistency in sex differences has been explained by the fact, that women’s responses to experimentally evoked pain may vary across the menstrual cycle, as hormone levels affect pain sensitivity35,92,109. Women have been tested in different phases of their menstrual cycle, but documentation of exact hormone concentrations is often lacking35. The
finding of central sensitization can be provoked to a larger extent in the forearm, than in the forehead when the same dose is applied has not been confirmed in other studies\(^9\). This result could be of importance when developing future diagnostic assessments in patient studies. We investigated to what extent capsaicin induced pain was influenced by menstrual phase in healthy women, before MCS patients were challenged. Both sexes were enrolled to determine if results were equal and would allow both sexes to be included and analysed as one group in the next study. Further, site-specific responses were evaluated.

Material and Methods

Subjects

Described in section 3.2 and Table 1.

Design

Two experimental sessions were conducted for every participant. Capsaicin induced pain by intradermal injections was randomly selected to start either before or after bronchial inhalation test, described in section 3.2. To achieve thermal stability of skin temperature, participants rested for 30 min after arrival, because exercise strongly influences cutaneous blood flow and colour\(^11\). During pain assessments the participant rested in a supine position in a quiet room (temperature: 21-22ºC).

Measurements of hormones

Described in section 3.2.

Intradermal capsaicin injection

Hundred microlitres capsaicin (100 \(\mu\)g/0.1 ml (= 3.3 mM) polyoxyethylene sorbitan monooleate, hospital pharmacy, Aalborg Hospital, Denmark) was injected intradermally using a 27-gauge disposable needle. The injection was made at an angle of approximately 15 degrees into both the volar surface of the forearm (one third distal to cubital fossa) and the forehead (2 cm above the eyebrow and 3 cm from the face vertical midline)\(^9\). Before the injections baseline assessment with von Frey hair was performed.

Assessment of pain

Capsaicin-induced pain intensity was recorded continuously every 2 sec on a 10 cm electronic visual analogue scale (VAS), with anchor points 0 = no pain and 10 = the worst imaginable pain. Data were collected in a computer for later analysis. Onset of pain, duration (time in minutes), and peak pain intensity, \(VAS_{\text{max}}\), were determined and area under VAS–time curve, \(VAS_{\text{AUC}}\), was measured by a skin prick test (SPT) area scanner, Handy Genius scanner® (Taiwan, KY-892030)\(^11\).

Assessment of pain distribution

The subjects were presented with face and forearm charts and asked to draw their pain distribution after capsaicin injections in order to obtain pain distribution patterns. Area determination was carried out by the SPT area scanner as above.

Assessment of flare

To assess the area of flare, transparent paper was placed on the skin, and the area with visual hyperaemia was outlined 5 min after the injection. As above the SPT area scanner carried out area determination.

Measurement of secondary pinprick hyperalgesia (SPPH)

A handheld von Frey nylon monofilament (No. 17, bending force 60.0 g, Somedic, Sweden) with a blunt tip was used to map the area of SPPH\(^9\), before and approximately 10 min after the injection. During this measurement the subject’s eyes were closed and the nylon filament was applied to the skin 6 cm away from the injection site and reapplied every 2 sec in 1 cm steps towards the injection site. The borders of SPPH were determined along 8 spokes radiating at 45-degree angle (to reduce inter- and intra-individual variability in study subjects\(^10,13\)); When sensation changed from a pricking sensation to a distinct pain, marks where made and transferred to a specially designed paper. As above the SPT area scanner did calculations of the areas.
Chapter 4

Figure 9 Flow charge

Statistical analysis

Comparison of forehead and forearm were made with Wilcoxon’s signed rank test (two-sided) for paired data. Comparisons between women luteal or menstrual with mean of visit 1 and 2 in men were made with Mann-Whitney’s rank test (two-sided) for unpaired data.

Log was expressed as geometric mean ± 1 standard deviation, all other results were expressed as mean and SD. Significance level throughout this study was P < 0.05. Statistical test were performed using SPSS software, version 15.0 (SPSS, Chicago, USA).

Results

Intradermal capsaicin injection

Capsaicin evoked pain and flare in both forehead and forearm (Table 4). Two women reported minor headache symptoms remaining for the rest of the day, and the majority of participants reported watery eyes and sensitivity to light, lasting 2 minutes after the injection in the forehead.

Pain intensity and duration

The pain was maximal at the time of injection and declined rapidly independently of sex. The \( VAS_{AUC} \) was correlated within persons (forehead as compared with forearm), independently of sex, R-square visit 1 = 0.72 (Figure 10 A). Pain intensity and duration was not significant within men (visit 1 compared with visit 2) and women (menstrual phase compared with luteal phase) when forehead or forearm was compared, respectively (data not shown). However, pain duration time intensity, \( VAS_{AUC} \) was significantly greater in the forehead as compared with the forearm in women in the menstrual phase, P<0.05 (Table 4).

Significant differences were found between sexes in \( VAS_{AUC} \) and \( VAS_{max} \) for both injections (forearm compared with forehead) at both sessions (1 + 2) (Figure 10 B and Table 4).

Pain area

The pain area did not change across menstrual cycle but women recorded larger pain areas than did men at both sessions (1+2) and for both injections (forehead and forearm) (P<0.05, Table 4). In men significant differences was found between pain distribution in the forearm and forehead at the first but not the second visit.

Secondary pinprick hyperalgesia

No significant differences were found in area of SPPH between sexes or between menstrual phases (Table 4). However, the areas on the forearms were significantly larger than on the forehead in both sexes at both sessions.

Visible flare

The skin turned bright red within five minutes of the injection and faded to the colour of the surrounding skin within 15 minutes. There was no difference between menstrual phases (Table 4). Women, in both phases, showed larger flare area in the forehead compared with men, although only significant at luteal phase (P<0.05, Table 4). For forearms, the same tendency was seen, though only significant at menstrual phase (P<0.05, Table 4). In both sex significant difference between injection sites (forehead compared with forearm) was found at both visits in men, and both phases in women, respectively P<0.01 (data not shown).
Table 4. Comparison of data from 13 male subjects (mean of visit 1 and 2) with data from 13 female subjects in luteal and menstrual phase

<table>
<thead>
<tr>
<th></th>
<th>Women Luteal (n = 13)</th>
<th>Women Menstrual (n = 13)</th>
<th>Men Mean of visit 1 and 2 (n = 13)</th>
<th>P Luteal (♂ vs. ♀)</th>
<th>P Menstrual (♂ vs. ♀)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forehead</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (max) (cm)</td>
<td>8.3 ± 1.8</td>
<td>8.4 ± 1.7</td>
<td>5.3 ± 1.9</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VAS (AUC) (cm x min)</td>
<td>15.5 ± 12.9</td>
<td>16.7 ± 12.7</td>
<td>5.4 ± 4.1</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>29.3 ± 12.5</td>
<td>25.6 ± 14.8</td>
<td>15.3 ± 9.4</td>
<td>0.002</td>
<td>0.07</td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>39.3 ± 28.0</td>
<td>28.8 ± 20.6</td>
<td>10.2 ± 6.1</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>Sec. hyp. (cm²)</td>
<td>5.1 ± 5.0</td>
<td>8.6 ± 13.2</td>
<td>3.2 ± 4.8</td>
<td>0.10</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Forearm**

|                      |                       |                          |                                   |                   |                      |
| VAS (max) (cm)       | 8.4 ± 2.1             | 8.7 ± 1.8                | 5.0 ± 2.4                         | 0.002             | <0.001               |
| VAS (AUC) (cm x min) | 12.5 ± 9.7            | 11.8 ± 9.6               | 4.6 ± 2.7                         | 0.001             | 0.02                 |
| Flare area (cm²)     | 41.1 ± 21.5           | 50.4 ± 19.4              | 34.3 ± 13.8                       | 0.40              | 0.02                 |
| Distribution (cm²)   | 43.6 ± 27.0           | 45.8 ± 31.6              | 20.0 ± 12.4                       | 0.02              | 0.03                 |
| Sec. hyp. (cm²)      | 16.1 ± 10.1           | 21.3 ± 13.7              | 14.8 ± 10.7                       | 0.68              | 0.23                 |

Mean ± Standard deviation (SD), P values between sexes are shown at visit 1 and visit 2.

**Hormones**

There was a significant difference between women (luteal phase) mean 0.795 nmol/L, SD± 0.433 nmol/L, as compared with both women (menstrual phase) 0.191 nmol/L; SD ± 0.159 nmol/L and men 0.113 nmol/L, SD ± 0.020 nmol/L, P<0.001 for estradiol level.

No differences were found between men and women at menstrual phase (P=0.26) in estradiol level. Progesterone did not vary significantly at any time between sexes (Table 1).

**Figure 10**

Intraindividual correlation of area under VAS-time curve following intradermal injection of 100 µg capsaicin into the forehead and forearm (N=26).

