””It is we who change as we learn to recognize what was formerly imperceptible””
Jean-Martin Charcot, 1892.

Ann Christin Gjerstad

Is Skin Conductance a predictor of arousal, noxious stimuli and pain in the sedated and anaesthetized patient?

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1. Acknowledgements

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2. List of abbreviations

ABP – arterial blood pressure

ANS – autonomeous nervous system

AVA – arteriovenous anastomoses

BIS – bispectral index

BMI – body mass index

CNS – central nervous system

CPOT – critical care pain observational tool

CSI – cerebral state index

DNA – deoxyribonucleic acid

EDA – electrodermal activity

EEG – electroencephalography

EMG – electromyelography

fMRI – functional magnetic resonance imaging

GSR – galvanic skin response

HPA – hypothalamic pituitary adrenal axis

HR – heart rate

HRV – heart rate variability

ICU – intensive care unit

JCAHO – Joint Commission on Accreditation of Healthcare Organization

MAAS – motor activity assessment scale

mAChR – muscarinic acetylcholine receptor

MRI – magnetic resonance imaging

mS – micro Siemens

mS/s – micro Siemens/seconds

NA – noradrenaline

NK – natural killer

NO – nitric oxide

NRS – numeric rating scale

NSCF – number of skin conductance fluctuation

NSRI – noxious stimulation response index

OAAS – observer´s assessment of anaesthesia and sedation

PET – positron emission tomography

PGP – protein gene product

PICU – paediatric intensive care unit

PSI – patient state index

PTSD – post traumatic stress disease

rCBF – regional cerebral blood flow

RE – response entropy

RE-Δ - response entropy minus state entropy

RSA – respiratory sinus arrhythmia

VACht – vesicular acetylcholine transporter

VAS – visual analogue scale

VIP – vasoactive intestinal peptide

SC – skin conductance

SE – state entropy

SPR – skin potential response

SR – skin resistance

SSI – surgical stress index

TCI – target control infusion

3. List of papers

The thesis in this dissertation were shed light on through the following papers:

Paper I:

Gjerstad AC, Wagner K, Henrichsen T, Storm H. Skin conductance versus the modified COMFORT sedation score as a measure of discomfort in artificially ventilated children. Pediatrics 2008; 122: 848-853.

Paper II:

Gjerstad AC, Storm H, Hagen R, Huiku M, Qvigstad E, Ræder J. Comparison of skin conductance with entropy during intubation, tetanic stimulation and emergence from general anaesthesia. Acta Anaesthesiologica Scandinavica 2007; 51: 8-15.

Paper III:

Gjerstad AC, Storm H, Hagen R, Huiku M, Qvigstad E, Ræder J. Skin conductance or entropy for detection of non-noxious stimulation during different clinical levels of sedation. Acta Anaesthesiologica Scandinavica 2007; 51: 1-7.

4. A historic view

The discovery of electrical influence in nerve and muscle action by Luigi Galvani (1737-1798) opened up for many new aspects of science, including the viewing of vital processes as basically electrical. The first development of electro dermal research was related to psychological factors in relation to electro dermal phenomenon's. Initially, Romain Vigouroux described changes in the skin electrical resistance. The German physiologist Ludimar Hermann was a pioneer on electro dermal research, using the skin potential. He developed the hypothesis that the excitation current was connected to activity of the sweat glands. In 1878, Hermann and fellow researcher Luchsinger made an important contribution, examining the nerve function of the cat. By cutting the sciatic nerve they found that the sweating and also the current stopped in the footpad. The cat was then injected with curare (acts by blocking neuromuscular transmission) and ventilated artificially while the sciatic nerve was stimulated, creating sweat secretion in the footpad. Atropine sulphate (acting as a muscarinic antagonist) was then injected causing both the secretion and the current to stop (1). Lader and Montagu in 1962 demonstrated that atropine abolished the skin conductance without affecting vasomotor activity monitored by pulse volume in human. On the other side, the vasomotor tone was abolished by bretylium (acting by blocking release of norepinephrine from nerve terminals) without affecting the conductance. They concluded that the skin conductance reflex could solely be attributed to the sweat glands (2). In 1972 Hagbarth et al, using skin resistance, more directly confirmed some of these findings. They located a skin nerve fascicle and impaled it with a metal microelectrode by looking for the characteristic, sympathetic efferent, spontaneous activity in the neurogram. After injection of 1% lidokaine (local anaesthetic drug probably acting by blocking spontaneous discharge from damaged sensory nerve terminals) proximal to the recording site, the spontaneous activity promptly disappeared. When the same amount of lidocaine was injected distal to the recording site, the spontaneous burst remained as before (3).

The first researchers on this field concealed their recording methodology by neglecting to make their method clear to the reader, and used the term Galvanic Skin Response (GSR), without taking into consideration that GSR refers only to skin resistance (SR) and skin conductance (SC). In 1966 the term electro dermal activity (EDA) was introduced, including the galvanic skin response (GSR) and the skin potential response (SPR), SPR being a measure of impedance (4). The nomenclature EDA was an attempt to subsume all methods of measuring the electric activity of the skin. Different methods and terminology are still in use.

5. Introduction

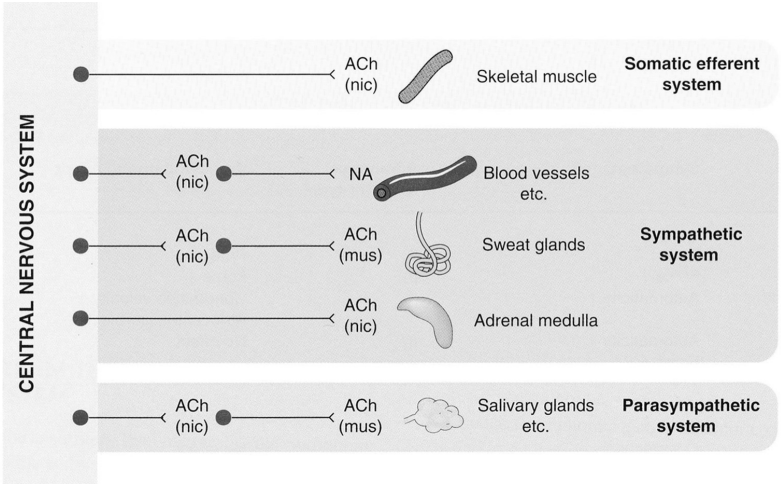
SC measures the activity in the sympathetic nervous system through sweat secretion obtained by electrodes placed in the palm of the hand or under the foot. Skin conductance has previously been used to measure arousal due to emotionally reactions. We hypotetisised that SC in clinical settings could tell us about arousal due to noxious stimulation and arousal that take place during emergence after surgery. Further, that the skin conductance activity would decline when the analgesic level rise.

The technology we used to measure SC was developed in 1995 by the Institute of Physics, University of Oslo. The equipment was further refined into the Skin Conductance Algesimeter®™, including a supporting computer software. Until December 2010, a total of 40 articles have been published using this device.

Nociception is necessary for the preservation of life. The reflex circuit described by Sherrington in 1906, in which activation of nociceptors through harmful stimulus lead to activation of the autonomous nervous system (ANS) and a stimulus triggered reflex withdrawal, was crucial for our later understanding of both the peripheral and central nervous system (5). Noxious stimulus can, through centres in the brain stem, rapidly influence heart rate (HR), arterial blood pressure (ABP) and respiration rate. In spite of the advantageous aspects of the nociceptive capability in humans, the negative effects of pain and stress are many. The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in USA has now officially recognized that pain is a major health problem and “patients have the right to appropriate assessment and management of pain” (6). According to the guidelines, pain should be the “fifth vital sign” to be recorded, along with temperature, HR, respiration rate and ABP. In Italy and France similar guidelines concerning pain management have recently been outlined.

Awake persons, with intact communication skills, are capable of communicating their pain verbally, or by the means of body language. The challenge of pain communication is more evident in artificially ventilated patients, in newborn term and preterm children, and in patients during surgery. Also in drowsy extubated patients after surgery, quantifying pain and balancing pain relief and the possible respiration depression from additional analgesics is a problem. Most objective methods to discover pain in these various groups are based on measurements of activation of the ANS, change in Electro Encephalo Graphi (EEG) activity, or the more subjective clinical observation scoring systems.

Figure 1



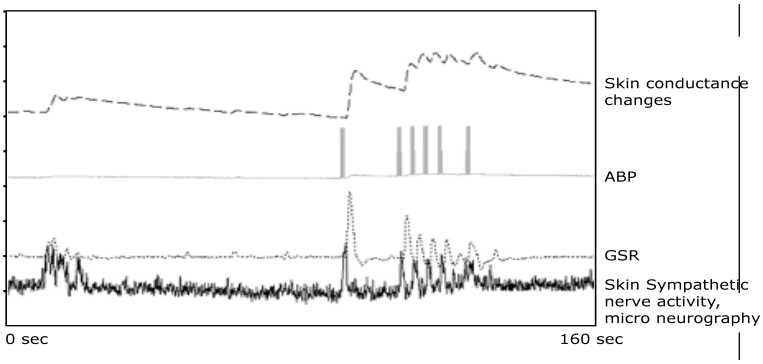
Transmitters in the autonomic nervous system. In this figure it is shown the sympathetic innervation of the sweat glands through acetylcholine acting on the muscarinic receptor.

The Skin Conductance Algesimeter®™ is based on measuring the conductance of sweat in the epidermis in the palms of the hands or under the feet during supposed arousal or noxious stimulations. This objective method attempts to establish, following a slightly different activation path – the release of acetylcholine – whether a patient is in comfort, or if the patient is experiencing pain or discomfort based on activation of the sympathetic nervous system (Figure 1).

To validate the association between SC measured by the technology from Med-Storm, SR, ABP and the activity in a sympathetic nerve, we asked professor Gunnar Wallin at Salhgrenska University Hospital, Sweden, to insert microelectrodes in the right nervous medianus of one of the investigators while making simultaneous recordings with microneurography. The compiled data (7, Figure 2), gave a close connection between galvanic SC and the activity in nervous medianus and an ensuring validation of the SC equipment.

The skin participates in the regulation of core body temperature and the water balance of the body. The sympathetic nervous system regulates the vasomotor, pilomotor and sudomotor effects in the skin. Sweating can be either an indication of arousal, or thermoregulation, and is, by activation of eccrine sweat glands, the physiological function underlying SC. The sympathetic branch of the ANS innervates the eccrine sweat glands, but acetylcholine is the chemical transmitter at the postganglionic synapse, acting on muscarinic receptors.

Figure 2



Experiment at Sahlgrenska University Hospital to explore the connections between SC, microneurography, ABP and the galvanic skin response. Each burst in the nervous medianus was accompanied by a skin sympathetic nerve activity, fluctuations in GSR, increase in ABP, and SC fluctuations in the SC equipment from Med-Storm.

6. The skin and eccrine sweat glands

6.1. Anatomy

The skin consist of three layers; epidermis, dermis and sub cutis. The superficial layer of the skin, the epidermis, evolves from the ectoderm. The ectoderm also gives rise to the sweat glands through epidermal proliferation. The epidermis is divided further from outer layer to inner layer: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. Epidermis is avascular and nourished by diffusion from the dermis. SC reflects the ionic conduction in the stratum corneum, which is largely determined by sweat duct filling. The underlying dermis develops from the mesenchyme (8).

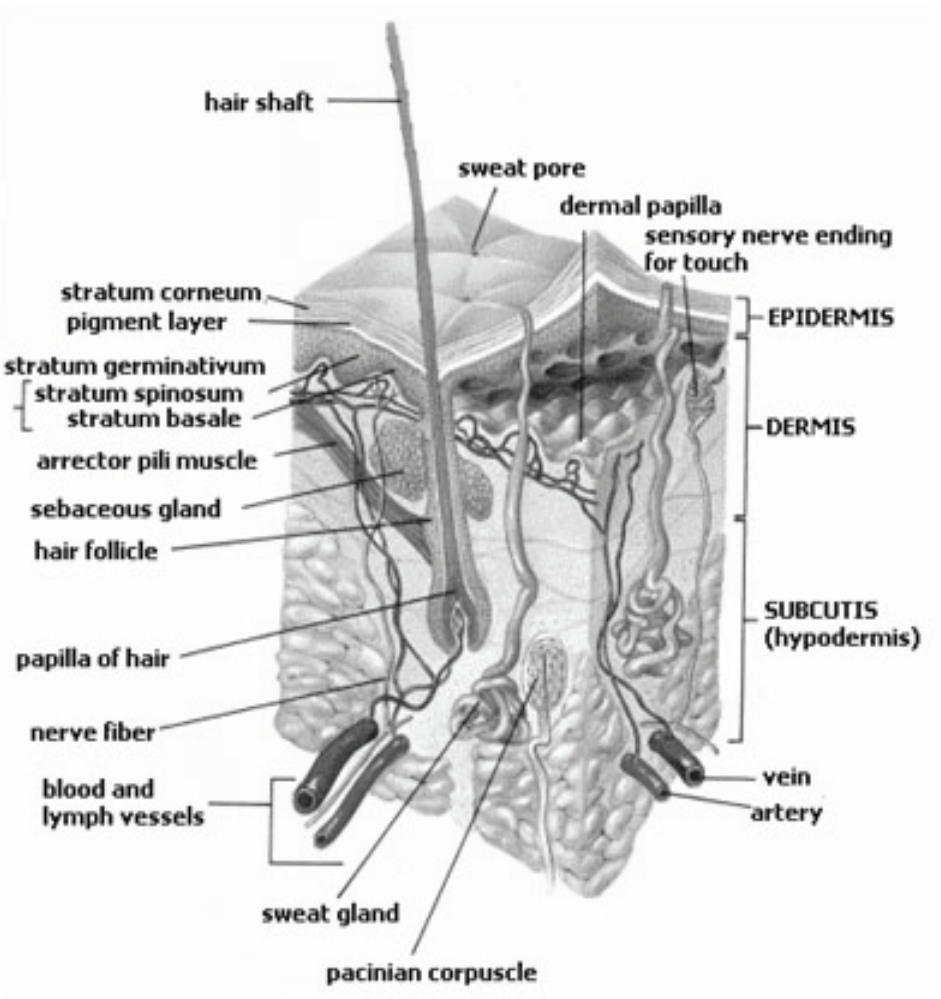
The vasculature of the skin in the palm of the hand and feet are arranged in horizontal layers, with deep plexuses in the sub cutis, atriovenous anastomoses (AVAs) in the transition from sub cutis to dermis, sub epidermal plexuses in the dermis and finally capillary network right beneath the basal layer of epidermis. Through AVAs blood is allowed to enter the venous circulation directly without passing the capillaries. The AVAs are found at approximately the same depth as the eccrine sweat gland tubules.

