Drug-enhanced Spinal Cord Stimulation for Neuropathic Pain

Studies on Neurochemical Mechanisms and Clinical Applications

Gastón Schechtmann, M.D.

Stockholm 2009
Cover image: Radiography of the lumbar spine with an implanted spinal cord stimulation system (in blue) and a pump for intrathecal drug delivery (in green). The picture was modified in Adobe Photoshop (Judith Socha).
ABSTRACT

Spinal cord stimulation (SCS) is an effective surgical treatment for neuropathic pain refractory to pharmacological therapy. For many patients suffering from this type of chronic pain, SCS is in fact the ultimate treatment option. However, 30 to 40 percent of well selected patients fail to obtain useful pain relief with SCS, and in some patients an initial good analgesic effect diminishes over time. Therefore, the need to improve the efficacy of SCS in these patients, prompted us to perform experimental research in order to advance the comprehension of the underlying mechanisms of SCS and further, to apply this knowledge clinically in order to develop new therapeutic strategies.

When SCS is applied to the dorsal columns, multiple neuronal networks may be activated and several neurotransmitters are released in the spinal dorsal horn. This neuronal response to SCS may be regarded as equivalent to in situ drug delivery to the spinal cord in physiological amounts.

The present thesis provides evidence that the analgesic effect of SCS partially relies on the activation of the cholinergic system because stimulation applied in rats with nerve injury produces a release of acetylcholine (ACh) in the dorsal horn. This effect occurred only in rats that in preceding experiments have been found to respond to SCS. It was also demonstrated that the SCS effect on signs of neuropathy was mediated via the activation of muscarinic, particularly M4 receptors. Intrathecal clonidine, a partial α2 receptor agonist, has proven to be an effective drug for both neuropathic and nociceptive pain. It is known that its analgesic action is in part related to an augmented spinal release of ACh, binding mainly to M4 receptors. In this thesis it was demonstrated that in nerve injured rats, concurrent administration of clonidine and SCS may potentiate the suppressive effect of SCS on mechanical hypersensitivity.

In a previous experimental study, it was shown that the administration of subeffective doses of a GABA\textsubscript{B} receptor agonist, baclofen, may enhance the effect of SCS. It is now demonstrated that also two anticonvulsants, gabapentin and pregabalin, in low and by themselves ineffective doses, may potentiate the suppressive effect of SCS on signs of neuropathy in rats. On the basis of these experimental studies, a double-blind, placebo-controlled clinical trial was performed, demonstrating that clonidine and baclofen in low intrathecal doses may enhance the analgesic effect of SCS in neuropathic pain, improving also quality of life. A long-term follow-up
of a group of patients with combined SCS and baclofen treatment revealed that they still enjoyed good pain relief.

The present studies contribute to the understanding of the mode of action of SCS and it provides a new therapeutic option to enhance the pain relieving effect of SCS by concomitant administration of low doses of intrathecal drugs for patients who obtain insufficient analgesia by SCS alone. This thesis also represents an attempt to translate knowledge from experiments performed in animals to clinical application.

**Keywords:** acetylcholine, baclofen, cholinergic analgesia, clonidine, gabapentin, neuropathic pain, nerve injury, pregabalin, spinal cord stimulation, intrathecal drug
LIST OF PUBLICATIONS

The present thesis is based on the following papers, which will be referred to in the text by their roman numerals as given below.


V. Schechtmann G, Lind G, Winter J, Meyerson BA, Linderoth B. Intrathecal clonidine and baclofen enhance the pain relieving effect of spinal cord stimulation: a comparative placebo-controlled randomized trial. Submitted to *NEUROSURGERY*

All previously published papers were reproduced with permission from the publishers.
© 2008 International Association for the Study of Pain (IASP) [I]
© 2004 International Anesthesia Research Society [II]
© 2002 and 2007 European Federation of Chapters of the International Association for the Study of Pain [III and IV]
LIST OF ABBREVIATIONS