**Figure 11**

Area under VAS-time curve following intradermal injection of 100 µg capsaicin into the forehead in women during menstrual and luteal phase and in men at visit 1 and 2. # indicates significantly higher responses in women than men.
Discussion

It has been shown experimentally that women rate identical pain stimuli more painful than men, but it is not evident if this difference is due to hormones\textsuperscript{95,101}. In the present study we found no correlation between oestrogen and progesterone level and pain sensitivity at different phases of the menstrual cycle in regularly menstruating women although SPPH tended to be larger at menstrual phase compared with luteal phase, at both sites (forehead and forearm). These findings are in contrast to Gazerani et al\textsuperscript{92,113} who found the vast majority of pain parameters to be affected by the menstrual cycle in the forehead.\textsuperscript{92} However, in accordance with Gazerani et al\textsuperscript{92,113} we found a significant sex differences in both pain intensity and distribution at both sites. Further, both area of SPPH and flare area was found to be significantly larger in the forearm as compared with the forehead, independently of gender.\textsuperscript{113} Additionally, the area of flare was larger in women than men in general\textsuperscript{92}, and the size of flare area were larger than area SPPH, at both sites\textsuperscript{92,113}. In other studies, it has been reported that the size of visual flare is smaller than the size of SPPH\textsuperscript{33,65}, when visual inspected. In many studies, the group of healthy volunteers are quite small, as in ours, and hence quite fragile to individual variations\textsuperscript{77}. Also, the ratio between men and women and age needs to be addressed, as this influence size of flare area\textsuperscript{71,113} and the discussion in this regard is often sparse\textsuperscript{33,65}. Further, in the study by Sumikura et al\textsuperscript{66} flare area and SPPH was measured along a line of 14 cm times 1 cm. However, the shape of flare area is irregular in size\textsuperscript{71}, which means that maximum length might not have been present within the chosen line of measurement. Moreover, the full extent of the flare area develops rapidly after capsaicin injection and begins to shrink within 5–10 min\textsuperscript{73}. It is possible that previous authors measured visually was mainly long-lasting central flare close to the injection site, and we measured the extensive vascular flush that appears very early after capsaicin injection\textsuperscript{73}. Finally, in some studies up to three injections were administrated randomly within a range from 0.01–100 µg\textsuperscript{44} in each forearm, and these injections might have influence each other, although stated otherwise\textsuperscript{44} as it has recently been demonstrated that contralateral hyperalgesia is induced by unilateral stimulation\textsuperscript{114}. Thus, more studies are needed to fully address the relationship between flare and SPPH. However, as an indication of the quality of our test methods, we found good agreement between sessions within subjects for both area of SPPH and flare. Further, a strong correlation was found in all subjects between pain intensity in the forehead compared with that in the forearm.

However, the study contains some pitfalls, as we recruited healthy volunteers by advertisement. This procedure might lead to selection bias. Firstly, since the study involved severe pain (comparable to a wasp sting for up to two minutes) it was somewhat difficult to recruit female participants, and it is our impression that the women who chose to participate were in better physical condition than average. Secondly, women were not included if they were taking oral contraceptives, which showed to be a major selection criteria. However, if these inclusion criteria did influence the study, we would expect that they would minimize rather than increase the significant differences found in this study between sexes. These significant differences might both be due to structural and biological factors (hormones and endogenous pain inhabitation system\textsuperscript{113}), social cultural factors (e.g. age, gender roles\textsuperscript{113}) and psychological factors (e.g. anxiety, depression, cognitive, and behavioural factors\textsuperscript{113}).

Conclusion

Pain intensity, duration and distribution were significantly greater in women than in men and were independent of menstrual cycle. Further, a strong association between pain intensity in the forehead and forearm within subjects was found. Finally, the different capsaicin-evoked sensory and vasomotor manifestations were confirmed, with larger area of SPPH and flare in the forearm, which make it suitable for test site for these parameters in future clinical studies. This difference is likely due to variation in innervations and neurovascular activity between anatomic sites.

4.3 Increased capsaicin-induced secondary hyperalgesia in patients with MCS (Paper II)

Background

Sensitization is the opposite of habituation, which means decrease in response to repeated exposures\textsuperscript{115}.  

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It has been hypothesized that response amplification in MCS may partly be caused by transcriptional and translational changes in the spinal cord and brain (e.g. thalamus, somatosensory -, insular -, and ACC) resulting in CS \[^{42}\], and partly, that sensitization in these patients involve the limbic circuit\[^{14,115}\]. In a recent study, sensitization effects were estimated in the limbic circuit as perceived intensity, reaction time, and event related potentials when exposed to CO\(_2\) and amyl acetate. \[^{115}\] It was shown, that patients with self-reported chemical sensitivity did not habituate to the same extent as controls and had difficulties ignoring the chemical exposures (attention bias) \[^{115}\]. However, contribution of the central nociceptive pathways, as shown in chronic pain conditions such as FM, CFS and CP, where CS plays a role\[^{18}\] has not yet been investigated in patients with MCS. An important test of the central pain amplification relies on detection TS\[^{116}\]. This technique reveals sensitivity to input from unmyelinated (C) afferents and the status of the NMDA receptor system that are implicated in a variety of chronic pain conditions\[^{116}\]. Further, SPPH as shown enlarged in many clinical studies\[^{39,40,67}\], with somewhat overlapping conditions to MCS\[^{18}\] needs to be investigated. The aim of this study was thus, to assess pain reactivity, SPPH, TS, and vasomotor responses in two groups of patients with symptoms related to odorous chemicals as compared with healthy female controls.

**Material and Methods**

**Subjects**

Subjects are described in section 3.3 and basal characteristics are shown in Table 2 and appendix 2

**Design**

All participants were asked about the presence of depression, FM, CP and CFS. The two patient groups also responded to a questionnaire on duration, specific initiating chemical exposure event and symptoms related to chemical exposure (appendix 2).

**Intradermal injection of capsaicin**

The administration of injections was as follows: Every participant was given one injection with 100 \(\mu\)l isotonic saline, at the flexor site of the right forearm, 1/6 distal to the cubital fossa. After measurements of pain assessments and secondary hyperalgesia 100 \(\mu\)l capsaicin (3.3 \(\mu\)M) was injected 1/3 distal to the cubital fossa in the same forearm. In the left arm, 100 \(\mu\)l capsaicin (33 \(\mu\)M) was injected similarly. In order to instruct individuals in outcome measurements for pinprick hyperalgesia individuals were tested for pinprick sensitivity with a von Frey hair prior to injections. Capsaicin-induced SPPH and TS were quantified as markers of abnormal central nociceptive processing and visual flare was used as a marker for peripheral response (neurogenic inflammation).
Measurement of Temporal summation (TS)

Repetitatively pinprick stimulation with a handheld von Frey hair (No. 17, bending force 60.0 g, Somedic, Sweden) was carried out in capsaicin (33 μM) sensitised secondary hyperalgesic area 3 cm proximal to the injection site. Skin was stimulated repetitively within an area of 1 cm² (these spots are within the receptive field size of primary nociceptive afferents5) at 2.0 Hz (2/s). The duration of stimulation was 60 s. Evoked pain during and after stimulation was scored continuously on an electronic VAS (0 = no pain, 10 = maximal pain) and collected on a computer. Maximum evoked pain, and duration of TS were calculated. Further, the slope coefficient within the first 4 second (8 repetitave pinprick stimuli) was calculated as a stimulus response function.

Other measurements

Pain intensity, distribution, visual flare, pinprick hyperalgesia, and questionnaire-based measurement of subjective pain experience, McGill score17,18 were done as described section 4.1 and in paper II, see Figure 12.

Results

The co-morbidity of depression was equivalent in both groups of patients with 27% and 25 % for EC and MCS patients respectively. However, the percentage of co-morbidity of FM, CP and CFS was extensively higher in the MCS group, with 25% of the group compared with 7 % of EC group. Individual data for duration and intensity of symptoms for EC and MCS patients are listed in Table 2 and appendix 2.

Intradermal capsaicin injection, Pain intensity and duration

Capsaicin injections evoked higher pain intensity and duration (VAS_{AUC}) in all groups for the highest concentration (33μM). However, Pain intensity and duration (VAS_{AUC}) was only significant in MCS patients compared with controls at the highest concentration of capsaicin (p<0.001, Table 6 and Table 2, paper II). Isotone saline evoked a shortly mild pain sensation lasting less than two seconds for all groups with an estimated VAS_{AUC} between 0.2 ± 0.1 cm x min for all three groups.

Table 6. Intensity and duration of pain, area of flare and hyperalgesia of controls, eczema patients with symptoms induced by odorous chemicals (EC) and patients with multiple Chemical Sensitivity MCS.