The human body has a total number of 1.6-4 million eccrine sweat glands (9). The density of eccrine sweat glands varies widely through the body; Sato et al found approximately 108/cm^2 on the forearm and 600 – 700/cm^2 on the palms and soles (9). The eccrine sweat glands are tubular structures deep in dermis and sub cutis with secretory ducts that open as small pores on the surface of the skin (Figure 3).

The lower portion of the eccrine sweat glands consist of a tightly coiled secretory structure consisting of three types of cells; dark basophilic cells that secretes mucous material, light acidophilic cells that is in charge of passage of water and electrolytes and smooth muscle cells that stiffens the sweat capsule to aid fluid expulsion. Surrounding these cells are myoepithelial cells whose contraction is thought to aid the expulsion of sweat (Figure 4). Laser fluorescence microscope techniques have shown that the eccrine sweat gland is innervated by nerve axon that run parallel to the sweat tubule and then encircle it. Nearby capillaries supply the fluid. Sweat ducts ascend toward the skin surface accompanied by longitudinally oriented nerve fibres and capillaries (10).

The apocrine sweat glands secrete a fluid rich in precursors of odoriferous substances and are mainly found in the armpits and in the genital area, are relatively large, and are always connected to hair follicles (11). The apocrine sweat gland consists of columnar secretory cells with a short and thick secretory duct.

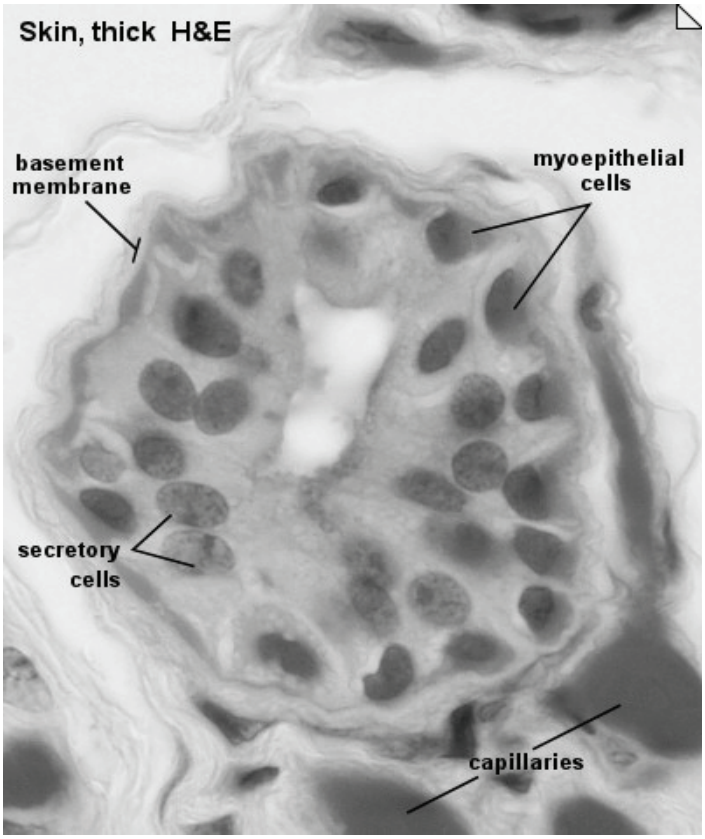
Figure 3



A transection through the human skin. The eccrine sweat gland is seen as a tubular structure deep in dermis towards hypodermis with excretory duct running through dermis and epidermis and the sweat pore which opens at the surface of the skin.

In the late 1980-ties, a third type of glands were discovered among the axillary glands, called the apoeccrine glands. The glands varies in size, but are mostly intermediate in glandular size and have like the eccrine glands a long sweat duct. When it comes to cellular types, the apoeccrine sweat glands resemble both eccrine and apocrine sweat glands. The apoeccrine sweat glands are not found before puberty and are only located in the armpits (9).

Figure 4



Histology section through the eccrine sweat gland stained with haematoxylin and eosin. Dark basophilic cells and light acidophilic cells are surrounded by smooth muscle cells and nearby capillaries.

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http://www.lab.anhb.uwa.edu.au/mb140/corepages/integumentary/integum.htm'

6.2 Physiology

The main function of the epidermis is to be a barrier against the outer environment. The epidermis also, when exposed to sun, facilitates the production of vitamin D3. The dermis and the sub cutis play a role in regulating temperature, in giving information to the central nervous system (CNS) through mechanoreceptors, thermo receptors and nociceptors, and in contributing to regulate ABP through the baroreflex system (12).

Eccrine sweat glands outnumber apocrine glands and are known to be responsive to arousal as well as sensory stimuli. The main function of the eccrine sweat glands is thermoregulatory. The eccrine sweat glands are especially dense on palmar and plantar surface of the hand and feet respectively, but on these locations they respond weakly to heat and here they play virtually no role in thermoregulation (13).

The apocrine sweat glands do not play a role in SC, but in contradiction to the eccrine sweat glands, they are under adrenergic influence. By immunohistochemistry and light microscopy, no nerve fibres are found near the apocrine sweat glands, but the apocrine glands present beta-receptors in the secretory part of the glands, suggesting activation through circulating catecholamines (11).

The apoeccrine glands are in vitro stimulated to extremely copious sweat secretion both by epinephrine and methacholine and might thus be activated both adrenergic and cholinergic (9).

The CNS control of the AVAs is related to body core temperature and is not related to local skin temperature (14). Norepinephrine vasoconstrictor fibres innervate the AVAs (14).

Anhidrosis is a decrease in or a lack of sweat secretion, due to e.g. autonome neuropathy, Guillain-Barre syndrome or congenital disorders, including ectodermal dysplasias. These rare diseases would probably affect SC.

Miscellaneous studies have suggested that, in children with various metabolic, genetic or chromosomal diseases, the length of the sweat duct and the coil volume affects the amount of sweat secreted from eccrine sweat glands. In children with cystic fibrosis (a disease known for abnormal sweat electrolyte composition), anatomical features of the eccrine sweat glands are normal. For these patients the conductivity of the skin is elevated, probably due to increased sodium chloride content. Children with trisomy 21, chronic renal disease and tetralogy of Fallot, even though they have normal sweat composition, carry an abnormal anatomy of the sweat glands (15).

6.3 Innervation and sympathetic activation

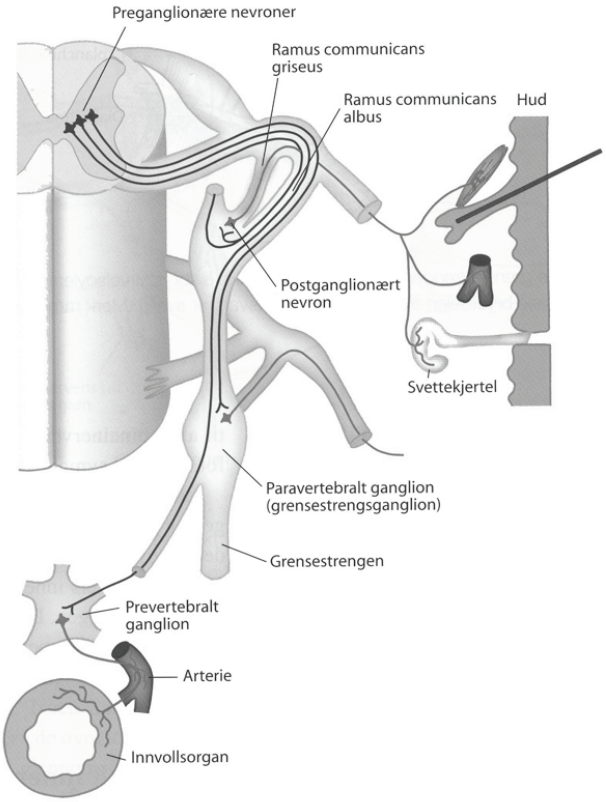
The sympathetic division of the ANS innervate the sweat glands. The exact pathways from the brain to the sweat glands are still not entirely understood, but animal studies draw a connection from hypothalamus via pons to the raphe nucleus regions of the medulla (9). From the medulla these fibres connect to the preganglionic sympathetic neurons. The preganglionic sympathetic neurons have their cell bodies located in the intermediolateral cell column in the lateral horn of the grey matter – from Th1 to L2 in the spine. The preganglionic fibres leave the CNS through the ventral root and enter the postganglionic sympathetic neurons in the paravertebral sympathetic chain. The autonomic ganglion releases acetylcholine and activates the postsynaptic nicotinic receptors. The postganglionic C-fibres run through ramus communicans, following the peripheral somatic nerves (9) to the effector organ – i.e. the eccrine sweat glands (Figure 5).

Immunofluorescence microscopy has provided a three-dimensional visualization of the sensory and sympathetic nerves in the skin. In research on humans, by using specific markers like dopamine β hydroxylase (DβH) (as a marker for noradrenergic fibres), protein gene product (PGP) (as a marker for nerve fibres) and vasoactive intestinal peptide (VIP) (as a marker for autonomic fibres), Donadio and colleagues found a rich supply of noradrenergic fibres in arteriovenous anastomoses, erector pilorum muscles and arterioles. Around sweat glands however, few adrenergic fibres were found (16). On the other hand, the sweat glands received a dense autonomic innervation with fibres running longitudinally or encircling the sweat tubules (16). In animal studies, by the use of immunohistochemical methods, and using VIP and the vesicular acetylcholine transporter (VACht) (as a marker for cholinergic fibres), these autonomic fibres have been acknowledged as cholinergic (17). Because of the close proximity of both adrenergic and cholinergic fibres to the eccrine sweat glands, assumptions have been made that circulating catecholamine's stimulate sweat gland activity (18). It seems however as if catecholamine's cannot elicit sweat secretion but that catecholamine's are, like acetylcholine, needed for triggering the final maturation of the glands and for inducing secretory responsiveness (18). A parallel development of adrenergic and cholinergic fibres in the paravertebral chain prenatal is observed. During development, a very early differentiation and segregation occur, leading to functional cholinergic innervation of sweat glands from early postnatal days (18).

The postganglionic sympathetic fibres use norepinephrine as transmitter and neuropeptide Y as co-transmitter to induce vasoconstriction in the microcirculation. Acetylcholine, together with VIP as a co-transmitter, is suspected to facilitate active vasodilatation, and in addition,

histamine and nitric oxide (NO) are probably involved in the vasodilator system of the microcirculation (19).

Figure 5



A drawn picture of the peripheral sympathetic nervous system. Preganglionic cellbodies in the intermediolateral cell column in the lateralhorn of the gray matter. The preganglionic fibres leave through the ventral root and enter the paravertebral sympathetic chain. Postganglionic fibers follow peripheral somatic nerves to the end organ, e.g. the eccrine sweat gland.
Hud - skin, Svettekjertel - sweat gland, Grensestrengen - paraverebral sympathetic chain, Arterie - artery, Innvollsorgan - internal organ, Preganglionære nevroner - preganglionic nevrons.

7. Skin conductance

7.1 Clinical use of skin conductance

Noxious stimuli influence SC (20 - 30, paper I and paper II), and SC is correlated with changes in norepinephrine levels in the blood during tracheal intubation (24).

Clinically, SC have been used to asses noxious or arousal stimuli in preterm and term infants (20, 21, 24, 28, 31 - 37, paper I), before introduction of general anaesthesia in pre- and not premedicated patients to see if analgesics or benzodiazepines influence the SC response (38, paper III), during intubation (23, paper II), during general anaesthesia (22, 23, 30, paper II), during emergence (39 - 41, paper II), to predict hypotension following spinal anaesthesia (42, 43), and to asses postoperative pain in children and adults (44 - 47).

SC provides a direct proportional representation of the number of sweat glands under the electrode involved in the response (48 - 50). SC yields a significantly shorter recovery time than skin resistance (49). Although, because the two are reciprocals, main findings in studies using skin resistance are transferable to studies using SC.

Sympathetic nerve stimulation gives synchronic eccrine sweating within 1 to 2 seconds after nerve stimulation, but does not change the blodflow in the skin (51, 52).

In a study by Jacobs et al, including patient with mild hypertension, baseline values were obtained for the derivate of the SC curve, systolic BP, diastolic BP and HR, all in the absence of medication before and during arousal stimuli. The arousal stimuli gave rise in the derivate of the SC curve, systolic ABP, diastolic ABP and HR (53). Secondly, the same authors performed a dobbelblinded placebo controlled crossover study including patients with mild hypertension using β-adrenergic blockade and measured the same variables. The presence of β-adrenergic blockade induced a reduction in systolic ABP, diastolic ABP and HR during arousal stimuli, whereas the derivate of the SC curve were not influenced by the β-adrenergic blockade (53). Wallin et al found similar effects; when the carotid sinus nerve was stimulated, muscle nerve sympathetic activity (measured by microelectrodes inserted into a muscle nerve fascicle), HR and ABP were reduced, along with no effects in skin nerve impulses (measured by microelectrodes inserted into a skin nerve fascicle) (54). Ledowski compared NSCF, the Bispectral Index (BIS), HR and ABP during emergence from general anaesthesia and found both the NSCF and the BIS monitor to distinguish clinical states during emergence better than ABP and HR (40, 41). Consequently, eccrine sweating is only weakly, if at all, influenced by arterial baroreceptors (51, 54). Thereby, SC is fairly robust towards circulatory and ABP changes, and also towards most adrenergic receptor active agents.