ACh  acetylcholine
CCI  chronic constriction injury (Bennett and Xie 1988)
CCK  cholecystokinin
CGRP  calcitonin gene-related peptide
CNS  central nervous system
CRPS  complex regional pain syndromes
CSF  cerebrospinal fluid
4-DAMP  4-diphenylacetoxy-N-methylpiperidine methiodide
DLF  dorso-lateral funiculus
DRG  dorsal root ganglion(a)
EAA  excitatory amino acid
ECF  extracellular fluid
GABA  γ-amino butyric acid
GAD  glutamic acid decarboxylase
HPLC  high-performance liquid chromatography
5-HT  serotonin
IASP  International Association for the Study of Pain
i.t.  intrathecal
i.v.  intravenous
LTM  low-threshold mechanoreceptive
M  muscarinic (receptor)
MCM  mecamylamine
MT  motor threshold
MT-3  muscarinic toxin – 3
NO  nitric oxide
PAG  periaqueductal gray
PNS  peripheral nervous system
RVM  rostral ventromedial medulla
SP  substance P
STT  spinothalamic tract
TENS  transcutaneous electrical nerve stimulation
VAS  visual analogue scale
WDR  wide dynamic range (neuron)
WT  withdrawal threshold
SCS and electrode implantation in patients 42
Lumbar punction - bolus administration of drugs 45
Pump implantation – Surgical aspects 45
Assessment of on-going pain and changes in quality of life (IV-V) 45

Results 47
Incidence of mechanical hypersensitivity in rats with mononeuropathy (I-III) 47
Release of ACh in an animal model of mononeuropathy (I) 47
The role of the different subtypes of ACh receptors in the SCS effect (I) 48
Drug enhanced SCS in rats: clonidine, gabapentin and pregabalin (II-III) 48
Baclofen and SCS in patients (IV-V) 49
Side effects of baclofen 50
Clonidine and SCS in patients (V) 51
Side effects of clonidine 51
Placebo effect (V) 51

Discussion 52
ACh release in a nerve injury animal model 52
Methodological considerations of microdialysis 53
Cholinergic and GABAergic mechanisms in the effect of SCS 54
“Drug-enhanced SCS”: additive, synergistic or potentiating effects 56
Intrathecal drugs: bolus vs. chronic administration 58
Pain assessment and changes in quality of life 59
Animal models of pain and clinical relevance 59
Translation research: From bench to bedside - from bedside to bench 61

Concluding remarks 63
Acknowledgements 64
References 67
INTRODUCTION

The experience of pain is inherent in all higher vertebrates and constitutes a fundamental step in the phylogenetic evolution of the animal species to survive (Garry et al., 2004; Kavaliers, 1988). In fact, pain responses represent an adaptive behaviour to prevent or minimize tissue damage. Even though pain constitutes the most common cause of suffering and disability in humans, it has not been easy to reach a consensus on how to define pain. Interestingly, there are indeed no “painful stimuli” since not all humans experience pain by the same stimulus magnitude (Sullivan, 2004). The classical example of a wounded soldier not feeling pain until being removed from the battle field may clearly illustrate such an idea. Pain is a subjective sensory experience, and this unpleasant sensation is always associated with an emotional and cognitive components. This duality of physical and emotional components of pain constitutes such a complex experience which makes it difficult to define (Perl, 2007). Accordingly, the International Association for the Study of Pain (IASP) has defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”.

In most cases, the treatment of pain is indeed very effective by means of pharmacotherapy, such as non steroidal anti-inflammatory drugs and opiates, and easy to accomplish, since the oral administration is by far the most common way to treat pain. However, the existing pharmacological treatments are not always efficacious and are sometimes associated with severe side effects. The successful outcome in most cases of this physiological, protective pain - called nociceptive pain - differs with respect to the complexity in the management of chronic neuropathic pain (see definition below). Neuropathic pain remains a major challenge, since the outcome of conventional pharmacotherapy is still unsatisfactory (for review see Cruccu, 2007; Jensen and Finnerup, 2007; Koltzenburg and Scadding, 2001).