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=29)</th>
<th>EC (N=15)</th>
<th>MCS (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capsaicin 3.3 μM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (max) (cm)</td>
<td>4.3 ± 3.0</td>
<td>4.5 ± 2.5</td>
<td>5.0 ± 2.9</td>
</tr>
<tr>
<td>VAS (AUC) (cmxmin)</td>
<td>0.7 ± 0.6</td>
<td>0.7 ± 0.5</td>
<td>1.0 ± 0.8</td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>4.9 ± 4.5</td>
<td>6.2 ± 3.7</td>
<td>5.3 ± 3.2</td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>5.5 ± 11.3</td>
<td>3.9 ± 4.0</td>
<td>4.8 ± 6.7</td>
</tr>
<tr>
<td>Sec. Hyp.(cm²)</td>
<td>4.3 ± 4.8</td>
<td>11.2 ± 9.8*</td>
<td>10.5 ± 8.2*</td>
</tr>
<tr>
<td>Sec. Hyp. Area - Flare area (cm²)</td>
<td>-0.7 ± 7.3</td>
<td>4.9 ± 11.3</td>
<td>5.2 ± 9.3*</td>
</tr>
<tr>
<td><strong>Capsaicin 33 μM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (max) (cm)</td>
<td>7.2 ± 2.8</td>
<td>6.0 ± 3.7</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>VAS (AUC) (cmxmin)</td>
<td>3.2 ± 2.3</td>
<td>4.0 ± 3.6</td>
<td>6.4 ± 3.8*</td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>19.0 ± 7.7</td>
<td>19.3 ± 7.3</td>
<td>17.3 ± 8.4</td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>18.8 ± 21.4</td>
<td>28.2 ± 31.4</td>
<td>19.5 ± 17.8</td>
</tr>
<tr>
<td>Sec. Hyp.(cm²)</td>
<td>17.7 ± 13.3</td>
<td>27.8 ± 16.2*</td>
<td>27.1 ± 9.3*</td>
</tr>
<tr>
<td>Sec. Hyp. Area - Flare area (cm²)</td>
<td>-1.3 ± 14.5</td>
<td>8.5 ± 18.9</td>
<td>9.8 ± 10.3*</td>
</tr>
<tr>
<td>Temporal summation (cmxmin)</td>
<td>0.6 ± 0.5</td>
<td>1.0 ± 0.9º</td>
<td>1.7 ± 1.1º</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD), *P < 0.05 for EC and MCS compared to controls, º P<0.05 for EC compared to MCS.
Secondary pinprick hyperalgesia

Injections with saline did not evoke SPPH in any of the three groups (controls, EC and MCS). The area of SPPH was increased in both EC and MCS groups (both concentrations of capsaicin) compared with controls (P<0.05, Table 6). The relationship between size of SPPH and likelihood of belonging to the MCS group was optimal at an area of SPPH above 10 cm² with a sensitivity of 50% and a specificity of 90% for capsaicin 3.3 µM (ROC analysis) (Figure 13).

Visible flare

No difference was found in visual flare areas as such between groups. However, a considerably larger difference between visual flare area and area of SPPH was found in all patients (EC + MCS) in comparison with controls, although significant for only MCS patients at both concentrations (Table 6).

Measurement of Temporal summation (TS)

Temporal summation was increased in both groups of patients, but VAS_{AUC} was significantly increased only for MCS patients compared with controls. In patients with symptoms elicited by odorous chemicals (EC + MCS) and co-morbidity of FM, pain or fatigue, pain continued after end of stimulation and was enhanced compared with patients without co-morbidity and to controls (P<0.01, Figure 14). The slope of the VAS curve to repetitiv stimulation within the first 4 seconds (8 stimuli) was further analysed as a stimulus response function, Figure 14 B, to determine the gain in the system. With median slope coefficient as 2.05 ± 2.15, 2.49 ± 1.85, 3.92 ± 2.62, and 4.45 ± 3.35 for controls, EC, MCS and MCS# respectively. This difference was significant only for MCS patients when pair-wise Mann-Whitney rank sum test was employed (P<.05).
Discussion

The results seem to indicate that alteration in response of the nociceptive pathways contributes to CSS in patients with MCS and EC. Further, that patient with MCS reacts with a stronger stimulus response to TS than EC and controls. Hence, the gain in pain system was stronger in MCS patients. These interpretations are in line with the response amplifications found in the limbic circuit, when patients with self-reported chemical sensitivity were exposed to CO$_2$.

The capsaicin concentrations were lower than reported in previous experimental pain studies of healthy subjects. Although high doses have been suggested for pharmacological profiling of novel anti-nociceptive agents, low doses might be preferable when assessing neurogenic responses in patients with chronic diseases. Our findings could support this notion, as we observed the most profound difference between patients (MCS and EC) and controls when comparing areas of SPPH after injections with the lowest concentration of capsaicin. Further, area of SPPH might be a valuable diagnostic tool in future, as the post-hoc analysis revealed a reasonably high sensitivity and a high specificity across patients groups (MCS and EC). In addition, the German Research Network in Neuropathic pain recently demonstrated that quantitative sensory testing (e.g. thermal, vibration, and pressure pain threshold) shows significant age dependency (> 40 years of age with P < 0.01) except for pinprick-evoked measurements (e.g. SPPH and wind-up). Absence of SPPH when applying placebo (isotone saline injection) as well as the reproducibility of SPPH, assessed in men at two occasions, that showed largely comparable results, also support the difference found between patients and controls to be noteworthy. The finding of increased TS/wind-up further emphasized evidence for abnormal central nociceptive processing in these patients. Wind-up presented in this study was inspired by the method applied in patients with neuropathic pain by Gottrup et al. Here it was demonstrated, that TS and aftersensation evoked in sensitized skin in nerve injury patients could be mimicked in capsaicin sensitized skin in control subjects. It was shown that there was no difference of maximum pain score, time to onset, and duration of aftersensation between groups.
Finally, evoked pain score was frequency dependent\(^{121}\). In contrast, another study demonstrated that patients with FM have both prolonged after sensation and abnormally increased TS when heat evoked as compared with healthy controls\(^{58}\). Furthermore, FM patients showed SH to the first thermal stimulus, which could only be demonstrated after 10 identical heat pulses in controls\(^{58}\). Both studies used VAS\(_{\text{auc}}\) as measurement-outcome for response stimuli function. In contrast, the German Research Network in Neuropathic pain suggested wind-up ratio as outcome measurements, as it has been demonstrated that injection of capsaicin induces a leftward shift in response function, corresponding to a decrease in pain threshold and increase of painfulness of suprathreshold stimuli by a factor 3-4\(^{57}\). However, the gain in wind-up ratio remained unchanged in the SH area. We found that both gain in pain as expressed by slope coefficient and VAS\(_{\text{AUC}}\) to be significant in MCS patients. Further that patients with co-morbidity did not ceased in pain – after end stimulation, an indication of prolonged after sensation in accordance with previous findings in patients with FM\(^{58}\). This last information would however, be overlooked if only ratio and not duration was taken into consideration. Although it has been suggested that neurogenic inflammation is part of the pathological mechanism triggering enhanced responsiveness of neurons within the dorsal horn followed by enhanced SPPH\(^{101, 119}\), flare area in the forearm in the present study was equivalent in size when comparing patients (EC and MCS) with controls. Whereas the size of SPPH and visual flare was equivalent in healthy controls, patients showed a considerably larger area of SPPH in comparison with visual flare. In the first study, the area of visual flare was significantly larger than SPPH, as already discussed in the previous section (4.2). This divergence in healthy controls could partly be due to capsaicin concentration used in this study is 100 times less than in the former study, partly that the mean age in present study is nearly twice as high hereby influencing the size of flare area as shown by Helme et al.\(^{71}\). The significant ratio seen in MCS and EC groups is probably due to the large SPPH presented in these patients in general. For validity of results it needs to be considered that in the present study MCS patients were invited to participate by letter; about half of the group accepted. The part that chose not to participate was very heterogeneous, some were taking medicine mentioned as exclusion criteria, two had experienced a stroke recently, one was epileptic, and one was pregnant. Further, some did not wish to participate because they felt too ill. The participants in the MCS group were mildly to moderately affected. It is possible that the differences between groups would have been even more significant if more severely ill patients had participated. Further, pain measurements were not done twice as in healthy volunteers thus, the reproducibility of these parameters in patients is unknown. In addition, the eczema group was recruited consecutively from patients attending a dermatological outpatient clinic for patch testing. We put only little effort in characterising the type of eczema as the focus of our investigation was on the possible association between capsaicin sensitivity and self-reported chemical sensitivity and not on the link between eczema and respiratory chemical sensitivity. This group was found to be very heterogeneous, and some patients had symptoms from the central nervous system in common with the MCS patients. As a result, distinct differences between MCS and EC groups could have been overlooked. More criteria to make groups homogeneous would have facilitated conclusions and have made results more reliable from the relatively small groups.

**Conclusion**

Central pathophysiological processes may at least be partly responsible for MCS and EC as in the case for CP conditions without identifiable peripheral sources of nociceptive input
Capsaicin-induced neurogenic inflammation