SC is likely to show low interindividual differences in children at rest without pain. In paper I the children were initially totally calm according to the Comfort sedation score. The NSCF had at the same time a median of zero. This should implicate a limited interindividual difference. In a study of 15 preterm and term born infants at behavioural state one (eyes closed, not moving, no pain) observed for 48 hours and obtaining SC six times for 30 minutes, the intraindividual variability was low (55). These findings are supported by studies using skin resistance. Skin resistance was measured repeatedly during nerve stimulation in awake adult subjects. Within the same individual limited differences were obtained between the measurements and also between the subjects, the differences were small (56).

It is, of course, difficult to estimate the level of pain during general anaesthesia if awareness does not occur, but our study with tetanic stimulation shows that skin conductance is sensitive to nociceptive stimuli during different analgesic levels of general anaesthesia (paper II). SC has also shown promising results in the ability to predict severe hypotension in the elderly (42), a common problem after administration of spinal anaesthesia. This results was not confirmed when SC was used to predict hypotension from spinal anaesthesia during caesarean delivery (43).

The sensitivity and specificity to discover nociceptive stimuli in anesthetized patients have both been estimated to 86% (22).

The sensitivity to discover moderate to severe pain in awake adults was from 77% to 89 %, and the specificity changed from 53 % to 74 % (42, 44, 45).

This method shows a high correlation with nociceptive and arousal stimuli down to 29 weeks gestational age (15, 33, 34, 37) and two studies even showed high sensitivity in preterm from a gestational age of 24 weeks (35, 36).

In one study the sensitivity and specificity to discover severe postoperative pain in children were respectively 56.3% and 78,4% (46), while the sensitivity and specificity to discover moderate postoperative pain in children were 51.9% and 71,0% (46). It must however be noted that this study exceeded the recommended sampling rate of 15 seconds and a monitor refreshment rate of 15 seconds for SC, using instead a refreshment rate of 60 seconds.

Another study on postoperative children, using the recommended 15 seconds sampling rate and a monitor refreshment rate of 15 seconds for SC, found a sensitivity of 90,3% and a specificity of 64,0% to predict moderate to severe pain (47). NSCF increases by the severity of postoperative pain (44, 45) and NSCF might, with 97% probability, predict the absence of moderate to severe pain in postoperative paediatric patients (47).

Despite this, these different studies show that, when compared to anaesthetized patients, the specificity to pain and nociceptive stimuli decrease in awake patients.
In an assessment of postoperative pain, patients quantified their pain on a numeric rating scale (NRS) from 0 to 10 rated by the patient, where 10 was severe pain and 0 no pain. The Skin Conductance Algesimeter®™ index showed possible pain at NRS > 4 and NSCF 0.27 and probable pain at NRS > 6 and NSCF 0.33 (45). After administration of a bolus dose of fentanyl to the patients recognized to be in pain, the NSCF fell significantly (45).
Different from the mean SC level, the NSCF is not influenced from environmental temperature. Thus, normal room temperature variations do not influence the NSCF (13).
As suspected, when reversal drug affecting cholinergic transmission is used after surgery, the SC method is unreliable when measured in a short time-window from the administration of the reversal drug (57).

7.2 Pharmacological considerations related to skin conductance

In theory, the nerve transmission influencing SC can only be altered at two sites; at the autonomic ganglion (acetylcholine/nicotine) or at the end organ (acetylcholine/muscarine) (Figure 6), but both muscarinic and nicotinic receptors are also present in the CNS.
Anticholinergica have the potential to inhibit the transmission of acetylcholine, synonyms are parasympatholyticum and antiparasympathomimmetics. Anticholinergica include muscarinic receptor antagonist and nicotinic receptor antagonist. Drugs that inhibit acetylcholine synthesis or release also decrease cholinergic transmission.
Cholinergics have the potential to enhance the transmission of acetylcholine. Other used name is parasympathomimetics. This group include muscarinic agonist, nicotinic agonist, and anticholinesterases.
Locally acting agents, with little systemic effects, do probably not influence SC. It is likely that some agents will influence the basal SC level, but will not in clinical doses inhibit the burst of SC after arousal or noxious stimuli. The latter is probably an important differentiation.
Five different muscarinic receptors are found and atropine has the ability to block all of them. The receptor in eccrine sweat glands are found to be the M3 mAChR (muscarinic acetylcholine receptor) (58, 59). Activation of the M3 receptor at the eccrine sweat gland induces excitation, i.e. secretion and contraction of smooth visceral muscles. Other sites for differential muscarinic receptors are the brain, peripheral neurons, gastric parietal cells, the

heart, smooth muscle and secretory glands. All muscarinic receptors are G-protein-coupled and acting fast – mediating their effects within seconds (60).

Since acetylcholine secretion activate muscarinic receptors and thereby eccrine sweat, muscarinic receptor antagonists are thought to modulate autonomic nerve transmission and the SC (2, 61 - 66). The most important muscarinic antagonists are (most common brand names in brackets); atropine, hyoscine/scopolamine (Buscopan), solifenacin (Vesicare), ipratropium (Atrovent), tiotropium (Spiriva), tropicamid (Tropikamid), biperiden (Akineton), pirenzepine (not registered in Norway), and glycopyrrolate (Robinul). These muscarinic antagonist agents are mostly non-selective. Atropine and hyoscine are the only two occurring in nature – atropine in nightshade and hyoscine in thorn apple. Both have capacity to penetrate the blood-brain barrier. Atropine is a competitive antagonist for the muscarinic acetylcholine receptor. Atropine does not, except in toxic doses, completely abolish cholinergic transmission during systemic administration (2). Inhalational drugs are usually partly absorbed into the circulation, and systemic effects can occur. Ipratropium is however poorly absorbed and thus have minimal systemic effects (67). Solifenacin is probably a selective M3 antagonist (68). Tiotropium is a muscarinic antagonist with a long half-life. Biperiden cross the blood-brain barrier and block the M1 receptor but also has some effects in the ANS (69).

Muscarinic agonists include acetylcholine and muscarine, but the only clinically used agent in this group is pilocarpine (Pilokarpine), used as eye drops in treatment of glaucoma (59).

There are three main types of nicotinic receptors; muscular, ganglionic and CNS types. Ganglionic nicotinic receptors occur in the autonomic ganglia and CNS types in the brain, whereas muscular nicotinic receptors occur at the neuromuscular junction.

Nicotinic antagonists working at the neuromuscular junction are widely used during general anaesthesia to produce paralysis. They include the non-depolarising blocking agents tubocurarine (rarely used), pancuronium (Pavulon), atracurium (Tracurium), cis-atracurium (Nimbex), mivacurium (Mivacron), vercuronium (Norcuron) and rocuronium (Esmeeron). These agents varies in duration of action between about 15 minutes and and 1-2 hours. Non-depolarising blocking agents do probably not influence SC, because the main nicotinic receptor site is at the neuromuscular junction producing transmission block. Depolarising blocking agents like suxamethonium (Anectine/Qeulicin/Scolin) are, because of a number of side effects (e.g. malignant hyperthermia), and are basically only used when a fast onset and/or short duration of effects is important.

Nicotinic antagonists working at the autonomic ganglia may influence SC, but are rarely in use due to side effects stemming from the widespread distribution of nicotinic receptors. An exception is trimetaphan (Arfonad), sometimes used to produce a controlled hypotension during anaesthesia (59).

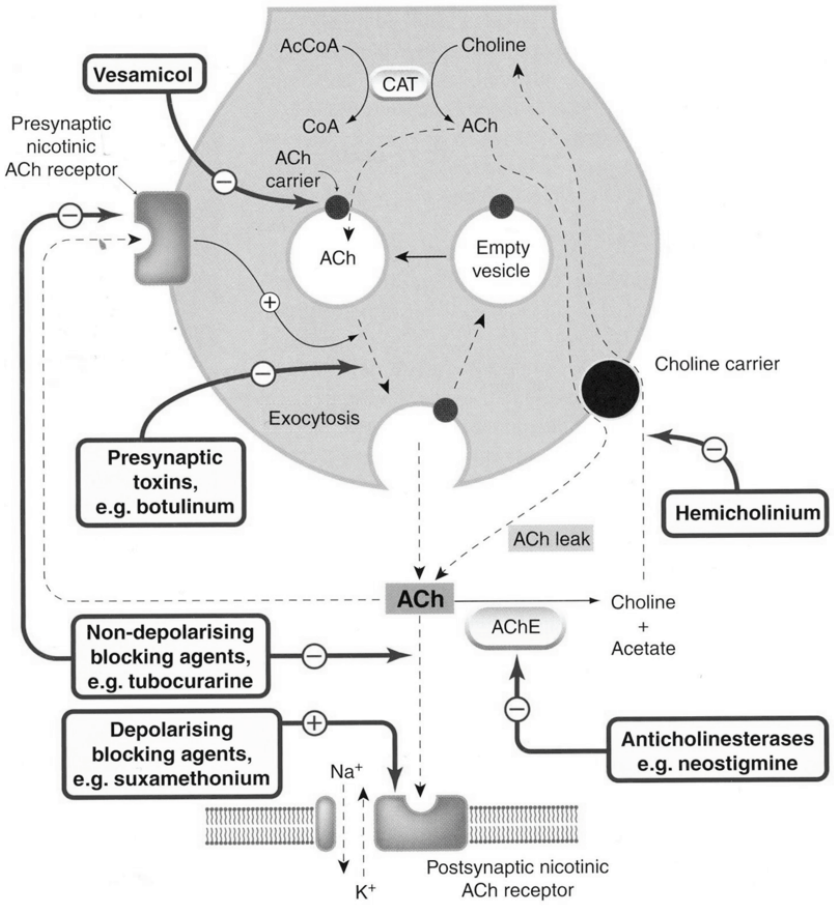
The most important nicotinic agonist is nicotine, used in nicotine replacement therapy. The effect of nicotine is complex and dependent on accumulated consumption. Initially it works excitatory at the peripheral ganglion, but a rapid tolerance is developed. If large doses are obtained, a ganglion block is produced. At CNS level, regular use leads to a substantial increase in the numbers of nicotinic receptors. Plasma nicotine concentration has a half-life of 10 minutes (70). In intensive care and during elective surgery, preoperative cigarette smoking does probably not affect NSCF, because of it´s short half-life.

Inhibiting acetylcholine synthesis or release presynaptically, may block the autonomic nerve transmission. The best-known drug is botulinium toxin, injected locally into muscles in treatment of muscle spasms or for cosmetic purposes. Injecting cosmetic botulinium toxin in the forehead could probably affect EEG measurements, implicating a myelographic component. Injecting botulinium toxin in the treatment of e.g. axillary-, palmar- or sole hyperhidrosis decrease skin conductance (71). Acetylcholine release could also be inhibited by aminoglycosides and magnesium (inhibiting calcium entry into the nerve terminals) - paralysis being an unwanted side effect.

Drugs inhibiting cholinesterase activity increase cholinergic transmission. Most commonly used drugs include neostigmine (Neostigmine, in treatment of myasthenia gravis or as a reversal agent after surgery), pyridostigmine (Mestinon, in treatment of myasthenia gravis), physiostigmine (Fysiostigmin, as eye drops in treatment of glaucoma) and donepezil (Aricept, in treatment of Alzheimer´s disease). Postoperatively, the NSCF is attenuated when reversal agents that inhibit cholinesterase e.g. neostigmine or block the muscarinic receptor e.g. glycopyrrolate, are used (57).

Adrenergic beta-antagonists reduce the release of NA from nerve endings, adrenergic alpha1-antagonists induce reduced sympathetic activity in the CNS, but neither of them is supposed to influence muscarinic SC. The two adrenergic alpha 2-agonists, clonidine (Katapresan) and alfa-metyldopa (Aldomet/Dopamet/Aldoril), might influence SC by acting in CNS more like analgesics substances. The analgesic effect is due to stimulation of inhibitory alpha 2-receptors in descending pathways in the dorsal horn of the spinal cord, inhibiting the release of nociceptive neurotransmitters (72).

Figure 6



In this figure the nicotinic cholinergic synapse. In the muscarinic cholinergic synapse, non-depolarising blocking agents are interchangeable with a muscarinic antagonist, e.g. atropine, and depolarising blocking with a muscarinic agonist, e.g. acetylcholine. At the muscarinic cholinergic junction the postsynaptic receptor must be a muscarinic acetycholin (G-protein-coupled) reseptor.

7.3 Central control of skin conductance

Different tools have been involved in exploring the CNS structures involved in activation of SC; SC changes during direct electrical stimulation of intracerebral structures (73), SC changes during functional Magnetic Resonance Imaging (fMRI) (74, 75) and SC induced changes in regional Cerebral Blood Flow rCBF as measured by Positron Emission Tomography (PET) (76). SC responses have also been measured in brain-damaged subjects (77).

SC responses are also related to emotional arousal. This knowledge has been used in psychophysiology. When subjects are gambling, lying, exposed to decision-making tasks, in fear or receive electrical shock of non-painful character, activity is seen in both SC, fMRI and PET scan (76, 78 - 81).

Impaired SC response is observed with damage to ventromedial frontal region and to the anterior cingulated gyrus, whereas extensive damage to the right inferior parietal region tends to abolish SC response (77). One study, using MRI, suggested that prefrontal cortex, pons and the amygdala area are involved in SC changes (75). By direct stimulation of the amygdala, anterior and posterior hippocampus or anterior cingulated gyrus (limbic structures) with intracerebral electrodes, strong ipsilateral and weak contralateral SC changes were evoked. This may be explained by the limited connections existing between the right and left limbic structures (73). With direct stimulation of frontal cortical convexities, bilateral SC response was observed to be either weak, absent or bilaterally equal, possibly because of quick intrahemispheric transfer (73).

SC and fMRI studies, on subjects performing biofeedback (the persons capacity to, by obtaining feedback of one´s own physiological response, influence the state of arousal), pointed towards a difference between neural systems controlling the level of basal sympathetic tone and neural systems controlling transient burst of SC. Increased activity in SC response was reflected in activity within striate and extra striate cortices, anterior cingulated and insular cortices, thalamus, hypothalamus and lateral prefrontal cortex (79). For persons able to control their state of arousal, there was a change – measured by the SC level – in the activity of the orbitofrontal cortex.