Neuropathic pain: definition

Whereas acute nociceptive pain arises when an organism has been injured, representing an adaptive function, chronic neuropathic pain does not have a positive influence on the healing process and, in fact, it constitutes a maladaptive response to a previous injury. Neuropathic pain, according to the IASP, is defined as “pain initiated or caused by a primary lesion or dysfunction in the nervous system” (Merskey and Bogduk, 1994). It occurs as a result of a lesion or dysfunction affecting the peripheral or central...
nervous system (CNS). Recently, it has been proposed that the definition should specifically state that the lesion or disease involves the somatosensory system from peripheral fibers up to the CNS, affecting the spino-thalamo-cortical pathways (Treede et al., 2008). In this revised definition, the ambiguous term “dysfunction” is replaced by the term “lesion or disease” and the nervous system is restricted to the somatosensory system. This modification has also been suggested in order to avoid the inclusion of other painful conditions such as muscular pain associated with spasticity and rigidity.

Neuropathic pain is classified according to the underlying aetiology or disease (e.g. herpes zoster, diabetes, multiple sclerosis), or to the anatomical lesion site (e.g. peripheral nerve, spinal cord, brain). A mechanism-based classification of neuropathic pain has also been proposed (Hansson, 2003; Hansson and Kinnman, 1996; Woolf et al., 1998; Woolf and Max, 2001) because the mode of action of pharmacotherapy is more related to the underlying molecular mechanisms and neurotransmitters implicated than to the aetiology (Sindrup and Jensen, 1999).

Neuropathic pain can be further characterized as spontaneous (stimulus-independent) and evoked (stimulus-dependent) pain. Spontaneous pain can be experienced as burning, lancinating, shock-like, shooting, etc. Evoked pain is usually classified according to the stimulus modality that induces pain, for example mechanical (dynamic or static), thermal (cold or heat) and chemical. Allodynia, evoked pain induced by non-noxious stimuli, and hyperalgesia, abnormally low threshold to noxious stimuli, are sometimes present in neuropathic pain syndromes.

Animal models of neuropathic pain and ethical aspects

Pain research using human subjects is accurate and reliable, but for obvious ethical reasons possesses certain limitations. Conversely, the use of laboratory animal pain models has inherent difficulties and controversial ethical aspects (Vierck et al., 2008). The main guidelines in animal research rely on the “three R concept” in which replacement, reduction and refinement in using animals have to be rigorously applied (Russell and Burch, 1959). In brief, the ethical concepts behind these guidelines are to replace, if possible, the use of live animals, to decrease as much as possible the number of them and finally to improve the welfare conditions during the experiments. These ethical aspects are, naturally, of utmost importance when applied in pain research (cf. IASP, 1983).

The use of animal models of neuropathic pain began in 1979 with the neuroma model (Wall et al., 1979), in which a complete transection of the
sciatic nerve was performed resulting in autotomy behaviour (self-mutilation of the digits). However, the acceptance of this model has been much controversial for ethical and interpretational reasons (Kauppila, 1998). Consequently, a shift towards models comprising only a partial nerve lesion became more widespread. The first model was the chronic constriction model (Bennett and Xie, 1988), in which loose ligatures around the sciatic nerve induce oedema resulting in tactile hypersensitivity or “allodynia” (similar to allodynia observed clinically). Lately, several models have been developed where nerve injury has been induced by ischemia, inflammation, partial ligation or section of the nerve, nerve roots or nerve branches that innervate the hind paw of rats (Chacur et al., 2001; Decosterd and Woolf, 2000; Eliav et al., 1999; Gazelius et al., 1996; Kim and Chung, 1992; Kupers et al., 1998b; Seltzer et al., 1990; Walczak et al., 2005).

All these mentioned animal models share a common feature: a partial denervation of the hind paw. Thus, these models avoid a complete deafferentation, allowing the assessment of tactile sensitivity such as with von Frey filaments. There are, as mentioned before, numerous rat models of nerve injury-induced pain described in the literature, each with somewhat different characteristics (for review see Koltzenburg and Scadding, 2001). Two of these models of neuropathy, the Seltzer model (Seltzer et al., 1990) and the Gazelius model (Gazelius et al., 1996) have been utilized in the studies included in this thesis and will be described in detail later in this chapter.