5.1 Measurements of neurogenic inflammation

C-fibres have two functions. They encode noxious stimuli and convey impulses to the CNS, as described and investigated in the previous chapter by measurements of SPPH and TS (CS). In addition, their actions induce local vasodilatation\textsuperscript{122}. This axon reflex, as described in chapter 1, section 1.8 can be activated by noxious stimulation of peripheral C-nociceptors, in particular the mechano-heat-insensitive nociceptors\textsuperscript{6}. Hereby, eliciting afferent potentials, which trigger the release of CRGP and SP, followed by vasodilatation and visible as a “flare” (neurogen inflammation)\textsuperscript{122}. The temporal and spatial profiles has been studied to elucidate mechanisms behind neurogen inflammation and CS, as these mechanisms are hypothesised to be associated\textsuperscript{123,124,130}. The quantification of flare can be done visually or objectively by use of e.g. laser Doppler, which planimetrically analyses vasodilatation, an indirect measure of CRGP release. However, a recent study suggests that when blood perfusion increases during vasodilatation the initial phase relies mainly on an increase in red blood cell concentration\textsuperscript{125} whereas, the subsequent perfusion increase is due to an increase in blood cell velocity\textsuperscript{123,125}. This initial change in microcirculation in skin was revealed by comparing laser Doppler with a new optical devise, the tissue viability imager (TiVi). Thus, to study the initial dynamics in neurogen inflammation in skin the tissue viability imaging might provide better information about temporal profile (erythema intensity). Further in contrast to techniques involving laser Doppler, the TiVi is insensitive to movement of tissue and has the same degrees of freedom as a standard photography\textsuperscript{123,124}. To minimise intra- and inter-individual variations, several factors need consideration. The skin plays a major role in thermoregulation, with about 85% of blood perfusion serving this function\textsuperscript{125}. The core temperature controls this regulation; however, local heating, too, results in vasodilatation, thus local as well as core temperature are of importance for skin colour\textsuperscript{125}. Therefore, measurements should be performed in a room with a temperature in the range of 19–23ºC, and exercise should be avoided up to four hours before experimental sessions in order to minimize the effect of these factors\textsuperscript{126}. Further, caffeine (more than 6 cups of coffee, approximately 500mg) and nicotine have been shown to reduce cutaneous blood flow due to vasoconstriction\textsuperscript{125}, whereas alcohol induces vasodilatation\textsuperscript{125}. In general, male skin colour has been found to be redder than female skin colour\textsuperscript{125}, and sex differences in regional cutaneous microvasculature in response to capsaicin injection have been found in the fingertip but not in the forearm\textsuperscript{127}. Further, age-related change of the cutaneous microvasculature and significant variations within persons at different anatomic sites have shown to be of importance\textsuperscript{128,129}. In addition, within the forearm, proximal areas have a lower blood flow than distal sites\textsuperscript{111}. Furthermore, body position should be standardised during studies to eliminate orthostatic variations, especially if tests are performed on the extremities\textsuperscript{125}.

The cutaneous microcirculation is organized as two horizontal plexuses: the upper plexus (the sub-papillary plexus) is situated 1–1.5 mm below the skin surface and supplies the skin vasculature of the papillary dermis, the lower plexus is at the derm-subcutaneous junction. Laser Doppler\textsuperscript{130} or tissue viability imaging\textsuperscript{131} measures microvascular responses primarily in the reticular dermis, whereas measurement by for example a thermo camera can detect microvascular responses in the subcutaneous tissue\textsuperscript{132}.

Erythema intensity measured by tissues viability imaging

In brief, the tissue viability imager (TiVi) uses linear polarisation light spectroscopy to quantify the red blood cell concentration (RBC\textsubscript{corr}) in the sub-papillary plexus of the cutaneous vascular bed\textsuperscript{123,133}. The TiVi-system consist of a digital camera, equipped with perpendicular-polarization filters in front of the flash and lens\textsuperscript{123,133}. This system instantaneous captures data from all image measure points at a maximal lateral resolution of about 50 µm (corresponding to 12 million measurements sites at maximum) and an image up data rate of 12 images pr. minute\textsuperscript{133}. When the flash is activated, the broad spectrum white light emitted becomes linearly polarised after passage of the first polarisation filter\textsuperscript{123,133}.
Chapter 5

Figure 15

(A) Operating principle of the imaging system, showing the polarisation gating method. Both 1 and 2 are polarisation filters. LP and RP represent linear and randomly polarized light respectively. (B) Cross section displaying the development of the TiVi value throughout the sequence of images. Note the spatial heterogeneity of the microvasculature over the entire area.

5.2 Capsaicin-induced neurogenic inflammation in patients with symptoms induced by odorous chemicals (Paper III)

Background

Involvement of neuropeptides in patients with MCS has been suggested in a number of studies. Further, one study found substance P, vasoactive peptide and NGF to be increased in plasma in MCS patients when exposed to volatile organic compounds. However none of these studies investigated neurogen inflammation in skin. The latter, is of further interest since it has been proposed that CS is the primary casual pathophysiological mechanisms for MCS. Moreover, that CS and cutaneous neurogenic inflammation (vaso-motor response) to be associated. Finally, that CS evoked experimentally by capsaicin is inflammatory. In contrast to experimentally measurements, there is a lack of consistency in clinical studies between CS and neurogenic inflammation. This inconsistency might be due to different application modes (intradermal versus topical) of the algogenic substances used to evoke the responses. In addition, age span is of importance in regards of both blood flow and visual flare. Further, in patients with RA, capsaicin based techniques have revealed that, while a selective increase of peripheral sensory fibres activity was apparent over involved joints, but a general up regulation was not found. Hence only flare area above inflamed joints were increased in size. However, in a similar set up SPPH was found...
increased in the forearms in RA patients, with normal peripheral sensory reactivity in this area. In view of these controversies, and the former observation of increasing plasma levels of neuropeptides in MCS patients, but not in eczema patients the present study, was planned to evaluate 1) the contribution of neurogenic inflammation in patients with MCS and eczema patients with airway symptoms elicited by odorous chemicals by assessing erythema intensity e.g. temporal profile and flare area 2) the relationship between erythema intensity when using the TiVi-system and flare.

Material and Methods

Subjects are described in section 3.3, 4.3 and basal characteristics are shown in Table 2 and appendix 2. Participants underwent two intradermal injections of capsaicin (3.3 μM and 33 μM) and one with saline as described above.

TiVi parameter setting

Tissue RBCconc was quantified by use of a commercial TiVi-system (TiVi600, WheelsBridge AB, Linköping, Sweden). Image analysis was made using the analysis software (TiVi Version 4.1, WheelsBridge AB, Linköping)

The camera was positioned about 30 cm above the object. The resolution was set to small and the Macro checkbox was checked. Size Reduction was set to Minor Size Reduction. Number of photos was set to 36, and captured with 5 sec. intervals, for the first 20 photos, then delay to 12 sec. for the last 16 photos. This setup was used to ensure that successive photos were captured at the maximal sampling rate of 12 photos per minute within the first minute, as it has previously been shown, that the full extent of flare develops rapidly after capsaicin injection.

In the analysis, the photos were first aligned using the integrated TiVi alignment tool. The area around the injection trauma was set to background colour by using the Reduced Photo option. By this arrangement image pixels around the injection trauma were blocked and not further included in the analysis. These arrangements resulted in photos of a size of about 630 x 470 pixels, a field view of about 30 x 20 cm and a lateral resolution of about 0.5 mm. Photo capturing was initiated just prior to the injection of the test substance. Thus the needle appeared in the first and in some case also in the second photo while the substance was injected. In the analysis these first photos were not included and the First Image was generally selected as the third photo in each sequence. Prior to drawing the region of interest (ROI), the last image was inspected with respect to the approximate extent of the flare reaction and used as a guide for drawing the ROI. The ROI was then drawn to include the flare area but excluding other possible local high RBC concentration sites further away from the flare and not being part of the reaction to the injection.

In the analysis the Express Wizard was used to automatically calculate the Erythema Intensity inside the ROI area. In the Express Wizard window, the Subtract First Image option was selected which subtracted the first image from all other images pixel-by-pixel. This arrangement made it possible to visualize and analyze only changes in RBC-concentration produced by the injected substance accounting for the normal RBC concentration in resting skin. The Set maximum Value Lower % was set to 80 and the Set Intensity Value % to 50 (default values). These settings produced a threshold value halfway in between the minimum and the maximum RBC concentrations of the entire sequence. The average Erythema Intensity of pixels above this threshold and within the ROI area was then automatically calculated by the Express Wizard and eventually presented in a diagram displaying Erythema Intensity versus time.

Results

Individual data for duration and intensity of symptoms for EC and MCS patients are listed in Table 2 and appendix 2.

Erythema intensity

Injections of isotone saline reduced TiVi index in a small spot equal to the injection trauma, induced by capsaicin. In order to avoid influence of this area injection trauma was set to background colour and no further analyses were performed. The erythema intensity was dose dependent Table 7 and Figure 16. Individual curves are shown in paper III, p 8 for erythema intensity versus time following the highest concentration of capsaicin (33 μM). The AUC displays large inter-individual difference in A.U values x time for all groups, controls compared with patients (EC and MCS).
Table 7. Erythema intensity and area of flare in controls, eczema patients with mucosal symptoms elicited by odorous chemicals (EC) and patients with Multiple Chemical Sensitivity (MCS)

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls (n=29)</th>
<th>EC (n=15)</th>
<th>MCS (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin 3.3 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema Intensity (A.U. x sec)</td>
<td>5817 ± 2874</td>
<td>6464 ± 3439</td>
<td>6477 ± 2689</td>
</tr>
<tr>
<td>Visuel flare area (cm²)</td>
<td>4.9 ± 4.5</td>
<td>6.2 ± 3.7</td>
<td>5.3 ± 3.2</td>
</tr>
<tr>
<td>Capsaicin 33 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema Intensity (A.U. x sec)</td>
<td>9003 ± 2894</td>
<td>9132 ± 3264</td>
<td>9657 ± 2667</td>
</tr>
<tr>
<td>Visuel flare area (cm²)</td>
<td>19.0 ± 7.7</td>
<td>19.3 ± 7.3</td>
<td>17.3 ± 8.4</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD). No significant differences was found between groups.