Mapped by fMRI, brain activity in anterior cingulated cortex, amygdala, thalamus and hypothalamus is observed during noxious stimuli inducing SC responses (74).

Despite the differences within the presented studies, it is clearly established that the production of SC responses is not merely a result of reflexes at brain stem level.

7.4 Changes in skin conductance with age, gender, weight and race

Gestational age seems to have little influence on emotional sweating. When it comes to gestational age, new born children may be divided into three main groups, preterm: < 37 weeks, term: ≥ 37 weeks, post term: > 42 weeks. The preterm children may again be divided into extremely preterm children: < 28 weeks or birth weight < 1000 gram, very preterm: < 32 weeks, and moderately preterm: 32-36 weeks. Very preterm children have a low waterloss from sweat in the palms and soles and a high skin waterloss. Inversely to the rest of the body, the waterloss from the palms and soles rises within the first month in both moderately and very preterm children. This is interpreted as the onset of emotional sweating (81, 82). The cutaneous sensory receptors are present in all cutaneous and mucosal surfaces by the 20th gestational week in the human foetus. This is preceded by development of synapses between interneuron's and sensory neurons in the dorsal horn of the spinal cord. By 30 weeks various types of neurotransmitters, laminar arrangements and synaptic interconnections thought necessary for nociception are completed in the dorsal horn of the human foetus and myelination of the pathways to the brainstem and thalamus is completed (83). Foetus down to 23 weeks gestational age, have during invasive procedures (e.g. prolonged needling during infusion into the intrahepatic vein), an increase in foetal plasma cortisol and the concentration of b-endorphin (84). At shorter duration of cannulation into the intrahepatic veins or by inserting the needle into the placental cord, similar hormonal stress response was not found (84). During heel incision in very preterm children, from 25 weeks gestational age, there is an increase in NSCF (15). When measuring mean SC level only, the rise is only significant in term children (21). A decrease in mean SC level and thus an increase in skin resistance is seen with old age (85), but although older persons have lower mean SC level at rest, there are no differences between the older and the younger persons when it comes to NSCF responsiveness (48, 86). This implicates that the age dependent changes are due to a change in the peripheral effector systems; e.g., a decrease in number of active sweat glands (48, 87). Further, the menstrual cycle provides a gender-related difference. There is a decrease of activity in sweat glands during the luteal phase of the menstrual cycle (88) and during administration of progesterone (89). SC level is found to be lower at rest in females than in males in all ages, although women shows greater responsitivity during physiological activation (86). With obesity, it is not known whether low sympathetic activity is linked to weight gain or if low sympathetic activity is a consequence of elevated body fat, and if these changes may influence SC. Krohnholm et al found no connection between Body Mass Index (BMI) and sympathetic activity in a sample of nearly 200 individuals, even though BMI by

others has been suspected to influence the activity of the sympathetic nervous system (90, 91).

When it comes to the influence of race on SC, findings are diverse. It seems like white children have higher mean SC level than black, but no significant difference is found between black and white when it comes to SC responses (92).

8. Nociception and pain

8.1 Definitions

In 1906, Sir Charles Sherrington brought to common use the term "nociception" and separated it from the term "pain" in his book "The integrative action of the nervous system: a centenary appreciation" – bringing vital new understanding on the organization of the CNS – (5).

In 2008, "The Kyoto protocol of IASP basic pain terminology" listed and defined a number of definitions concerning pain (93);

Pain: "an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage".

Nociceptive stimulus: "actual or potentially tissue damaging event transduced and encoded by nociceptors". It is important to notice that these stimuli may cause pain, but also that "not all noxious stimuli are adequate stimuli of nociceptors" in order to cause pain.

Nociceptive pain: "pain arising from activation of nociceptors".

Neuropathic pain: "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system".

Sensitization: increased responsiveness of neurons to normal stimulation.

Allodynia: "pain due to a stimulus which does not normally provoke pain".

Hyperalgesia: "increased response to a stimulus which is normally painful".

The term "suffering" is more vague and refers to the affective conscious experience of unpleasantness and aversion.

8.2 Pathways for noxious stimuli

There are a number of subclasses of nociceptive pain stimuli, but the most commonly used classification is into the cathegories of thermal, mechanical and chemical. Another way of sub classifying nociceptive pain is as superficial somatic, deep somatic and deep visceral.

Aδ-fibre nociceptors have a high conduction velocity (2-30 m/s) (94) and are responsible for the sharp, pricking, first pain (95). C-fibre nociceptors have a slower conduction velocity (< 2 m/s) and are responsible for sustained burning pain and second pain sensation (94). Aδ-fibre nociceptors show adaptation to repeated heat pulses in younger patients (96). The difference in conduction velocity is due to differences in myelinisation.

Nociceptive afferent fibres perceiving pain are located as free nerve endings. The transmission from potential peripheral tissue damage to electrical activity in nociceptors is

complex and yet not fully understood, but the NMDA receptor, the vanilloid receptor and the Acid Sensing Ion Channels are identified as playing different roles (97). The neurons enter via the dorsal root and synapse in the grey matter of the dorsal horn and then decussate in the anterior white commissure to the contra-lateral side of the spine. Here the axons of the lateral spinothalmic tract ascend the length of the spine through medulla oblongata and the formatio reticularis and synapse in thalamus with tertiary sensory neurons. The third neurons send their axons towards the primary somatosensory cortex (98). In the spinal cord afferent signals are modulated via inter-neurons. There is also communication between higher level neurons of the CNS and the lower level autonomous reflexes, influencing e.g. ABP, HR and emotional sweating in response to pain. Some spinal neurons do not end in lateral spinothalmic tract and some of these smaller pathways are thought to interpret the experience of pain (94). Newer scientific research is focused on finding ways of inhibiting the transmission of nociception in the spinal tract, avoiding the many side effects of traditional analgesics.

The C-fibres are responsible for a dorsal-horn neuron activation termed temporal summation or wind-up – a progressive, frequency-dependent increase in the excitability of nociceptive neurons (99). Temporal summation occurs when repeated stimuli become increasingly painful in spite of unchanged stimulus intensity. This is a normal variety, but may play a central role in development of chronic pain (100 - 102). Women have a lower threshold for temporal summation (103) as well as patients with fibromyalgia (101, 102). Through different techniques, including MRI, it has been established that this is a process involving most pain related brain areas (74, 102, 104).

8.3 Physiological activation due to pain and distress:

Both physiological and psychological stressors may disrupt homeostasis in the body. Stressful events can e.g. induce rise in plasma catecholamine's, glucocorticoids, neurotransmitters, neuropeptides and cytokines, mainly by activation of the hypothalamic-pituitary-adrenal (HPA) axis system and the sympathetic nervous system (105 - 112). Macrophages and lymphocytes have receptors present for catecholamines, glucocorticoids, neurotransmitters and neuropeptides (108, 109). Pain, stress and depression can induce suppression of the immune system through altering their functions. Studies of Natural Killer (NK) cells have shown a surgery-induced suppression of NK cell activity and enhancement of lung metastasis (113 - 115). NK cells are a type of lymphocyte who participates in the rejection of tumour cells and viruses. Surgery can result in immune suppression through compromising cellular immunity by affecting the balance between Th1 and Th2 cells (116, 117), decreased cell

mediated immunity (118, 119), and by alterations in NK cell activity (120 - 123). Morphine in analgesic doses could prevent these effects (113). In rat experiments, pain relief pre- and postoperative provide protection against the decrease in host resistance against metastasis (124). At high doses however, opioids also suppress the immune system (125, 126) and make the body more susceptible to metastases (127) and infections (128). Opioids may also cause addiction, respiratory depression, urinary retention, obstipation, nausea, vomiting, hallucinations, itching, dizziness and sleeping disturbances.

Long time administration of analgesic and sedative medications during a prolonged stay at an intensive care unit (ICU) may result in acute withdrawal syndrome (129) and a possible increase in morbidity (130).

Chronic stress is associated with a suppressed specific immune response in the NK cell and the antigen (131, 132). A chronic activation of the sympathetic nervous system and the HPA axis are seen in melancholic depression (109, 112). People with high levels of perceived stress have a greater risk of rhinovirus infections (133), and persons with low levels of NK cells are more prone to infections in the upper respiratory tract (134). Foetal activation of the HPA axis is also associated with growth retardation (135), and activation of the sympathetic system and the HPA axis participates in stress suppression of the immune-inflammatory reactions (107, 136, 137) as seen in long-term excessive exercise (138). Low levels of NK cells and T-cells, impairment of the DNA repair and increased cancer risk are seen in individuals after separation and divorce (139, 140).

8.4. Pain in infants

It was for a long time believed that, due to neurological immaturity, neonates had no conscious cortical experience of pain (141). As a consequence, newborns often received no analgesic or anaesthetic medication during invasive procedures, including surgery (142 - 146). In neonates without anaesthesia during circumcision, plasma cortisol was elevated during and after the intervention (147, 148).

It was observed that boys, at the age of 4-6 month, during vaccination showed more pain than girls during vaccination. Tests were done to see if the pain response during vaccination differed for boys having been circumcised without anaesthetics at the age of 5 days or less. The circumcised boys, month after being exposed to the surgery, showed a stronger pain response during routine vaccination – only to be explained by a sensitization to pain from the circumcision (149). Increased hormonal responses to surgical stress in neonates have been associated with increased incidence of postoperative complications and even deaths (150 -

152). In extremely preterm children (birth weight less than 800 grams) at 8 months corrected age, a significantly higher basal HR is found compared to term born children at 8 months (153). Extremely preterm children are also found to have higher cortisol level and higher sustained cortisol levels after exposure to noxious stimuli at 8 months corrected age compared with very preterm children and term children (154). Neonates in the PICU find non-invasive routine procedures stressful, and are also more exposed to painful skin-breaking procedures (33, 155, 156). Still, vein puncture does not seem to be a strong enough or long lasting enough stimuli to result in rise of plasma levels of cortisol, epinephrine or norepinephrine (157). Tracheal suction and routine procedures in respiratory distressed neonates results in prolonged hypoxemia. In respiratory distressed neonates exposed to the same procedures after receiving pain relief with opioids, the duration of hypoxemia is shorter. Pain relief with opioids did not influence ABP, HR, cortisol level or glucose level in respiratory distressed neonates (158). It is reported that neonates receiving morphine are long term protected against cardiac reactivity to pain (159). A connection has been established between previous tissue damage from surgery and a raised demand for intraoperative fentanyl, higher Comfort score, and higher plasma levels of catecholamine's when the surgery again affects the same dermatome as prior surgery (160). During painful procedures in premature infants, increased intracranial pressure has been measured (161 - 164) and there are also indices that this could result in severe intraventricular haemorrhage (165).

During nursing procedures lasting for about 10 minutes, neonates showed a significant increase in nor adrenaline levels, but during heel prick, just a no-significant increase occurred (156). The SC increased during heel prick, but was not reduced by oral sucrose and milk unlike crying time and behavioural state (166). This could indicate a difference in behavioural response to noxious stimuli and a physiological response to noxious stimuli.

8.5 Pain during hospitalisation and intensive care

After the implementation of the JCAHO standard in the United States in 2001, Vila et al performed a study to evaluate the safety with the use of numeric pain rating scales as guidance for opioid delivery. According to their findings, the patient satisfaction improved, but at the same time, the adverse drug reactions due to opioid over sedation increased (167). Long after having been discharged from an intensive care unit, the patients can struggle with post-traumatic stress syndrome (PTSD) and depression (168 - 171). This can be associated with the number of days spent with sedation and neuromuscular blockade (168). Daily

interruption in sedative medication may reduce the incidence of PTSD (169). It is challenging to remove artificial ventilation and sedation in the ICU patient, and in a trial performed by Girard et al they attempted to find a better routine practice (172). The control group went through daily trials with spontaneous breathing. The intervention group had daily trial with spontaneous breathing together with daily interruption of all sedatives and analgesics used for sedation to look for signs of spontaneous awakening without signs of serious adverse effects (e.g. pain, agitation). Girard et al concluded that lives could possibly be saved if daily combination of sedation and ventilator weaning are completed for mechanically ventilated and sedated patients (172). Patient with a memory of delusions from a stay at the ICU, but without any real memory of the event, are more prone to develop PTSD related symptoms (170). On the other hand, high daily doses of fentanyl equivalents, lorazepam equivalents and neuromuscular blockers are associated with withdrawal during drug weaning (129), prolonged ventilation, delirium and death (129, 173).

8.6 Pain during general anaesthesia

From a patient point of view, one of the most feared complications in the operating theatre, is awareness or conscious experience of pain during surgery, basically occurring most frequently when neuromuscular blockers are used and the patients are not able to move as an important signal of distress.

In a study by Sandin et al, nearly 12000 patients who had undergone general anaesthesia were included, and 18 cases of awareness were identified (174). Of the 18 patients experiencing awareness, 14 were paralysed with muscle relaxants, which was a predictor for the patient experiencing the awareness as a more serious event. 7 of the 18 patients who reported awareness reported intraoperative pain (174). In a study by Sebel et al 25 cases of awareness were identified in a multicentre study including 19500 patients undergoing general anaesthesia, of these 25 patients, 19 received muscle relaxants and 7 reported to have experienced pain during the event (175).

Recent reviews estimate the incidence of awareness with recall to be in the range of 0.13–0.18 % (174, 175) with additional 0.24% cases of possible awareness (175). A Finnish study showed the incidence of awareness to be 0.4% (176). During cardiopulmonary bypass, awareness incidence has been reported as high as 1.14%, possibly because of the complexity associated with this kind of surgery, difficulties in avoiding periods of light anaesthesia, and fear of haemodynamic effects of high dose anaesthesia (177). Light levels of anaesthesia are also separately identified as being a risk factor for intraoperative awareness (176). In obstetric

patients undergoing general anaesthesia for caesarean section, the incidence of awareness was 0.26% and among paediatric patients as high as 0.6% (178, 179), probably due to lower doses of anaesthetics in order to protect the children against unwanted side effects.