The perception of pain: neurophysiological aspects
The perception of nociceptive signals depends on complex and integrated processes at different levels in the nervous system (Millan, 1999). The organization of this network will be briefly described in this chapter. Pain is a sensorial experience similar to touch, pressure, proprioception, vision and hearing. Normally, pain is transduced by Aδ and C fibers and the nervous system has the ability to recognize different sensory pain modalities - mechanical, thermal, chemical. The perception of pain is finally the result of a refined complex processing in the brain as a consequence of circumstances such as a specific stimulus, the environment and the individual threshold or tolerance for pain.

Certain peripheral tissues, such as the skin and deeper in muscles or joints, possess nociceptors, defined by the IASP as “receptors sensitive to noxious stimulus or to a stimulus which would become noxious if prolonged”. Thus, in the presence of harmful stimuli, nociceptors are
activated. The primary sensory neurons, whose cell bodies are situated in the dorsal root ganglia, are responsible for the transmission of signals to the CNS. There are three types of nociceptors – mechanical, thermal and polymodal. The latter are activated by high-intensity mechanical, thermal (cold and heat) and chemical stimuli. The inputs from mechanical and thermal nociceptors are conducted by small-diameter, thinly myelinated Aδ fibers, while polymodal nociceptor signals are transmitted through small-diameter non-myelinated C fibers (for review see Julius and Basbaum, 2001). There is as well another kind of nociceptor, called silent nociceptors located principally in the viscera and joints (Cavanaugh et al., 2006), which are not activated until being involved in an inflammatory process.

Nociceptive primary afferents are pseudounipolar neurons, which have the soma located in the dorsal root ganglion (DRG) and a bifurcated axon with a peripheral branch innervating the skin, muscles or joints and a central branch that terminates in the spinal dorsal horn.

On basis of the cytological characteristics, the dorsal horn can be divided into six layers (laminae of Rexed) (Rexed, 1952; Willis and Coggeshall, 1991) where lamina I is the most dorsal and superficial. The painful stimuli, conducted by Aδ and C fibers primary afferents, reach the superficial laminae in the spinal cord (i.e. laminae I and II for C fibers and laminae III and IV for Aδ). In the dorsal horn, there are three basic types of somatosensory neurons that receive information from the periphery: nociceptive specific (NS), low-threshold mechanoreceptive (LTM) and wide dynamic range (WDR) neurons (for review see Willis and Coggeshall, 1991). Lamina V contains predominantly WDR neurons, which receive input from Aβ fibers carrying innocuous sensory information, as well as indirect nociceptive afferents from Aδ and C fibers. The convergence of nociceptive and non-nociceptive inputs in lamina V constituted a key observation in the conceptualization of the gate control theory (Melzack and Wall, 1965), discussed later in this chapter. Furthermore, the WDR neurons are modulated by descending pathways from supraspinal nuclei (Millan, 1999; Willis, 1988).

Nociceptive neurons, located in the superficial laminae of the dorsal horn, project further to different nuclei of the sensory thalamus. This sensory relay structure is situated in the ventral part of the thalamus, formed by three nuclei (i.e. ventro-lateral, ventro-medial and ventro-posterior nucleus). The latter nucleus is subdivided into the ventral posterior medial (VPM), the ventral posterior lateral (VPL) and the ventral posterior inferior (VPI) nuclei. The sensory thalamus projects further to a wide range of regions in the brain such as the primary and secondary
The spinal cord - ascending pathways

Nociceptive information is transmitted via the spinal cord to the brain via several pathways, briefly described below (for review see Almeida et al., 2004; Dostrovsky and Craig, 2006).

The spinothalamic tract (STT) is the principal ascending nociceptive pathway, conveying also temperature information. This tract, formed by the axons of the NS and the WDR neurons, crosses the midline and ascends to the VPL nucleus of the thalamus. The STT constitutes an important target of a neurosurgical pain procedure - called cordotomy, by which this tract is destructed resulting in unilateral pain abolition. The analogous trigeminal pathway involves trigeminal ganglion cells, whose axons descend through the spinal trigeminal tract to the spinal trigeminal nucleus. This nucleus contains the second-order neurons, which cross the midline to join the spinothalamic tract and ascend to the VPM nucleus of the thalamus. In turn, VPL and VPM project to the postcentral gyrus, the primary sensory cortex.