Figure 16 Erythema Intensity versus time following, median values

A Capsaicin 3.3 μM

B Capsaicin 33 μM

C Capsaicin 3.3 μM

D Capsaicin 33 μM

Increase in Erythema Intensity above baseline in arbitrary units, A.U., plotted against time. The dose dependent significant increase was independent of groups (controls, eczema patients with mucosal symptoms elicited by odorous chemicals (EC) and patients with multiple chemical sensitivity (MCS)). Areas under curves were compared (A) The erythema intensity was not significantly different between groups although a faster but insignificant response was observed in the two groups of patients (EC + MCS) (B) No differences were found between groups at the highest concentration. (C) The erythema intensity was correlated negatively with age, p< 0.05 between young and older (3.3μM), although not significant between young and middle (p>0.08). (D) No significant differences were found between age groups at the highest concentration.
Chapter 5

Post-hoc assessments of the influence of age on erythema intensity

Erythema intensity increased with young age at the lowest (3.3 μM), but not the highest (33 μM), concentration.

Visual Flare

Visual flare was independent of group (controls as compared with patients). Further, flare area was dose dependent and positively correlated to young age at both concentrations (Figure 17).

Correlations between Flare and erythema intensity

Erythema intensity did not correlate with the area of visual flare in patients (EC and MCS) nor in controls when each group was analysed separately or when analysed in age groups (younger, middle and older).

Discussion

We measured cutaneous vascular response to capsaicin injections using a new optical measurement technique – the tissue viability camera. The TiVi camera detected a dose dependent increase in erythema intensity in all groups. However, there was no difference in response in patients (EC and MCS) as compared with controls.

CRPG released from peripheral c-nociceptors induces vasodilatation (increase in RBC_{conc} and flare\textsuperscript{122}. These reactions are therefore a surrogate of c-nociceptors activation\textsuperscript{122}. In addition, it has been hypothesized, that mechanical hyperalgesia (e.g. SPPH and TS) develops as a consequence of peripheral nociceptive action and should be based on central sensitization. In accordance with these assumptions, we would have expected the erythema intensity to be exaggerated in patients with MCS proposed to be part of the CSS\textsuperscript{18}. However, there are several pitfalls in this study, which might have contributed to lack of difference between the groups.

First of all, we did not ask if the patients were specifically irritated in the skin (other than exclusion criteria at injection side), when exposed to odours chemicals – this might had lead to another way of separating subgroups – as the individual curves was clearly divided into high and low responders, although this was also the case for controls (Figure 1, paper III). Secondly, we did not record more than one baseline photograph from each person – this might have improved measurements, as we observed a large inter-individual variation in baseline erythema intensity prior to capsaicin injection. Thus and intra individual viability might also be expected. However, it was shown recently that short-term variation was limit to below 2% in TiVi units recorded at different skin sites\textsuperscript{133}. Third, we did not compare this new method to more established methods used to quantify neurogen inflammation such as laser Doppler or Thermo camera. A comparison was not possible in the present setup as pictures were taken every 5 seconds for the first minute – and it was thus not possible to fit other equipment in between. However, a recent study compared the TiVi and laser Doppler methods, and found ED\textsubscript{50} to be insignificant\textsuperscript{123}. Further, the result suggested that when blood perfusion increases in skin, the initial phase are mainly dependent on the change in RBC\textsubscript{conc}, which is later followed by increase in velocity of the RBC as perfusion.
increases. In light of the latter it was of interest to study the reaction rate of erythema intensity (RBCcon) within the first 30 seconds – as laser Doppler previously found the flow to increase within the first minute. Although the response was faster at the lowest concentration in patients, this difference was insignificant compared with controls. Interestingly this difference was not present at all at the highest concentration. Finally in other clinical studies with RA, FM and vulvodynia similarly suggested to be part of the CSS – results are not consistent between groups. Likewise, the flare response was found to be insignificant between patients and controls, nor did we find any relation between flare area and erythema intensity. The latter could be due to different time equilance between flare measured after 5 minutes and erythema intensity measured after 210 seconds. However, we were unable to analyze the hole sequence of pictures due to artifacts and “out of focus” partly explained by patients were unable to stay still for five minutes. Further we observed that the camera was quite sensitive to heavy breathing. Also it would have been of interest to compare erythema intensity with TiVi area – however despite many attempts to do so, we could not get any reliable values. We have no explanation for this incidence. Finally, a small difference in e.g. flare would probably necessitate that more patients needed to be included. Thus results might also be due to lack of power.

**Conclusion**

Patients (MCS and eczema) with symptoms induced by odorous chemicals did not show any different peripheral neuro-vascular responses as compared to controls. Although further investigations needs to be carried out.
Correlations between sensitivity to capsaicin in skin and airways

“TRP channels play an important role as multifunctional cellular sensors. They are involved in many fundamental cell functions suggesting, that the investigation of their role in human pathophysiology and disease will become an urgent priority in biomedical sciences”, Nilius et al., 2007. The TRPV-1 sub family is expressed on sensory fibres in various organ systems, such as the skin, airways, gastrointestinal, and the bladder. In chronic hypertussive cough states, normally innocent stimuli that would not under normal circumstances initiate a response, evokes cough. This reaction parallels alterations during chronic pain state, characterized by hyperalgesia and allodynia. Further, this interpretation, is in line with a recent study, whereby comparison of supramedullary pathway revealed significant overlap between the brain region activated following both peripheral somatosensory and airway sensory nerve stimulation of the TRPV-1 channel with capsaicin. The mechanism underlying alterations in nociceptive and airway sensory pathways has been described in details in the previous chapters. However, even though peripheral sensitization involves hyper excitability of sensory neurons due to e.g. changes in phenotypes of the receptor, and increased neurotransmitter expression in both pain and airways the proposed shared alterations in the CNS is less clear. For instance, CS in pain pathways involves enhanced efficiency of excitatory synaptic transmission pathways within the CNS. Notably, in the dorsal horn of the spinal cord at the synapses between the sensory nociceptors-fibres and second order relay neurons. Whereas, it is less clear whether insults that can induce a hypertensive state due to changes in the presynaptic inputs to the nTS, also produces postsynaptic changes indicative of CS. The latter, has been supported by a study in guinea pigs where animals were exposed to tobacco smoke. It was shown, that increased firing in the nTS relay neurons paralleled the increased C-fibres responsiveness to capsaicin, due to increased expression of CRGP and SP in the peripheral nerve endings.

Background

The symptoms of MCS share similarities to other unexplained illness such as FM, vulvodynia, sensory hyperreactivity and CFS. In chapter three to five, the sensitivity to capsaicin was investigated in both skin and airways. First, healthy controls including both genders were examined twice, in order to investigate reproducibility in men and the influence of menstrual cycle on various parameters in women. Secondly, patients with either MCS or EC were compared with age-matched controls. The experimental sensations were carried out on exactly the same time during the day for each provocation in both studies. This was done, in order to avoid any influence of diurnal hormone fluctuations during the day on pain, and cough sensitivity. By stimulating the TRPV-1 receptor in skin and airway we thus aimed to see if there would be any correlation between various modalities such as LogC5, SPPH, TS, Flare, and erythema intensity in the two studies mentioned above.

Material and Methods

Subjects

Subjects are described in section 3.2, 4.2, and basal characteristics are shown in Table 1, in regards of study 1. Subjects are described in section 3.3, 4.3, and basal characteristics are shown in Table 2 and appendix 2, in regards of study 2.

Statistics

Relationship between sensitivity to capsaicin (logC5) and pain assessments, in women at menstrual phase and in men were examined in stepwise multiple regression analysis. Relationship between sensitivity to inhaled capsaicin (logC5) and pain assessments, in patients (EC and MCS) and controls were examined in stepwise multiple regression analysis. Significance levels throughout this study were P < 0.05. Statistical tests were performed using SPSS software, version 15.0 (SPSS, Chicago, USA).
Chapter 6

Results

The relationship between cough threshold to capsaicin (logC5) as the dependent variable and SPPH, TS, pain intensity and duration, visual flare, and erythema intensity as independent variables was examined by stepwise multiple logistic regression analysis (Wald’s Test) (Table 8-12). Primary outcome variables are presented in section 3.2, 3.3, 4.2, 4.3 and 5.2. The first two tables (Table 8 and 9) present data from the first study (13 healthy women and 13 healthy men), data from women in the luteal phase are not shown. A weak negative correlation between threshold log C5 and area of secondary hyperalgesi was found in men.

Table 8. Relationship between sensitivity to capsaicin (logC5) and pain assessments, in females at menstrual phase (N=13) examined in stepwise multiple regression analysis

<table>
<thead>
<tr>
<th>Parameters in models</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution (cm²)</td>
<td>0.52</td>
<td>0.36</td>
<td>0.39</td>
<td>0.51</td>
</tr>
<tr>
<td>Sec. Hyp (cm²)</td>
<td>0.48</td>
<td>0.40</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>VAS (AUC) (cm×min)</td>
<td>0.77</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows P-values for the significance of each parameter in each model with decreasing number of variables. The least significant variable at step 1 (Flare area with highest P-value) is omitted at step 2, where the least significant (VAS(AUC)) is omitted, etc. At step 4 the last remaining variable (Distribution) is insignificant, and the stepwise analysis shows that none of the variables are significantly correlated with logC5.

Table 9. Relationship between sensitivity to capsaicin (logC5) and pain assessments, in males (N=13) examined in stepwise multiple regression analysis.