8.7 Postoperative pain

Postoperative pain is a major complaint in patients after surgery. In addition to be unpleasant, postoperative pain is also shown to increase the morbidity; i.e. increased immobilization with risk of pulmonary complications, thromboembolism, cardiovascular morbidity and infections (180).

Considerable efforts have been made to reduce the incidence of postoperative pain, still the occurrence of moderate to severe postoperative pain is high (181). As much as 10-50% of the patients experience persistent pain after acute postoperative pain, and 2-10% develop severe chronic pain (182). Meta-analyses have confirmed that postoperative pain control with epidural anaesthesia decreases the incidence of pulmonary morbidity compared to systemic opioids (183).

In general, a multimodal approach with a mixture of different drugs and methods have a better effect-side-effect profile than very high doses of single-drugs, such as the opioids. Examples of multimodal pain therapy include a mixture of paracetamol, NSAIDs, glucocorticoids, and local anaesthesia wound infiltration (184).

Postsurgical pain can be associated with opioid-induced hyperalgesia and with thoracotomy. After high-dose remifentanil a larger allodynia surface is reported after surgery and even increased incidence of chronic pain. With the use of less remifentanil and additional epidural ropivacaine analgesia during thoracotomy, the incidence of allodynia and chronic pain was reduced (185).

A major challenge to pain evaluation and treatment, also in the postoperative setting, is to get an objective evaluation of the patient's pain. So far the VAS scales and verbal scales seem to be the best-documented methods, but there is a potential for more objective postoperative nociceptive stimuli measurements, such as heart rate variation, puls plethysmography and SC (44, 45, 47, 57).

9. Methods of sedation and nociceptive stimulation detection

9.1 Clinical methods

A number of scales are used for assessing the level of sedation and pain in patients. Some are developed for mechanically ventilated patients, some for awake patients, some for children and some for the elderly.

The COMFORT score was developed to assess distress in mechanically ventilated children aged 0 to 18 years (186) and both the inter-observer agreement and internal consistency are high (186). It consisted originally of two physiological items (mean arterial pressure and HR) and six behavioural items (alertness, calmness, respiratory response, physical movement, muscle tone and facial tension). The physiological and behavioural items are found to constitute separate components, at least in children aged 0 to 3 years (187). In paper I, the modification was to exclude HR and BP, thus including only alertness, calmness, respiratory response, physical movement, muscle tone and facial tension. Crying was included only if the child was extubated before the "after" measure.

Observer's assessment of anaesthesia and sedation (OAAS) scoring was introduced to assess sedation with benzodiazepines (188). In paper III, the OAAS scale was used to classify the hypnotic levels of the patient. The modification, based on Struys et al (189), consisted of adding a level 0, to indicate *no response* to painful stimulus. The categories were responsiveness, speech, facial expression and eye appearance. Each category was originally scored in 5 dimensions. The OAAS scale was found to have a high discriminatory power for the different levels of sedation (188).

The Ramsey scale, widely used because of its simplicity, was developed to measure drug-induced sedation in adults, focusing on responsiveness and sleepiness (190). The patient's level of consciousness is divided into six categories. The scale is commonly used postoperatively. The Ramsey score have a good correlation with BIS in sedated children (191). Like all sedation scores based on verbal and/or motor response, the Ramsey score has limitations when muscle relaxants are used (191).

The Critical Care Pain Observational Tool (CPOT) was developed to assess pain in intubated conscious and unconscious ICU patients (192). CPOT ranged into one of four categories including compliance with the ventilator. It has been used both on conscious and unconscious intubated patients with an acceptable reliability and validity. It has now been translated and validated in Swedish (unpublished data) to use as a pain observational tool in adult intensive care units.

Motor Activity Assessment Scale (MAAS) was developed in the state of Utah for assessing sedation/agitation among mechanically ventilated adult patients, and is probably valid and reliable for this use (193). It differs from other sedation scales by including evaluation of agitation.

The Visual Analogue Scale (VAS), widely used for assessing pain in awake subjects, requires patient cooperation, but may also be used for evaluating sedation. The 10-cm ruler scale is probably not useful in monitoring artificial ventilated patients in intensive care. It has proven valid for both acute and chronic pain (194).

9.2 EEG based methods

Accelerated by the introduction of "the bispectral analysis of the electroencephalogram" in 1994 (195), several other methods have been developed for detecting pain in anaesthetised and artificially ventilated patients. BIS evaluates the state of arousal rather than the quantification of pain. The EEG mainly shows four types of waves; beta waves (dominating during wakefulness), alpha waves (dominating during rest), theta waves (in the transition to sleep) and finally delta waves (during deep sleep or anaesthesia). The BIS algorithm is based on these wave patterns, but the exact algorithm is still a well-kept secret. The use of BIS monitoring during anaesthesia lower the use of both volatile and intravenous anaesthetics (196, 197), but in some situations BIS may be inaccurate, due to electromyography activity (198). Using the BIS monitor has provided advantages in avoiding awareness. Although, its ability to do so in a day-to-day clinical setting has been disputed (175, 199 - 201). A large-scale study showed a 77% reduction in incidence of awareness with the use of BIS monitoring during surgery (202). In patients with high risk of awareness the reduction in awareness with the use of BIS was even higher, 82% (199).

Entropy measurements, like State Entropy (SE) combined with Response Entropy (RE), launched by Datex Ohmeda in 2004, also use the EEG technology. Entropy is a measure of the uncertainty associated with a random variable and can be used to quantify the irregularity of the stochastic EEG signals (203). Entropy spectral analysis is used to decompose a complex signal into simpler parts. Entropy can be measured in different ways. A frequency spectral analysis relates signal amplitude with frequency, time spectral analysis relates the signal amplitude with a defined time. The Datex Ohmeda S/5 Entropy Module combines the time spectral analysis with frequency spectral analysis. As the electrical activity of the brain is reflecting its function, the interpretation of the EEG may be used to monitor the level of

sedation, emergence and pain, followed by awakening. Entropy is measured in EEG from the forehead of the patient, with the signal also including an electromyelographic component (EMG), previously seen as an artefact. The facial muscles, are compared to other muscles, less sensitive to neuromuscular blocking medication and EMG signals is still present during light and intermediate levels of anaesthesia (204). The EMG activity implicated in RE was thought to be an indication of pain. When the suppression of EMG is complete, RE and SE is equal. If pain is present, RE will be higher than SE. The SE range from 0 to 91 and the RE from 0 to 100. SE in the range 40 to 60 will contribute to an optimal depth of surgical anaesthesia. SE 0 to 40 provide deep surgical anaesthesia, whereas SE 60 to 91 provides light surgical anaesthesia. Several studies, comparing Entropy and BIS, show that the two methods act in a similar way in sedated patients (205), but show a large intra-individual and inter-individual variability in the indices (206), resulting in difficulties in using them as guidelines for drug administration (207). Response Entropy (RE), in the study of Valjus et al (208), did not prove useful in detecting pain, but it is thought effective in measuring level of sedation (209).

When nitrous oxide (NO) or ketamine are used for sedation or anaesthesia, neither BIS, RE nor SE are reliable, because the EEG shows an “aroused” pattern with these agents (210, 211).

Patient State Index (PSI) is based on spectral analysis and burst suppression patterns in the raw EEG, as is Bispectral Index, but with a different algorithm. PSI has a range from 0 to 100 with a recommended range during general anaesthesia between 25 and 50. Studies comparing BIS and PSI show a variety of results, but overall the PSI seems to be able to measure the level of sedation (212 - 214).

The Narcotrend Index is also based on computerized analysis of EEG, but has incorporated the age-related EEG changes into the algorithm (215). The Narcotrend Index has been evaluated as a depth of sedation monitor and has also been shown to be comparable with the Bispectral Index (216, 217) using the same scaling, ranging from 0 (maximal deep general anaesthesia) to 100 (awake).

The Cerebral State Index (CSI), attempting to make an EEG based algorithm for monitoring sedation level at a lower cost than the Bispectral Index monitor, are likewise scaled from 0 to 100, aiming at surgical anaesthesia between 40 and 60. When it comes to measuring level of sedation, CSI generally mimics BIS, but it is believed that BIS, due to better separation of the myogenic signal components, overall is slightly more reliable (218, 219).

BIS variability has recently been investigated and is again taking the EMG composite into consideration. The EEG variability is quantified from the standard deviation of BIS (sBIS), the standard deviation of EMG (sEMG) and a weighted combination of BIS, sBIS and sEMG. The BIS variability reflects changes in remifentanil infusion during spinal surgery and the BIS variability might thus reflect nociceptive stimuli (220).

9.3 Other methods

Heart rate variability (HRV) measures autonomic control of the sino-atrial node (221). In normal HRV, three frequency components stand out; low frequencies, mid frequencies and high frequencies. The high frequency component is mediated by fluctuations in ventilation, the mid- and low frequencies is mediated by the sympathetic and parasympathetic nervous system (222). The high frequency component is thought to be exclusively under parasympathetic control. This high frequency HR correlation with respiration is termed respiratory sinus arrhythmia (RSA). Induction of anaesthesia is associated with a reduction in HRV dependent on anaesthetic agent (221, 223). RSA have shown to decrease during introduction of anaesthesia in children (222), to correlate with changes in propofol anaesthesia level in adults (224), and to be able to predict emergence from general anaesthesia in adults (225).

Pupillometry is a method in which dilatation of the pupillae is recognized to be a sign of noxious stimuli (226). Pupillometry have been used to evaluate the level of analgesia in anaesthetized children (227), and is also found to covariance with SC during arousal stimuli (228). The dilatator pupillae muscle is innervated by adrenergic fibres from the sympathetic nervous system, whereas the sphincter pupillae is innervated by cholinergic fibres from the parasympathetic nervous system. A dilatation is thus an indication of sympathetic activation.

The surgical stress index (SSI) is one of the newer methods in nociceptive stimuli detection. This method is a combination of normalized heart beat interval and plethysmographic pulse wave amplitude and seems so far promising in detecting surgical nociceptive stimuli and influence from analgesic drug concentration (229 - 231). The pletysmograph measures changes in pulsatile blood volume in the finger as an expression for autonomic induced variations in circulation.

The Noxious Stimulation Response Index (NSRI) is another novel method to monitor noxious stimulation. The NSRI is thought to predict the likelihood of a response to a noxious stimulus based on effect-site concentrations of opioids and an anaesthetic (232).

Pharmacogenomics, finding the individual patients genetic response to a drug, is an alternative approach towards predicting and treating pain. Pharmacogenomics ideally guides the choice of the best drug-treatment available for the individual patient. Genotyping for cytochrome P-450 enzymes for drug-metabolism is one approach in this direction. The CYP2D6 have been investigated in particular. The CYP2D6 is subject to genetic polymorphism – i.e. has allelic variants commonly occurring in the population. The allelic variant of the CYP2D6 of the patient will tell if the patient is a poor metabolizer, a normal metabolizer or an ultra-rapid metabolizer of, among other substances, various opioids. Genetic testing for enzymes and drug metabolism might improve pain related drug treatment and reduce adverse drug effects (233).

10. Aims of the thesis

Our hypothesis were:

Hypothesis 1)

Skin conductance can be used in clinical practice as a measure of pain and noxious stimuli.

A) Skin conductance can detect sympathetic activation during suction from trachea in artificially ventilated children.

B) Skin conductance will rise in patients exposed to known painful stimulation as intubation and tetanic impulses.

C) When increasing the level of analgesia during a standardized nociceptive stimulus, the skin conductance response will attenuate.

Hypothesis 2)

Skin conductance can be used during induction of anaesthesia as a measure of the transition from fully awake to the loss of consciousness.

A) Skin conductance will be able to discriminate between different levels of sedation from fully awake to asleep during propofol induction of general anaesthesia.

B) There will be a measurable difference in response to noxious stimulation when adding remifentanil on top of already administrated propofol.

Hypothesis 3)

Skin conductance can be used as a measure of the transition from general anaesthesia to emergence.

A) Skin conductance will similar to the EEG monitors be able to predict emergence from general anaesthesia.

11. General summary of papers

11.1 Paper I:

Introduction:

We wanted to use skin conductance (SC) as a measure of pain in artificially ventilated children. The aim was to examine how changes in skin conductance, arterial blood pressure (ABP) and heart rate (HR) were associated with changes in the modified COMFORT sedation score during suction from trachea.

The theory was that nociceptive stimulation would induce an outgoing sympathetic nervous burst to the skin, the palmar and plantar sweat glands would fill, and this would create a SC fluctuation.

Methods:

Twenty children, aged from one day to eleven years, all artificially ventilated and circulatory stable, participated. The data were obtained prior, during and ten minutes after endotracheal suction. The number of SC fluctuations (NSCF), the amplitude of SC fluctuations (ASCF), the mean SC level, ABP, HR and the modified COMFORT sedation score were recorded and tested from prior to during, and from during to after suction from the trachea.

Results:

NSCF, mean SC level, ABP and the modified COMFORT sedation score increased during suction in the trachea ($p<0.01$), in contrast to HR and ASCF. Only the NSCF from prior to during and from during to after endotracheal suctioning correlated with changes in the modified COMFORT sedation score ($r^2 = 0.61$ $p = 0.000$ and $r^2 = 0.46$ $p = 0.001$).

Conclusion:

The NSCF correlated with the increase in the modified COMFORT sedation score during endotracheal suction. Skin conductance rose in patients exposed to a known nociceptive stimulation and can thus tell us about skin sympathetic activation.

11.2 Paper II:

Introduction:

We wanted to find out if skin conductance would rise in adult patients exposed to known nociceptive stimuli, such as intubation and tetanic impulses, and if skin conductance could predict emergence at the end of surgery and stop of anaesthetic drug administration. In particular, the purpose of the study was to examine if NSCF and RE-*Δ* correlate with signs of clinical stress during intubation and tetanic noxious stimulation and to elucidate how fast and accurate the entropy and NSCF reacted during emergence from general anaesthesia.