<table>
<thead>
<tr>
<th>Parameters in model</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sec. Hyp. (cm²)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>0.08</td>
<td>0.10</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>VAS (cm x min)</td>
<td>0.16</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows P-values for the significance of each parameter in each model with decreasing number of variables. Despite being significantly correlated with logC5 at step 1-3, Sec. Hyp. is only borderline correlated with LogC5 at the last step.

Table 10-12 present data from the second study (29 healthy women, 15 eczema patients and 16 patients with MCS) separated into the three groups. Although a significant negative correlation between threshold log C5 and area of SPPH was seen in the first 5 steps in healthy women, this relation was not significant in the last step. In Table 11, negative significant associations were found between log C5 and both pain drawn area and flare area in patients with eczema and airway symptoms. In table 12 no significant correlations were found between Log C5 and various pain parameters and neurogenic inflammation in patients with MCS.
Table 10 *Relationship between sensitivity to inhaled capsaicin (logC5) and pain assessments, examined in stepwise multiple regression analysis in healthy controls (N=29)*

<table>
<thead>
<tr>
<th>Parameters in models</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Sec. Hyp (cm²)</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>*VAS(AUC) (cm x min)</td>
<td>0.08</td>
<td>0.08</td>
<td>0.06</td>
<td>0.06</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Flare area (cm²)</td>
<td>0.63</td>
<td>0.58</td>
<td>0.49</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tem. Sum (cm x sec)</td>
<td>0.42</td>
<td>0.43</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erytema int. (A.U. x sec)</td>
<td>0.58</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The table shows P-values for the significance of each parameter in each model with decreasing number of variables. Despite being significantly correlated with logC5 at step 1-5, Sec. Hyp. is not significantly correlated with LogC5 at the last step.

Table 11 *Relationship between sensitivity to inhaled capsaicin (logC5) and pain assessments, examined in stepwise multiple regression analysis in eczema patients with airway symptoms (N=15)*

<table>
<thead>
<tr>
<th>Parameters in model</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare area (cm²)</td>
<td>0.09</td>
<td>0.06</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>0.10</td>
<td>0.05</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Tem. Sum (cm x sec)</td>
<td>0.20</td>
<td>0.18</td>
<td>0.17</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Sec. Hyp. (cm²)</td>
<td>0.61</td>
<td>0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erytema int. (A.U. x sec)</td>
<td>0.71</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (AUC) (cm x min)</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows P-values for the significance of each parameter in each model with decreasing number of variables. It is seen that Flare area and Distribution gradually show decreasing P-values fra step 1-4, and remain significant at the last step.

Table 12 *Relationship between sensitivity to inhaled capsaicin (logC5) and pain assessments, examined in stepwise multiple regression analysis in patients with multiple chemical sensitivity (N=16).*

<table>
<thead>
<tr>
<th>Parameters in models</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare area (cm²)</td>
<td>0.37</td>
<td>0.36</td>
<td>0.29</td>
<td>0.15</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>Sec. Hyp (cm²)</td>
<td>0.17</td>
<td>0.15</td>
<td>0.10</td>
<td>0.13</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Distribution (cm²)</td>
<td>0.42</td>
<td>0.34</td>
<td>0.27</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (AUC) (cm x min)</td>
<td>0.40</td>
<td>0.38</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erytema int. (A.U. x sec)</td>
<td>0.60</td>
<td>0.54</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tem. Sum (cm x sec)</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows P-values for the significance of each parameter in each model with decreasing number of variables. The stepwise analysis shows that none of the variables are significantly correlated with logC5.
Chapter 6

Discussion

Among the variables tested for association with logC5 presented in this thesis only lower airway symptoms (Table 3, section 3.3 p 16) sec hyperaemia (table 8) and flare area and distribution (table 9), exhibits significant association to the cough threshold. However, since correlations are not consistent to e.g. peripheral sensitization or CS and not consistent within groupings, precautions should be taken when interpreting these results.

The divergence between results of these two studies, and the strong correlation between cough-related higher brain networks and those involved in the processing of pain previously found in another study, might have several explanations. First of all the overlap found in the previous study was between two different groups of patients provoked independently. Secondly, the comparisons made previously were between thermal pain stimulation and capsaicin inhalation, whereas in the present thesis both skin and airway stimuli were provoked by capsaicin. Third, the uses of function brain imagining in humans have some limitations. For instance it is difficult to precisely identify the key brain regions involved in either voluntarily cough or the urge-to cough. Further, controlling functional magnetic resonance imaging studies is often difficult and data sets will usually contain unwanted activations (e.g. pulmonary or cardiovascular dynamics) resulting in both motor and sensory activations that are not entirely related to the primary effect stimulus. However, there is growing evidence that cough is not just a simple reflex response to airway irritation, but must be considered as a complex series of events. Thus it is likely that multiple primary and secondary sensory pathways are involved and are processed in the brainstem. Further, that higher brain networks contributions to shape the response similarly to the pathways involved in pain. Although, no correlation between pain pathways and cough threshold were entirely evident in the presented studies further clarifications is needed. In particular, it would be of interest to investigate, whether a correlation between sensitivity in pain and airways exist if other methods had been applied, e.g. fixed-time inhalation test. In fixed-time inhalation test provoked cough cannot be inhibited adequately, in patients with airway symptoms to odorous chemicals which are likely due to CS.

Conclusion

Overall, no consistent correlations were found between capsaicin provoked pain in the skin and cough threshold in the airways in either healthy participants or patients.
In this thesis I have focused on patients who experienced airway symptoms and/or general symptoms after exposure to odorous chemicals, which are normally regarded as non-toxic. It was demonstrated that patients with either MCS or EC have enhanced capsaicin sensitivity in both skin and airways. Further, the results indicate that CS contributes to pathophysiological-mechanism of MCS, as shown by increased SPPH and TS in these patients.

MCS is a complex topic that has raised many questions to which there are no simple answers. William Osler once wrote “the study of medicine begins with the patients and ends...with the patient.” However, the lack of symptom constellation and a well-defined and consistently used case definition makes it difficult to portray a “typical” MCS patient. Further, lack of consistency has impeded the epidemiological and clinical research, as stated in chapter 1. Thus, data is still needed to clarify prevalence, natural history, aetiology, diagnoses, and management.

In order to prioritize, research issues prompt to clarify the aetiology of MCS. Dyer and Sexton divided the field into four levels. Hereby, research’s based on highly plausible and readily testable hypotheses were levelled as having highest priority. Consequently, neurogenic inflammation, learning/conditioning, chronic sinusitis, and mucous membrane irritation were suggested as special areas of interest. In addition, Arnetz proposed that investigations should involve patient groups with similar symptoms, but different diagnoses. In particular, research should address concurrent environmental stressors that trigger the limbic system of the brain, by assessing biological and psychological data. In line with these proposals neurogenic inflammation, mucous membrane irritation, and CS were investigated in this thesis. Furthermore, investigations were conducted in two different groups of patients, experiencing similar kind of symptoms when exposed to odours chemicals, but diagnosed as MCS or EC patients respectively.

Exposure to occupational chemicals and odorous is part of daily living. The majority of patients (58%) included in this thesis had some kind of work related initiating chemical exposure— for detail see appendix two – however, it was far from all, where such clear relationship exists. This is in accordance with previous findings where approximately 40 % could not identify the initiating stimulus.

It is well established, that responses in patients with MCS are unlikely to be explained by immunological/allergic reactions as being the main mechanism. In a recent study, patients with respiratory symptoms related to perfumes released more histamine when basophiles were incubated with perfume, by non IgE mediated reactions, as compared to healthy controls. In both groups, incremental concentrations of perfume showed a positive and significant dose-response effect on the release of histamine. We could not find any significant difference on the release of histamine between patient groups and healthy controls when applying the same method described by Elberling et al. in the present thesis; however, we did find a dose-response effect in all groups (unpublished data). Further, in this thesis the presence of asthma did not influence the relationship between lower airway symptoms elicited by odorous chemicals and cough threshold when challenged with capsaicin inhalations. This might have been expected if the mechanism of elicitation of asthma and MCS had been identical. In contrast, lower airway symptoms have previously been shown to of importance when applying capsaicin fixed-time inhalation test in a comparable group of EC patients. In addition, atopic patients was shown to have increased cough sensitivity to capsaicin in general, as compared to non-sensitive atopic subjects and healthy controls.

These results further indicate another mechanism behind the hypersensitivity to chemicals and odours in asthma and allergy. It is likely, that the exaggerated bronchial response seen in both MCS and EC patients with lower airway symptoms is due to a peripheral sensitization caused by increased sensitivity of the cough receptors. The latter explanation is supported by the finding of an increased expression of TRPV-1 on C-fibres in patients with chronic cough irrespective of cause. Further, the increased expression was significantly correlated with the cough sensitivity when challenged with capsaicin. However, it needs to be
addressed whether the expression of the TRPV-1 is increased in the airways in patients with lower airway symptoms related to odorous chemicals. New evidence has shown that higher brain motor pathways might influence descending inputs to the brain stem components of basic cough reflex sharing some of same pathways as pain perceptions. However, we could not find any consistent correlations between various pain parameters and cough sensitivity in either healthy volunteers or in patients with symptoms elicited by odorous chemicals, as discussed in chapter 6. Although it is likely that the respiratory mucosa could act as both a target, whereby odours initiates inflammation and an effector organ (e.g. cough) for patients with chemical irritations in the lower airways. MCS patients have symptoms from so many other organs, indicating other mechanisms than peripheral sensitisation to be involved as well.

In order to establish reliable tools for investigating pathological mechanism in patients, studies were first conducted in healthy volunteers as described in section 3.2 and 4.2. This provide the opportunity to test the influence of hormone level and sex differences supposed to be of importance. However, there was no influence of either sex or menstrual cycle on the cough threshold in healthy volunteers. This result was subsequently confirmed when applying the same methods in patients with symptoms elicited by odorous chemicals. Throughout the thesis, capsaicin was used as a sole stimulant in order to investigate the pathophysiological-mechanisms in both skin and airways. It could be argued that additional stimulations with non-allogenic inflammatory mediators (e.g. bradykinin or PGE2) and allogenic, non-TRVP-1 agonists like protons should be included as well, at least in skin. However, as we aimed to compare the sensitivity in the airways with skin to reveal any possible relationship between nociceptive and airway sensory pathways it was not desirable to combine various agents in this setup. For instance, in vitro electrophysiological studies has demonstrated markedly increased responses of single vagal C fibers to capsaicin, after perfusion with bradykinin. The TRP ion channels are important for multi organ systems in their interactions with the environment. The correlation between levels of TRPV-1 channel expression and disease symptoms, and/or enhanced responsiveness in the CNS, gut, and airways when experimentally provoked by capsaicin has already been described in previous chapters. In addition, capsaicin was already demonstrated to provoke cough in MCS patients with lower airway symptoms, as to EC patients, and has been extensively used in pain studies. However, until now it remains unsolved, how increased sensitivity to capsaicin and intolerance to odorous chemicals are connected. One study even excluded capsaicin in a systematic review of provocation studies. This puzzle might be solved by the recent finding of enhanced responsiveness to capsaicin in mice after exposure to sulfur containing malodrant vapors. This odorous primarily attacks the olfactory nerve. Thus, physiologically based interaction may exist between olfactory and trigeminal nerves. Additionally, previous studies by the same group have shown the sensory irritation response to be absent in TRPV-1/- knockout mice, confirming the role for the TRPV-1 channel in mediating the sensory irritation response to capsaicin. Yet, results need to be confirmed in human studies.

In the animal study mentioned above, the observed responses was concluded not to be due to conditioning (associative learning), as responses were measured during the first exposure. Neural sensation (non-associative learning) has been contrasted with conditioning, as a model when chemicals trigger a multisystem response. The term sensitization refers to the development over time of an exaggerated, pathological response to a stimulus that was originally innocuous or “subthreshold”. It is a broad term use to describe both reactions at a neural level and mechanisms in psychiatric illness. Neural sensation has been divided into kindling and non kindling mechanisms. The term kindling originally referred to a very specific type of sensitization process, the development of behavioral seizures, in response to repeated electrical or chemical stimuli. Non kindling is considered to be a time dependent sensitization with progressive amplification of responses. However, the word kindling is now more widely used to convey the concept of the development of persistent hypersensitivity. Animal studies have shown that kindling shares features with MCS including cross sensitization to chemicals. Measurements of neurotransmitter receptor density and neuroanatomical lesion studies have implicated the hippocampus, part of the limbic system, as an important structure in kindling. The limbic...
circuitry is also recognized as being particular sensitive to environmental stressors, as described in chapter 4. With the recent finding of patients with self-reported chemical sensitivity not being able to habituate to CO2 exposure recorded as event-related potentials further suggests enhanced sensitization and CS to play a role in these patients. Pain signals are also received and interpreted in the limbic structures as well as somatosensory areas of cortex (localization, duration and intensity of pain) and ACC (emotional responses to pain). The processing of pain signals in limbic structures may attenuate or promote central mechanisms and contribute to the development of symptoms in MCS by kindling, as suggested by Rainville et al. In the present thesis, this was done by using intradermal injections of capsaicin - well known to induce pain in a dose-dependent manner. From the study in healthy volunteers it could be concluded that a strong association between pain intensity in the forehead and forearm exists. The area of SPPH was not significantly different between sexes and none of the parameters measured was affected by menstrual cycle. Likewise, no association was found between pain and hormone level in patients with symptoms elicited by odorous chemicals and neither was there an association in controls. However, in these patients, intradermal injections of capsaicin evoked increased area of SPPH and TS response across groups (EC as well as MCS). In addition, patients with co-morbidities of FM, CFS and/or CP were found to have enhanced TS response compared with MCS patients without such co-morbidities. This is the first study showing abnormal pain processing of sensory stimulation in MCS patients. Different environmental stressors, including psychosocial stress, act as initiators of the limbic systems. However, psychological factors are not necessarily the primary cause of the occurrence of pathological pain hypersensitivity. This might also apply for MCS. In our study, the affective dimension of subjective pain experience was not profound in either group of patients compared with controls. The result is in agreement with a recent study in vulvodynia-affected women, using a score that has previously been shown to be associated with both the sensory and affective component of pain. The score did not predict pain response in this study. CS measured as an increased capsaicin-induced SH has also been found in other diseases including rheumatoid arthritis, and FM. However, in our patients with symptoms elicited by odorous chemicals the area of SPPH was unaffected by the presence of co-morbidity of FM, chronic fatigue, and/or CP. Thus, this result reflects a common dysfunction in their central processing of pain stimuli. These findings are in accordance with a recently proposed nosology for conditions sharing CS as the underlying mechanism, termed central sensitivity syndrome (CSS). The area under curve for TS (pain intensity along time) was significantly different between MCS patient and controls, moreover, it increased significantly with the presence of co-morbidity of FM, CFS and CP compared with patients without co-morbidity. This further confirms that although TS and SH are both related to CS, they are differently facilitated in the nociceptive pathways. This result is in accordance with findings in healthy volunteers where SH was found to be heterosynaptic facilitated and TS was found to be homosynaptic facilitated. However, it is uncertain whether TS, like SH, is a general phenomenon in diseases proposed to be part of the CSS. Although, TS is suggested to be a key to the underlying pathological mechanisms in patients with FM.

The role of peripheral neurogenic inflammation still needs to be investigated in both skin and airways. In the latter, previous study suggested that neuropeptides could play a role in patients with lower airway elicited by scents and chemicals. In skin inflammation has been suggested to contribute to the pathological mechanism triggering enhanced responsiveness of neurons within the dorsal horn followed by enhanced SH and TS as stated in chapter 5. However, both groups of patients (MCS and EC) produced a normal peripheral skin response to capsaicin compared with controls. No consistency has been found in clinical studies between CS and peripheral sensitization either, when measuring flare area as the vasomotor response. It could be that only the skin area overlying inflamed tissue or joints has a peripheral up-regulation of small fibre activity, as demonstrated in RA patients. Thus, from the above findings it is concluded that CS would be a feasible pathophysiological mechanism explaining many of the CNS symptoms presented in this group of patients. Further, this also explains the lack of a dose-response relationship that is mandatory for conventional toxicological understanding. Chemical stimulus does not necessarily need to be of noxious intensity, for CS to be present, because it is the amplification in
signalling within the limbic and somatosensory circuit that generate the noxious response.\textsuperscript{42,45}

**Clinical relevance and future perspectives**

The results suggest that the capsaicin single breath inhalation test is suitable when diagnosing lower airway symptoms. However, it should be noted that side effects might occur, MCS patients reported several symptoms related to the central nervous system which last for days after the provocation. Further, bronchial challenge cannot stand alone as a diagnostic tool in the future, as only about half the MCS patients reported lower airway symptoms in the studies described in the present thesis. This number is above average compared with a major survey, where only one third of the patients reported lower airway symptoms.\textsuperscript{3}

In order to study CS, intradermal injections with capsaicin are preferable in many ways. First of all this method has been used widely in other clinical studies including overlapping conditions to MCS. This provides the opportunity to compare results to healthy volunteers, who often react differently. Secondly, capsaicin induce pain in a dose dependent manner, which has also been acknowledged in patients.\textsuperscript{33,64} Third, the area of SPPH induced by capsaicin might be considered as a future diagnostic tool, as the specificity of this test was recorded as high. However, more research needs to be done especially with other stimulants.

In future studies it should be addressed whether our finding of increased SPPH in patients with MCS can be reproduced and whether this phenomenon occurs persistently or intermittently. Further, efforts should be directed towards determining the concentration of capsaicin with highest sensitivity and specificity regarding SPPH. Also, SPPH should be correlated to other modes of stimulations. In a recent study by Hillert et al, significant activation of the anterior cingulated cortex (ACC) was found, when MCS patients were stimulated with the pure olfactory odorant vanillin and recorded by PET scan.\textsuperscript{55} In parallel a reduced activation of the olfactory region was found. The author concluded that patients were not suffering from neural sensitization. However, it could alternatively be explained by top-down regulation of odorous response via the cingulate cortex.\textsuperscript{55}

Further, other quantitative measurements of CS should be investigated including responses to heat, cold, mechanical or electrical stimuli as well as noise, light and odour combined with electrophysiological testing.

The finding in our post-hoc analysis of patients with co-morbidity of FM, CP and CFS having an enhanced TS compared with patients without co-morbidity calls for further prospective studies in larger groups and subgroups. This might add valuable information to the ongoing discussion whether MCS is a single clinical entity.