Methods:

20 women scheduled for gynaecologic laparotomy were included. During intubation in remifentanil and propofol general anaesthesia the NSCF and RE-*Δ* were correlated to a clinical stress score. The clinical stress score consisted of summarizing one point for each reactions: large muscle movements, coughing, eye opening, sweating in the forehead, tears, face muscle reaction, and systolic ABP > 130 mmHg. Then, after a washout period, 2 series of tetanic stimuli were given, the first with remifentanil (R+) and the second without remifentanil (R-) infusion. The tetanic pre-stimuli periods were compared to the tetanic post-stimuli periods and R+ was compared to R-. During emergence the responses of the entropy and skin conductance were related to the time of extubation.

Results:

We found that NSCF correlated well with the clinical stress score during intubation, $r^2 = 0.73$ ($p < 0.0005$). RE-*Δ* had a weaker correlation, $r^2 = 0.33$ ($p = 0.007$). During tetanic stimuli the NSCF pre stimuli level was lower than the post stimuli level ($p<0.001$), and the NSCF R+ response was lower than the NSCF R- response ($p = 0.002$). The RE-*Δ* did not show similar differences. During emergence the RE reacted before NSCF and SE ($p = 0.003$).

Conclusion:

NSCF was better than RE-*Δ* to measure clinical stress during intubation and was sensitive to tetanic stimuli at different opioid analgesic levels, different from RE-*Δ*. Both modalities were able to predict emergence at the end of surgery, but RE was fastest.

11.3 Paper III:

Introduction:

We wanted to examine if skin conductance could be able to discriminate between different levels of sedation from fully awake to asleep by use of propofol, and if there would be a change when adding remifentanil to the already administrated propofol dose. The goal of this study was to evaluate if SC and entropy variables would differentiate the responses to a loud sound stimuli, at different sedation levels before induction of general anaesthesia.

Methods:

20 women due for gynaecological laparotomy were included. Modified Observer's assessment of anaesthesia and sedation (OAAS) scoring was used to classify the patient hypnotic levels. 98 dB white sounds were given in five sessions; at OAAS level 5 without propofol, at OAAS level 4-3, 3-2 and < 2 with propofol. At OAAS level 3-2 remifentanil was added, and at OAAS level < 2 the patients received both propofol and remifentanil. The OAAS level 5, 4-3 and 3-2 were obtain before intubation, and OAAS level < 2 was obtained 30 minutes after the start of surgery.

Results:

RE and SE showed a steady decline from OAAS level 5 to level < 2 ($p < 0.01$) and D-SC discriminated between all the different OAAS levels ($p < 0.01$). RE-Δ did not discriminate between any of the OAAS levels ($p = NS$).

Conclusion:

RE, SE and the D-SC level discriminated sound response similarly between the different sedation levels.

12. Material and methods

12.1 Study design and approval.

The regional Ethics Committee for Human Studies approved the study-protocols.
The study designs were prospective, clinical trials. In one study (paper II), the patients were randomly divided in two groups. All clinical trials were preformed with one investigator handling the monitoring equipment (H.S) blinded to the other investigator (A.C.G) who took care of the evaluation of the patients with OAAS (paper III), COMFORT sedation score (paper I) and online stress score (paper II).
To standardise, the two clinical trials in adults (paper II-III) included white women scheduled for gynaecological laparotomy. The same anaesthesiologist (R.H) performed the anaesthetical procedure in a standardized way in all patients. In the clinical trial (paper I) in the paediatric intensive care unit (PICU) all the subjects were artificially ventilated and circulatory stable as judged by the same anaestheologist (K.W). The SC was measured during the same standardized procedure in all patients, i.e. suction from the trachea.

12.2 Subjects

In paper II and III the patients gave written informed consent, before they where taken to the operating theatre. In paper I the parent or parents gave informed written consent in advance of the measurements and the nurse in charge of the PICU decided when suctioning from trachea was due. Subjects with diseases that could disturb the SC like dysfunctional eccrine sweat glands, neuropathies or neurological diseases were excluded. Subjects should in a period of four hours prior to the study neither be on psychoactive drugs, nor have used atropine or other anticholinergic. They should not have a pacemaker. In paper III patients with hearing impairment where excluded. In paper II and III all the patients were in ASA physical status I-II. All the children in paper I was artificially ventilated and circulatory stable.

12.3 Medication

In paper I, 8 children did not have any on-going infusion of hypnotics when we did our measurements, 3 received thiopental and 9 midazolam. The children received different amounts of morphine, fentanyl and remifentanil as analgesics, some with paracetamol in addition. One child did not receive neither hypnotics nor any analgesics.
In paper II, all patient received 4 mg cis-atracurium and a remifentanil effect target control infusion (TCI) of 2 ng/ml before intubation with at least one bolus dose of remifentanil in

addition to facilitate intubation. Half of the patients received an effect propofol TCI of 2 μg/ml and the other half an effect propofol TCI of 4 μg/ml right before intubation in order to separate the sedation levels during intubation. If necessary from the patient's reaction, a propofol TCI increase of 0.3 μg/ml was applied. Tetanic stimuli were given in three sessions. The first time with remifentanil TCI at 2 ng/ml, the second time after a four minutes washout period when the remifentanil concentration was supposed to be < 1 ng/ml and the third time again with remifentanil TCI at 2 ng/ml.

In study III the patient received 7.5 mg midazolam before entering the operating theatre. Propolfol were given preoperatively starting at an effect TCI of 0.5 μg/ml and then gradually adjusted to achieve the different sedation levels. A remifentanil effect TCI of 2 ng/ml was added when required by the protocol. The last sound stimulus was given during steady-state anaesthesia after intubation provided by a propofol TCI of 5.0 μg/ml and a remifentanil TCI chosen to maintain systolic ABP between 80 and 130 mmHg.

12.4 Skin conductance equipment

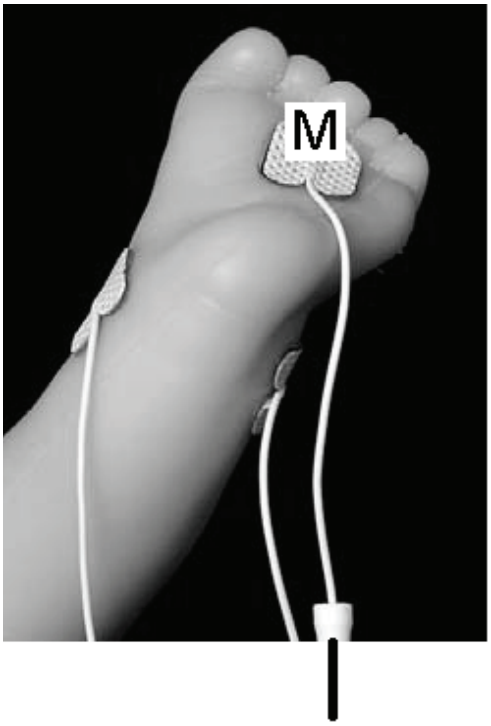
SC activity was measured by alternating current at 88 Hz. Low frequency electrical conductance reflects the ionic conduction in the stratum corneum, which is largely determined by sweat duct filling. An applied voltage of 50 mV and a three-electrode system were used. The three-electrode system comprises a measuring electrode (M), a counter current electrode (C), and a reference voltage electrode (R), which ensures a constant applied voltage across the stratum corneum beneath the M electrode. At the approximately same areas the electrodes are placed plantar (Study I, Figure 7) or palmar (Study II and III, Figure 8).

In study I, non-disposable electrodes were used and conductive paste from the National Hospital Pharmacy, Norway, containing 6 g hydroxyethyl-cellulose 700, 0.58 g NaCI, 0.1 g methylparahydroxybenzene, 0.1 g propylparahydroxybenzene, 2 g alcohol 96%, distilled water up to 100 g, was applied to improve electrode conductance. During study II and III disposable electrodes as shown in figure 8 and 9 were implemented. Lately, the choice of electrode gel has been suspected to influence the SC results, especially when a moist gel with low viscosity is used. Our gel was a moist gel and one of two recommended gel-types at that time. Probably the NSCF is not as influenced by gel-type as the SC level. Even though, mostly due to increase in the amplitude with a wet gel it could be that a greater NSCF was achieved especially in study I (234). It is not known if the disposable electrodes latter used

contained a wet gel or a gel with high viscosity, because the content is still concealed by the manufacturer.

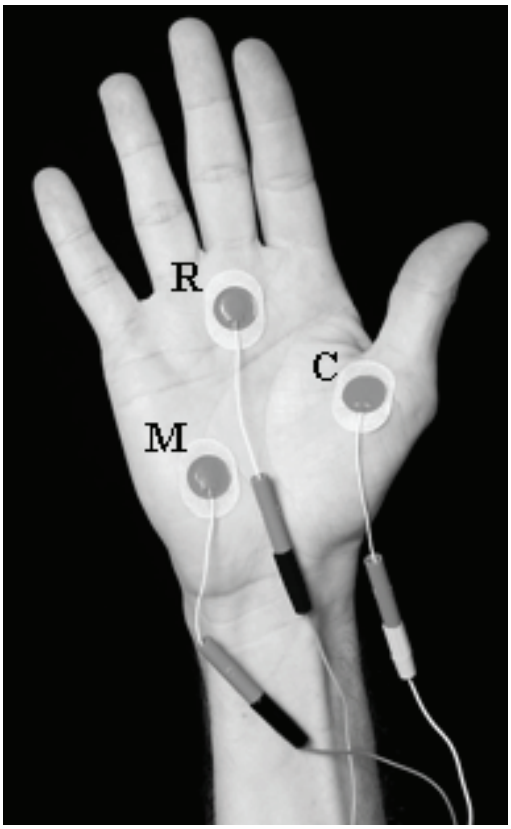
The SC data were stored on-line using a portable computer, and were analysed off-line by means of a software analysis package. The sample frequency was 50 Hz and the resolution was 12 bits. The software program for sampling and analysing SC was developed in Labview, National Instruments (235). The program contained a function that enabled us to define a threshold for the minimum amplitude, the minimum width of the wave and the maximum slope of SC. The software analysis program could also analyse smaller segments of the recorded data. Med-Storm Innovation AS commercially developed the apparatus and the software program.

Figure 7



The three-electrode system used together with the Skin Conductance Algesimeter®™ in children under one year. The measuring electrode is placed under the foot; the countercurrent electrode on the medial side of the foot and the reference electrode could as here be placed at the lateral ankle or at the dorsal side of the foot. Due to the increasing thickness of epidermis under the foot with age, the hand is a more suitable electrode placement site in older children.

Figure 8



The three-electrode system used together with the Skin Conductance Algesimeter®™ in children over one year and in adults. The measuring electrode is placed on the hypothenar eminence; the countercurrent electrode on the thenar eminence, and the reference electrode may be placed as here beneath the third phalange or at the dorsal side of the hand.

12.5 Skin Conductance measurements

Different measurements concerning the skin conductance have been in use. The basal SC level can be used for extracting other variables such as mean skin SC level, the derivate of the mean SC level (D-SC) and the amplitude of skin conductance fluctuation (ASCF). The SC fluctuations are defined from the amplitude, the slope of the SC wave, and the width of the SC wave, and are summarized as number of SC fluctuations (NSCF).

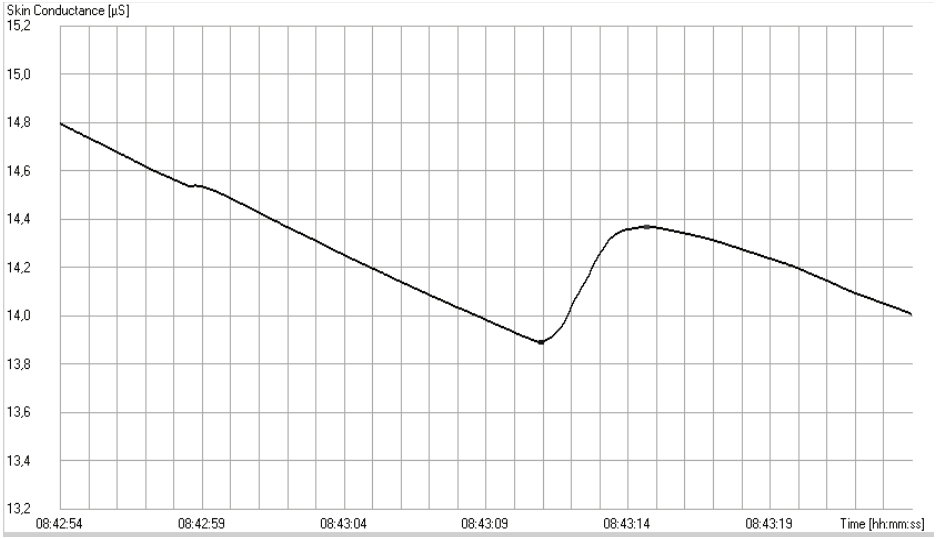
The basal SC level is influenced both by properties of the skin such as membrane permeability and the degree of moisture in stratum corneum, and activity in the sympathetic nervous system. Mean SC level usually rise slowly initially during recording (28), which is thought to be due to excess sweat accumulation under the electrodes (34). Arousal stimuli or noxious stimuli cause a rapid deflection or fluctuation from baseline. A fluctuation is defined by the amplitude, slope, duration, and width. The amplitude is calculated from the valley before the slope to the height of the peak defined by the turning points where the derivate are zero. If new SC fluctuations develop before the immediately preceding SC fluctuation has returned to baseline, the amplitude of the second fluctuation will be smaller.

Recommended analysing interval of NSCF and mean SC level is 30 seconds. The typical SC fluctuation wave will have a rapid slope rise and a slower slope fall by visual inspection (Figure 9).