Finally, Woolf and colleagues suggested that pain diagnosis and therapy should be mechanism based and that the pain assessment tools should be sufficiently sensitive and advanced to provide such mechanistic information.\textsuperscript{146} The proposal of the central sensitivity syndrome is very much in line with this way of diagnosing diseases.\textsuperscript{18} This would be beneficial in respect to hypotheses testing compared with the existing (descriptive) classifications established by Cullen and others.\textsuperscript{2,3,6}

If CS is the main key to patho-physiological mechanisms in MCS, it would also be of interest to treat these patients N-methyl-D-aspartate (NMDA) receptor specific antagonist, as enhanced receptor function is thought to follow kindling. However, more knowledge and research are needed before prevention and treatment can be obtained.
References

(1) Davidoff AL, Keyl PM. Symptoms and health status in individuals with multiple chemical sensitivities syndrome from four reported sensitizing exposures and a general population comparison group. Arch Environ Health 1996; 51(3):201-213.


(9) Johansson A. Airway sensory hyperreactivity linked to capsaicin sensitivity. Sahlgrenska Academy, University of Gothenburg, Sweden: 2008: 4-45.


Kimata H. Effect of exposure to volatile organic compounds on plasma levels of neuropeptides, nerve growth factor and histamine in patients with self-reported multiple chemical sensitivity. Int J Hyg Environ Health 2004; 207(2):159-163.


Appendix 1

The capsaicin concentrations used in this thesis for intradermal injection are listed below.

Concentration used in section 4.2:
- Molecular weight of Capsaicin = 305.416 g/mol
- Mass injected = 100*10^{-6} g
- Volume injected = 0.1*10^{-3} L
- Stock solution 100*10^{-6} g / 0.1*10^{-3} L = 3.3*10^{-3} M

Concentrations used in section 4.3 and 5.2:
- Molecular weight of Capsaicin = 305.416 g/mol
- Volume injected = 0.1*10^{-3} L
- Stock solution 100*10^{-6} g / 0.1*10^{-3} L = 3.3*10^{-3} M, as used in section 4.2
- Dilution (33*10^{-6} M): Stock solution was diluted 1:100
- Dilution (33*10^{-7} M): Stock solution was diluted 1:1000

Similar concentrations quoted by scientists with the field:

<table>
<thead>
<tr>
<th>Author</th>
<th>Capsaicin</th>
<th>Measure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanlon et al. 2006</td>
<td>Intradermal 10 μl 0.1, 1, 10, or 100 microgram</td>
<td>Pain scores were recorded at 0, 5, 10, 15, and 60 minutes on a visual analog scale from 0 to 100. Areas and intensities of mechanical allodynia (form brush stimulus) and pinprick hyperalgesia (von Frey test) as were flare areas</td>
<td>Capsaicin produced dose-dependent increases in spontaneous pain, intensity of mechanical allodynia, area of SPPH, and the flare area</td>
</tr>
<tr>
<td>Foster et al. 2004</td>
<td>Intradermal 10 μl of 0.1% (10 μg) capsicain</td>
<td>Spontaneous pain level, SPPH, surface area of dynamic allodynia, Laser-Doppler, regional skin temperature</td>
<td>Greater spontaneous pain, punctate hyperalgesia and dynamic allodynia, in vulvodynia-affected women. Capsaicin blood flow did not differ between cases and controls by anatomic site</td>
</tr>
<tr>
<td>Morris et al. 1998</td>
<td>20 μl of capsicain in solution (0.03 mg/ml) (capsicain diluted in ethanol) was applied</td>
<td>Area of SPPH</td>
<td>The area of secondary hyperalgesia was found to be substantially increased in both the FM and RA groups compared with controls.</td>
</tr>
<tr>
<td>Joliffe et al. 1995</td>
<td>Intradermal injection of capsicain 0.05 μg in 10 μl normal saline</td>
<td>Axon reflex vasodilatation was measured using laser Doppler flowmetry.</td>
<td>The results show a selective increase of capsaicin induced vasodilatation in skin overlying joints in patients with rheumatoid arthritis.</td>
</tr>
<tr>
<td>Simone et al. 1989</td>
<td>Intradermal injection of capsicain 0.01, 0.01, 1, 10 and 100 micrograms</td>
<td>The relationship between capsaicin dose and the magnitude and duration of pain was determined using the method of magnitude estimation. In addition to pain, capsaicin produced a flare and mechanical hyperalgesia</td>
<td>The lowest dose of capsaicin to produce an area of mechanical hyperalgesia was 0.1 micrograms. An area of hyperalgesia was present within seconds following injection. For doses of 10 and 100 micrograms, the area of hyperalgesia grew to reach a maximum within 5 and 7 min following the injection and gradually decreased, disappearing within 15 and 37 min, respectively. Capsaicin doses of 1, 10 and 100 micrograms produced successively greater areas of flare. The results demonstrate that humans can scale the magnitude of pain produced by capsaicin in a dose-dependent fashion</td>
</tr>
<tr>
<td>No</td>
<td>Age</td>
<td>Working situation</td>
<td>Possible environmental trigger</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>fv3</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fv5</td>
<td>23</td>
<td>Hairdresser / Hairdresser</td>
<td>Hair dyes</td>
</tr>
<tr>
<td>fv6</td>
<td>43</td>
<td>Librarian / Librarian</td>
<td>No identified initiating stimulus</td>
</tr>
<tr>
<td>fv10</td>
<td>56</td>
<td>Technical assistant / Technical assistant</td>
<td>Mould fungus</td>
</tr>
<tr>
<td>fv14</td>
<td>45</td>
<td>Laboratory technician / Laboratory technician</td>
<td>No identified initiating stimulus</td>
</tr>
<tr>
<td>fv18</td>
<td>62</td>
<td>Nurse / Nurse</td>
<td>Patch testing of perfumes in a dermatological clinic</td>
</tr>
<tr>
<td>fv28</td>
<td>57</td>
<td>Secretary / Secretary</td>
<td>Giant lily-of-the-valley</td>
</tr>
<tr>
<td>fv32</td>
<td>44</td>
<td>Cook / Sick leave</td>
<td>cooking fumes</td>
</tr>
<tr>
<td>fv34</td>
<td>57</td>
<td>Teacher / Clerk</td>
<td>Chloride water</td>
</tr>
<tr>
<td>fv42</td>
<td>27</td>
<td>Hairdresser / Sick leave</td>
<td>Hair dyes</td>
</tr>
<tr>
<td>fv44</td>
<td>46</td>
<td>Opera singer / Opera singer</td>
<td>No identified initiating stimulus</td>
</tr>
<tr>
<td>fv50</td>
<td>56</td>
<td>Teacher / Teacher</td>
<td>No identified initiating stimulus</td>
</tr>
<tr>
<td>fv51</td>
<td>45</td>
<td>Hairdresser / Sandwich maker</td>
<td>Hair dyes</td>
</tr>
<tr>
<td>fv52</td>
<td>49</td>
<td>Nurse / Zone therapist</td>
<td>Mould fungus</td>
</tr>
<tr>
<td>fv59</td>
<td>53</td>
<td>Educator / Educator</td>
<td>No identified initiating stimulus</td>
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<tr>
<td>No</td>
<td>Age</td>
<td>Working situation Former / Present</td>
<td>Possible enviromental trigger</td>
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<td>-----------------------------</td>
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<tr>
<td>f9</td>
<td>51</td>
<td>Hairdresser / Retired</td>
<td>Hair dyes</td>
</tr>
<tr>
<td>f16</td>
<td>49</td>
<td>Head of a primary school / Sick leave</td>
<td>Mould fungus</td>
</tr>
<tr>
<td>f17</td>
<td>59</td>
<td>Office manager / Sick leave</td>
<td>Installation of large print next to the patient’s office</td>
</tr>
<tr>
<td>f20</td>
<td>55</td>
<td>Therapeut and teacher / Sick leave</td>
<td>New bed</td>
</tr>
<tr>
<td>f21</td>
<td>59</td>
<td>Nurse / Nurse</td>
<td>Flowers</td>
</tr>
<tr>
<td>f22</td>
<td>47</td>
<td>Salesclerk / Housewife</td>
<td>Car scent</td>
</tr>
<tr>
<td>f24</td>
<td>64</td>
<td>Graphic design / Retired</td>
<td>Working with a form of tissue paper with a certain type of glue</td>
</tr>
<tr>
<td>f31</td>
<td>54</td>
<td>Computer teacher / Sick leave</td>
<td>New electronics in a computer</td>
</tr>
<tr>
<td>f33</td>
<td>45</td>
<td>Physiotherapist / Retired</td>
<td>Working with aromatic massage oils</td>
</tr>
<tr>
<td>f36</td>
<td>57</td>
<td>Salesclerk / Hairdresser</td>
<td>Hair dyes</td>
</tr>
<tr>
<td>f41</td>
<td>50</td>
<td>Building painter, Salesclerk / Sick leave</td>
<td>Aromatic massage oils</td>
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<td>f43</td>
<td>32</td>
<td>Hairdresser / Hairdresser</td>
<td>Hair dyes</td>
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<td>f46</td>
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<td>Aircraft exhaust</td>
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<tr>
<td>f53</td>
<td>63</td>
<td>Office manager / Retired</td>
<td>Mould fungus</td>
</tr>
<tr>
<td>f54</td>
<td>51</td>
<td>Engineer / Engineer</td>
<td>Mould fungus</td>
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</tbody>
</table>