The slope of the SC fluctuation is defined as the derivate of the mean SC. By manual counting of the number of waves and corresponding this to a pre-set minimum amplitude and slope, the NSCF is calculated by the software program. By using this method with the equipment used in this study, the recommended minimum amplitude is 0.02 mikroSiemens (μS) and the maximum slope 2 mikroSiemens/second (μS/s) for both adults, infants and preterm (37, 235) for the definition of one fluctuation. With a smaller amplitude minimum at 0.01 μS, some noise will also be counted as SC fluctuations by the software programme, with a larger amplitude minimum at 0.05 μS some of the SC changes is lost with the equipment used in this study. Still, if some peaks are observed manually which are not counted with the threshold of 0.02 μS, a threshold of 0.015 μS is used instead (235). This was done for three children in study I. The width of the waves should be at least one second for adults and unlimited for infants and preterm (37, 235). When a steady decline in skin conductance can be suspected, like during the induction towards anaesthesia, the derivate of the mean SC level (D-SC) can be measured for 15 seconds periods. This algorithm was used in paper III.

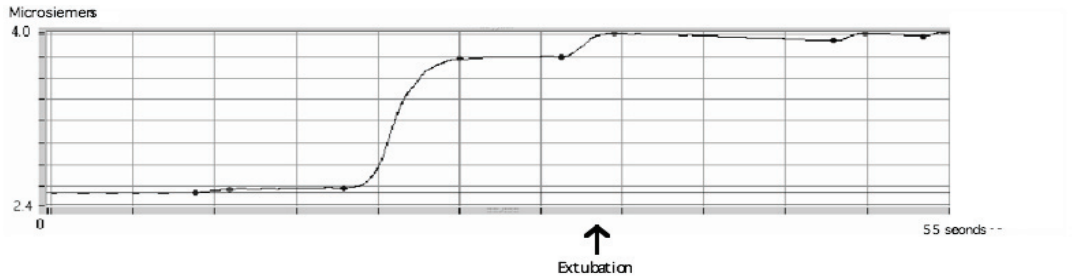
The derivate of mean SC level have been compared to BIS during emergence from general anaesthesia and they both performed similarly (40), while another study found BIS to have higher prediction probability but slower response time than NSCF (41). To assess emergence, the derivate of the mean SC level might be insufficient because the slope of the SC curve is too steep. A combination of a mean increase of the SC level of at least 0.1 μS within 20 second together with at least 2 NSCF was used for assessing emergence in the equipment used for this study (Figure 10).

Figure 9



A magnification of the skin conductance fluctuation as seen in the Skin Conductance Algesimeter®™. A typical SC fluctuation starts with a steep onset from baseline and a relatively flat recovery line. Minimum amplitude was in most patients set at 0.02 mS and the maximum slope at 2 mS/s.

Figure 10



A curve in the Skin Conductance Algesimeter®™ seen during emergence from general anaesthesia. The slope of the SC curve during emergence is steep, and we used a mean increase in SC level of at least 0.1 μS within 20 seconds and in addition at least 2 NSCF to define emergence during study II.

12.6 State Entropy and Response Entropy measurements

State Entropy (SE) and Response Entropy (RE) are EEG-based methods trying to quantify the EEG readings. Entropy is a description of the irregularity of the signal. When the regularity of the signal is at its maximum, the entropy value is zero, when the signal increase in irregularity the entropy value is increasingly higher than zero (203). SE measure cortical activity calculated from EEG frequencies ranging from 0.8 to 32 Hertz. At higher frequencies the EMG signal dominates and RE measures both EMG and EEG signals ranging in frequencies from 0.8 to 47 Hertz. In the presence of EMG activity, the RE index is larger than the SE index, the difference between them is denoted as RE-Δ. The SE value range from 0 to 91 and the RE value range from 0 to 100, and are arranged so that RE becomes equal to SE when there is no EMG signal present (203). To achieve different sedation levels during tetanic stimuli in paper II, the target SE was set at SE 30 in half of the patients and at SE 60 in the other half of patients. In paper II, an increase of RE to 80 or an increase of SE to 70 was recognized as emergence.

12.7 Other technical equipment

In paper II and III entropy measurements were used. The EEG signal was collected with a disposable Entropy Sensor (Datex-Ohmeda Division, GE Healthcare) composed of self-adhering flexible bands holding three electrodes applied to the forehead. Two Datex-Ohmeda S/5 Anaesthesia Compact Monitors furnished with entropy, Neuro Muscular TOF-Guard (NMT), and hemodynamic measurement modules obtained the Entropy measurements. The data record times of the SC variables were synchronized with the S/5 AM clock. The sampling rate was 400 Hz for SE and RE. All the data were collected on the same laptop we used for the SC variables.

In paper II tetanic stimuli were given using NMT. Two stimulating electrodes were placed above nervus ulnaris on the distal forearm, the mechano sensor was placed from the thumb to the index finger. Noxious stimuli were given manually using a NMT in tetanic 50mA level for 5 seconds and then 3 consecutive stimuli for 1 second with 10 seconds interval in between the stimuli. According to the protocol, the tetanic stimuli should have been given three times after intubation. As the surgeons entered the operating theatre right before intubation, the third tetanic stimuli were often given too early to try to “stick to time-schedule” in the operating theatre. Afterwards, the third tetanic stimuli were not included in the statistics, due to the insecurity concerning actual remifentanil concentration.

12.8 Statistical methods

Based on previous pilot studies, sample size was estimated by a statistician at Oslo University Hospital, Rikshospitalet, in study I. In study II and III a statistician connected to Datex Ohmeda calculated the sample size. As a consequence, we hope to have avoided type II errors in the statistical calculations. In all three studies, we received assistance in deciding what statistical methods to use by statisticians at Oslo University Hospital, Rikshospitalet. As we could not be sure the variables were normally distributed, in paper I, II and III we used Wilcoxon non-parametric test for two related samples, and in paper II Whitney U non-parametric test for assessing equality in two independent samples. The significance level was in all three studies set at 0.05 to avoid type I errors. The tests were not Bonferroni corrected, due to the controversy concerning this method (236). All statistical test were performed using SPSS 12.0 (SPSS Inc., Chicago, IL)

In paper I we used linear regression analysis to see if the SC parameters were correlated to a modified Comfort sedation score from *before* to *during* endotracheal suctioning and from *during* to *after* endotracheal suctioning. The modified Comfort sedation score was used as the independent variable. Wilcoxon nonparametric test was used to see if the difference between the variables measured *before*, *during* and *after* endotracheal suctioning were significant.

In paper II we used linear regression analyses to compare maximum NSCF and maximum RE-Δ response with a maximum clinical stress score for 1 minute after intubation in remifentanil and propofol general anaesthesia. A 30 seconds pre-stimuli level prior to intubation was also compared with a 30 seconds post-stimuli period after intubation for NSCF and RE-Δ, using the Wilcoxon non-parametric test.

After intubation three series of tetanic stimuli were given; one serie with remifentanil (R+), a second serie without remifentanil (R-), and a third serie with remifentanil (R+), (the third tetanic stimuli was not implied in the statistical analysis, see section 12.7). The mean of the two 30 seconds pre-stimuli periods were compared with the mean of the two 30 seconds post-stimuli periods using Wilcoxon non-parametric test for the clinical stress score, NSCF and RE-Δ. The NSCF and RE-Δ responses during the first R+ were then compared to the NSCF and RE-Δ responses during R- using Wilcoxon non-parametric test. To see if the level of SE influenced the SC reaction to tetanic stimuli we used Mann-Whitney U-test.

During emergence the time point the tube left the trachea was denoted as emergence. The SC variables, SE 70, SE 80, RE 70 and RE 80 were compared for time-difference to the extubation point using Wilcoxon non-parametric test.

Paper III compared Entropy variables and SC variables at different clinical levels of sedation. The different levels of sedation were evaluated according to the OAAS score, starting at awake patients without propofol, passing through light sedation with propofol and then at deeper sedation levels, first with propofol and then at the same level with remifentanil added. Finally, deep sedation with propofol and remifentanil before intubation was compared to measurements 30 minutes after surgery start. All comparisons were performed using the Wilcoxon non-parametric test in paper III.

13. Results

Hypothesis 1: Skin conductance can be used in clinical practice as a measure of pain and noxious stimuli.

Hypothesis 1A: Skin conductance can detect sympathetic activation during suction from trachea in artificially ventilated children.

We found that 61% ($r^2 = 0.61$, $p < 0.0005$) of the rise in SC during suction in the trachea was explained by the rise in the modified Comfort score. After suction in the trachea, 46% ($r^2 = 0.46$, $p = 0.001$) of the variation in NSCF was explained by the variation in the modified Comfort sedation score. As mentioned before, arousal before a new noxious stimulus could lead to higher arousal when repeated. When the eleven children, having been through surgery the same day as the measurements, where investigated (without one statistical outlier), 79% ($r^2 = 0.79$, $p < 0.001$) of the rise in SC could be explained by the rise in the modified Comfort sedation score. When using linear regression analysis, values over 50% is considered as a major influence between the dependent and the independent variables. There were no significant correlation between the other variables and the modified Comfort sedation score. The modified Comfort sedation score was different both from before to during ($p < 0.0005$) and from during to after ($p < 0.0005$) suction from trachea, and so was NSCF ($p = 0.001$ and $p = 0.004$) and ABP ($p < 0.0005$ and $p = 0.002$). Mean SC level was significantly different just from before to during ($p = 0.002$) and the other variables were not different neither from before to during nor from during to after suction from trachea. 16 of the children had no NSCF reaction during the measurements before suctioning from trachea, and 4 children had no NSCF reaction during suction from trachea. 8 children were totally calm according to the Comfort sedation score before suctioning from trachea. Only one child had no reaction in the Comfort sedation score during suctioning from trachea, and this child had also no reaction according to NSCF, ABP and HR.

Hypothesis 1B: Skin conductance will rise in patients exposed to known noxious stimulation as intubation and tetanic impulses.

The mean of the two pre-stimuli NSCF levels with and without remifentanil was compared with the mean of the two post-stimuli NSCF levels with and without remifentanil to find an overall alteration in NSCF during tetanic stimuli. Entropy and a clinical stress score were also recorded during the pre- and the post-stimulus periods.

The mean of the two pre-stimuli RE-Δ levels with and without remifentanil was likewise compared with the mean of the two post-stimuli RE-Δ levels with and without remifentanil to find an overall alteration in RE-Δ during tetanic stimuli.
Of the 20 patients, 9 had a reaction to intubation according to the clinical stress score, and 11 had a reaction to intubation according to the NSCF. The correlation was $r^2 = 0.73$ ($p < 0.0005$). 7 patients had a rise >10 in the RE-Δ during intubation, and the correlation to the clinical stress score was $r^2 = 0.33$ ($p = 0.007$).
16 patients had an overall reaction to tetanic stimulation according to NSCF and had a mean pre-stimuli NSCF level that was significantly lower than the mean post-stimuli NSCF level ($p = 0.001$). The mean RE-Δ level was not significantly altered by tetanic stimulation.
There was a non-significant tendency in NSCF being higher during tetanic stimuli in the SE 60 group than in the SE 30 group, both with and without remifentanil. A post hoc power test showed that another 20 patients had to be included to find a significant difference.

Hypothesis 1C: When the level of analgesia is increased during standardized nociceptive stimulus, the skin conductance response will attenuate.
NSCF and RE-Δ during the two sessions of tetanic impulses with and without remifentanil were compared to find if an analgesic agent would influence these variables.
Without remifentanil, 16 patients reacted with a rise in NSCF during tetanic stimulus, and 14 patients had a higher NSCF during tetanic stimulation without remifentanil compared with tetanic stimulation with remifentanil ($p = 0.002$). Under remifentanil infusion, 9 patients had a rise in NSCF during tetanic stimulation. The RE-Δ could not differ between tetanic stimulation given with or without remifentanil.

Hypothesis 2: Skin conductance can be used during induction of anaesthesia as a measure of the transition from fully awake to the loss of consciousness.

Hypothesis 2A: Skin conductance will be able to discriminate between different levels of sedation from fully awake to asleep during use of propofol.
Number of skin conductance fluctuations (NSCF) discriminated significantly between the OAAS levels from 5 to 3-2 (level 5 to 4-3, $p = 0.002$, level 4-3 to 3-2, $p = 0.024$).
Amplitude of skin conductance fluctuations (ASCF) discriminated between OAAS level 5 and 4-3 ($p < 0.000$).

Mean SC level discriminated between OAAS level 4-3 and 3-2 ($p < 0.000$). Mean SC level showed further decrease when remifentanil was added.
The derivate of mean skin conductance level (D-SC) discriminated between all levels of sedation, from OAAS level 5 to 4-3 ($p = 0.000$), and from OAAS level 4-3 to 3-2 ($p = 0.002$). D-SC showed further decrease when remifentanil was added.

Hypothesis 2B: There will be a measurable difference when adding remifentanil on top of already administered propofol.
Number of skin conductance (NSCF) did not discriminate when remifentanil was added at OAAS level 3-2 between propofol alone, and propofol and remifentanil together. Neither did NCSF discriminate between OAAS level 3-2 with remifentanil and OAAS level < 2.
Amplitude of skin conductance (ASCF) discriminated between OAAS level 3-2 with remifentanil and OAAS level < 2 ($p = 0.046$). This last p-value is not significant if a Bonferroni correction is performed.
Mean SC level discriminated between OAAS level 3-2 without remifentanil and OAAS level 3-2 with remifentanil ($p = 0.000$), and between OAAS level 3-2 with remifentanil and OAAS < 2 ($p = 0.001$).
The derivate of mean skin conductance level (D-SC) discriminated between OAAS level 3-2 with and without remifentanil ($p = 0.006$), and also between OAAS level with remifentanil and OAAS level < 2 ($p = 0.009$).

Hypothesis 3: Skin conductance can be used as a measure of the transition from fully sedated to emergence from general anaesthesia.

Hypothesis 3A: Skin conductance will similar to the EEG monitors be able to predict emergence from general anaesthesia.
RE 70 was reached faster than the changes in the SC variables ($p = 0.002$), RE 80 was also reached faster than the changes in the SC variables ($p = 0.03$). Still, SC variables showed emergence with a mean value of 14 seconds before extubation, SE 70 was reached with a mean value of 24 seconds before extubation, and RE 70 was reached with a mean value of 50 seconds before extubation. It seems fair to assume that the SC variables were similar to SE 70 during emergence, but that RE 70 is a better measurement when it comes to emergence with the SC method used in this study.

14. General discussion

We aimed at finding out if skin conductance could be used to monitor nociceptive responses in artificially ventilated children, in patients ahead of surgery, in patients during surgery in general anaesthesia and in patients during emergence from anaesthesia. We found that skin conductance could detect clinical discomfort during intubation, detect decreased tetanic response when the opioid analgesic level increase, and discriminate between different levels of sedation (OAAS score) during anaesthetic induction with intubation and subsequent surgery. Skin conductance could also detect nociceptive stimulation in children. All these findings can be explained by the emotional sweat activity being a part of the sympathetic nervous system.

To overcome the risks of to strong hormonal and physiological activation by nociception in patients not being verbally communicating we need a method of identifying nociceptive stimuli in an objective, sensitive and specific way. There is also a need for detecting signs of overdose from anaesthetic and sedative acting drugs, as may be suspected when there is no response detectable to significant nociceptive stimulation. This is the background for our studies.

For the surgeons a major concern is unwanted responsiveness of the patient during surgery, such as movement and coughing, which can eventually lead to bleeding and injury of the patient. The patients concern during an operation is the possibility of experiencing of or awareness. To follow up these needs, a correct and individually tailored level of anaesthesia and analgesia is important. In paper II, during anaesthesia, not all the patients reacted to noxious (tetanic) standardised stimulus as measured by the NSCF. This has later been confirmed in other studies, thus the individual response to tetanic stimulus is suspected to be associated to genes involved in pain perception (30). Through twin-studies it has been suspected that genetic factors contribute to some of the variability in pain sensitivity (237), and this suspicion is supported by an ethnic difference in pain sensitivity (238).

During tetanic stimulation, in paper II, the patients were given two sessions of tetanic stimuli; the first with remifentanil and and the second without remifentanil. There was a significantly lower pre-stimuli NSCF level than post-stimuli NSCF level in both sessions. The NSCF change was significantly attenuated by adding remifentanil, whereas the entropy and the clinical stress score did not show similar discrimination.

The reflex responses to noxious stimuli is successively suppressed during general anaesthesia, from pain experience suppression, movement suppression, breathing suppression, heart rate-

and arterial blood pressure suppression, sweating suppression and finally the suppression of the hormonal stress response (239). High doses of analgesic are needed to suppress the release of epinephrine from the adrenal medulla, while the release of nor-epinephrine from the peripheral sympathetic nerve is even more resistant (240). This means that the sweat secretion is still intact when most other measurable signs are suppressed, and explains why skin conductance is still present under tetanic stimulation during anaesthesia and opioid analgesia. Still, the NSCF is zero when the patients have enough or to much analgesia. If the level of analgesia is reduced when the patient has too much analgesia, the HR and ABP may increase before the NSCF deviates from zero. The reason why may be that HR and ABP is regulated on brain stem level different from the skin conductance that probably also are regulated from cerebellar levels. Thus, the SC may not be a tool for distinction between too much and adequate level of anaesthesia, such as the BIS and entropy, but may still be a sensitive and rapid warning sign of too little anaesthetic effect.

In properly sedated patients the clinical goal is to have a clinical stress score of zero. Thus, the NSCF may be a future possible and sensible tool for assessing individual needs of analgesia. During surgery two of our patients showed high entropy values, without any other signs of stress or awakening, and no change in skin conductance. Propofol doses were increased, without any reduction in entropy measurements. This was interpreted as a false positive entropy measurement of physiological stress. Also with BIS there are problems of false negative and positive measurements. BIS have earlier showed failing ability to predict awareness (201) and there are cases where BIS is unable to detect the level of sedation because of electromyography activity (198).

In general, activity of adrenergic receptor agents or changes in circulation or room temperature do not influence skin conductance (3, 13, 51, 54). In our results in paper I, the HR and ABP were less sensitive and specific than SC in identifying nociceptive stimuli. This supports the assumption that HR and ABP is often unreliable measurements of drug effects in protection against stimulation during anaesthesia and sedation.

In paper II, the patients received 4 mg Cis-atracurium to facilitate intubation. As assumed, the nicotinic antagonists Cis-atracurium did not influence skin conductance and there was a high correlation between NSCF and the clinical stress score during intubation. Moreover, because emotional sweating is activated by muscarinic receptors it is probably not influenced by nicotinic antagonists acting at the neuromuscular junction.

During emergence, the SC variables were able to predict emergence, but both RE and SE reacted before the skin conductance variables. This is supported by findings by Ledowski et al

when the BIS monitor predicted emergence with higher prediction probability than the SC variables (39, 41). Even though, in another study they found NSCF and BIS to perform similarly during emergence (40). The SC index algorithm has recently been changed in order to better discover emergence from anaesthesia. The changes are from the two peaks and the increase in the derivate of the mean SC level, to an increase in the relative area under the curve (www.med-storm.com). A new study should be performed to figure out if the new algorithm in the SC monitor would discover emergence from anaesthesia earlier than the algorithm available for this thesis.

It has not yet been established if there is a difference in skin conductance when different anaesthetic drugs are used, but previous studies indicate that benzodiazepines can induce a lower number of skin conductance fluctuations activities than propofol in the initial sedation phase before full sedation (38, 241). The skin conductance level has also been shown to decline by benzodiazepines in awake subjects (242, 243). Benzodiazepines have no direct analgesic effects but may act in a way which makes the subjective perception of pain weaker as well as they have a sedative effect. In our study in paper III with non-noxious stimuli in the period before intubation, all the patients got 7.5 mg midazolam as premedication before entering the operating theatre, and in our study in paper I, with artificially ventilated children, 9 patients received midazolam. Under these procedures the skin conductance reaction correlated to the OAAS score and the COMFORT score, respectively. This might be explained by the assumption that the benzodiazepines depress the skin conductance activity, in parallel with sedation as reflected in the depression of the OAAS score and the COMFORT score as well. However, we still found a significant correlation between sound stimuli and NSCF and SC level, and also between suctioning from trachea and the NSCF. The interpretation is that sedation will reduce the sympathetic activity per se, but this reduction will be counteracted by stimuli resulting in arousal. Another explanation might be newer and better technical equipment that through better technical interpretation of the fluctuation will detect smaller fluctuations.

Artificially ventilated patients are of special concern. They are often not able to communicate and some patients are, for different reasons, kept sedated over a longer time span. Long time experience of pain has negative effects on the immune system and is believed to alter hormonal pathways. Therefore, it is of importance to find the right balance between relief from pain or discomfort and the right level of sedation and analgesia.

The Neonatal Pain-Control Group has raised the issue on investigating underlying factors concerning biological pain responses and analgesic safety in neonates (244).

The specificity of responses to pain and noxious stimuli decrease in awake patients compared to anaesthetized patients (44 - 47, 57). In one clinical trial (paper III) we used white sound delivered by earphones to awake patients and kept the operating theatre otherwise quiet during introduction of sedation and anaesthesia. Still, uncontrolled arousal due to sudden visual stimuli was of course possible, especially at lighter sedation levels. In awake patients, emotional stressors other than pain should be taken into account when the SC is measured and interpreted. Such stressors may be intellectual tasks, nausea, vomiting, anxiety etc. When SC is used to monitor awake patients, it should be a tool to identify a general aroused state of the patient (highly sensitive), whereas the source of arousal may be complex and a sum of many different stimuli. The clinicians have to use their clinical judgement to determine if the increase in the measured index stem from pain or from emotional stressors. In anesthetized patients it seems that both the sensitivity and specificity to nociceptive stimuli are higher (22). During surgery in paper II, diathermy influenced the SC measurements. Electrocoagulation is known to interact with the SC measurement, but improvements have been performed in the commercial available equipment delivered today in order to reduce this source of electrical noise.

The main source of artefacts in SC signals is movement of the electrodes, either because the patient moves or because someone gets in contacts with the electrodes. In paper I, detachment of the electrodes was noted, although as a minor problem. Wrapping the electrodes eliminates movement artefacts (245). Sticky disposable electrodes also protect against movement artefacts.

In our studies, we used a variety of ways to describe the skin conductance measurements. Standardising the use of parameters would probably be beneficial. In general, NSCF seems to be the best measure of activation of the sympathetic nervous system. Changes in the derivate of the mean level of skin conductance can also give additional information, especially when there is a transition from awake to anaesthetised state or from anaesthetised to awake state. Likewise, ASCF shows a decrease in the transition from awake state to an anaesthetised state, but it is probably not the best measurement for detecting noxious stimuli during anaesthesia. The main overall problem is to distinguish between stress and pain, especially amongst awake subjects. It is not clear if this distinction actually is relevant when discussing the negative effects exerted by the body. There are almost hundred years of documentation of skin conductance as being a measure of mental arousal. There are reasons to believe that all sorts of rise in attention would raise the numbers of skin conductance fluctuations. In anaesthetised

patients much of this problem of low specificity is eliminated and the method is more accurate, with higher sensitivity and specificity.

By exhibiting its action of measurement on the sympathetic action of acetylcholine-muscarine, the skin conductance method offers a different approach compared with most other devices. Most other methods are basically based on EEG or effects exhibited by adrenalin/noradrenalin release. However, many anaesthetics also alter the typical clinically measurable signs of pain, like rise in pulse, elevated blood pressure and rise in respiration frequency (246 - 250) in an unspecific way, not related to perceived nociception alone.

15. Conclusion

Our clinical trials were to small to generalize to a larger population, but gave an indication that this might be a prospect. In practical use, skin conductance is more reliable in discovering noxious stimulation than blood pressure, heart rate and EEG monitors. This was tested when the patient was nearly unconscious before intubation, during artificial ventilation, during surgery and during emergence. Our studies give us reason to believe that changes in skin conductance could be a measure of noxious stimuli. In this area few viable competitors exist.

Hypothesis 1: Skin conductance can be used in clinical practice as a measure of pain and noxious stimuli.

Measuring noxious stimuli in sedated and anaesthetized patients is difficult, and no gold standard exists. As a consequence of this, we tested the SC against both entropy measurements as a representative of an objective measuring system, and against three clinical scoring systems as representatives of the subjective measuring systems. Skin conductance showed similar results as both the objective and the subjective scoring systems and is usable for measuring noxious stimuli in clinical practise.

Hypothesis 1A: Skin conductance can detect sympathetic activation during suction from trachea in artificially ventilated children.

There was a clear rise in skin conductance fluctuations during suction from trachea that correlated well with the modified Comfort sedation score. The Comfort sedation score was sensitive to suction from trachea in almost all of the children, while the skin conductance reacted to suction from trachea in a little less children. As suction from trachea is regarded as an arousal stimuli influencing sympathetic activation, we found that skin conductance could detect sympathetic activation in artificially ventilated children.

Hypothesis 1B: Skin conductance will rise in patients exposed to known noxious stimulation as intubation and tetanic impulses.

Skin conductance correlated with a clinical stress score during intubation. About half of the patients had a rise in both skin conductance and the clinical stress score during intubation. More than ¾ of the patients reacted to tetanic stimulation according to skin conductance. This indicates that skin conductance is a measure of arousal due to noxious stimuli.

Hypothesis 1C: When the level of analgesia is increased during standardized nociceptive stimulus, the skin conductance response will attenuate.

We found a rise in NSCF during a noxious stimulus under propofol anaesthesia, which was inhibited when an analgesic agent (remifentanil) was added. When supposed noxious stimuli in anaesthetized patients give a measurable physiological reaction and that measurable physiological reaction is lowered by analgesics, we may assume that the measurable reaction is an expression of noxious stimuli.

Hypothesis 2: Skin conductance can be used during induction of anaesthesia as a measure of the transition from fully awake to the loss of consciousness.

If the operating theatre is kept with as little noise as possible, skin conductance during induction of anaesthesia may be used as a measure of sedation. Anxiety, noise and light may be confounders to such measurements.

Hypothesis 2A: Skin conductance will be able to discriminate between different levels of sedation from fully awake to sleep during use of propofol.

These findings suggest that NSCF and D-SC are the most sensitive SC variables when measuring level of sedation from fully awake to sleep during use of propofol.

Hypothesis 2B: There will be a measurable difference when adding remifentanil on top of already administered propofol.

Mean SC level and D-SC are the most sensitive SC variables to detect the adding of an analgesic agent, like remifentanil, during arousal stimuli from when the patient is about to lose consciousness to fully anaesthetized.

Hypothesis 3: Skin conductance can be used as a measure of the transition from fully sedated to emergence from general anaesthesia.

Visually, the emergence curve is easily seen on the Skin Conductance monitor.

Hypothesis 3A: Skin conductance will similar to the EEG monitors be able to predict emergence from general anaesthesia.

Skin conductance was able to predict emergence, but the RE measurements reacted before the skin conductance variables.

16. Future perspective

The main area of use for the Skin Conductance Algesimeter should be in detecting noxious and arousal stimuli in sedated and unconscious patients, both adults, children and premature. To draw safer conclusions, larger scale studies should be performed directed at these topics. The EEG monitors can provide information about sedation level both before surgery, during surgery and during emergence. The “dream-team” would be an EEG monitor with the SC variables included.

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List of errata

p.7 In the list “Abbrevations” the capital letters is changed to lower-case letters.

p.7 “electroencephalogram” should be read “electromylography” in line 13.

p.9 The correct title of the journal is ”Acta Anaesthesiologica Scandinavica”, not ”Acta Anaesthesiology Scandinavia”

p.17 ”these glands various” is corrected to ”the glands varies” in line 2.

p.19 In line 20, reference 15 should be reference 14. Reference 20 has wrongly been given the number as reference 15. Reference 15 is changed to reference 20 in the reference list. This leads to a change in numbers of the references from reference 15 to 20 where reference 16 is 15, reference 17 is 16, reference 18 is 17, reference 19 is 18 and reference 20 is 19.

p.23 ”baroreceptor´s” is changed with ”baroreceptors” in line 31.

p.29 ”involved in SC activation” is corrected to ”involved in activation of SC” in line 2.

I

II

III