The spinomesencephalic tract is formed by axons of neurons of lamina I and V and projects to the mesencephalic reticular formation and periaqueductal gray matter and indirectly to the amygdala, part of the limbic system. It is thought that this tract contribute to the affective component of pain, leading to negative emotions (Willis and Westlund, 1997).

Other ascending pathways are also involved in pain processing such as the cervicothalamic tract, the spinoreticular and the spinohypothalamic tracts. This latter pathway has projections to the hypothalamus and it is thought to contribute with cardiovascular and neuroendocrine responses.

The functional anatomy of ascending nociceptive pathways and how they are integrated with several forebrain areas such as the sensory cortex, the thalamus, the hypothalamus, the anterior insula, the anterior cingulate has been extensively studied (for review see Craig, 2003). Although each specific pathway contributes to a particular component of the experience of pain, it is the complex constellation of multiple pathways which is responsible for the multidimensional pain experience.
Descending pain modulation

When the “gate control theory” was introduced, the knowledge about descending modulation of pain could not be imagined as it is understood today. Descending systems from supraspinal structures can selectively modulate afferent painful information inducing both inhibition and facilitation at the spinal cord level. The descending inhibition can be exerted by different mechanisms, targeting primary afferent fibers, dorsal horn interneurons or spinal second-order neurons that project supraspinally. It is well known that electrical stimulation of specific areas of the midbrain such as the periaqueductal gray (PAG) or the rostral ventromedial medulla (RVM), including the nucleus raphe magnus and the adjacent reticular formation, induces antinociception (Liebeskind et al., 1973; Oliveras et al., 1975) (for review see Millan, 2002). The RVM contains two groups of neurons, “ON-cells” and “OFF-cells”, which modulate pain transmission. The “OFF-cells” constitute a descending inhibitory system responsible for the attenuation of nociceptive afferents. Conversely, the “ON-cells” exert a facilitatory function on nociceptive transmission at the spinal cord level (Fields and Heinricher, 1985; 1989; Fields et al., 1991). The functional connection between these two supraspinal modulatory structures, the PAG and the RVM, is critical in pain modulation (for review see Fields et al., 2006). The PAG axons project only indirectly to the dorsal horn and use the RVM as a relay, while the RVM axons project directly to the superficial laminae of the dorsal horn. As a consequence of the connectivity from the PAG, via RVM to the dorsal horn, a lesion or a reversible inactivation of the RVM by lidocaine suppresses the analgesia induced by PAG stimulation (Fields et al., 1991).

The descending pain controlling systems are principally serotoninergic and noradrenergic (Todd and Koerber, 2006). The serotoninergic pathway originates in the raphe nuclei in the medulla and terminates mainly in laminae I and II (Ruda et al., 1982), while noradrenaline containing axons derive from cells in the locus coeruleus and project more diffusely in the dorsal horn, but with some dominance in laminae I and II (Westlund and Coulter, 1980). Other supraspinal nuclei which give rise to descending pathways that directly or indirectly innervate the dorsal horn, either inducing inhibition or facilitation, include the hypothalamus, the nucleus tractus solitarius (Morgan et al., 1989), the dorsal reticular nucleus (Zhao et al., 1999) and the anterior cingulum (for review see Fields et al., 2006; Millan, 2002).

The mechanisms behind the inhibitory control of descending pathways in the dorsal horn are difficult to elucidate due to several factors.
For example, the release of serotonin (5-HT) may act on many different 5-HT receptor subtypes, which in some cases can induce inhibitory and in others facilitatory influence on the pain related neuronal network. The location of these receptor subtypes on either excitatory or inhibitory interneurons, primary afferent neurons or projecting neurons defines the eventual effect of the released neurotransmitter. Thus, the complexity of the mechanisms involved in pain pathways demands an accurate knowledge of neurotransmitters involved, co-localization with other neurotransmitters, receptor subtypes and finally the neuronal circuitry (Millan, 2002).

Neuropathic pain: Peripheral, spinal and supraspinal mechanisms and the role of the dorsal columns

Most of the knowledge on mechanisms underlying neuropathic pain conditions is based on nerve-injury animal models. Lesions in peripheral nerves induce changes in the anatomical synaptic architecture and lead to a state of hyperexcitability which constitutes a main feature behind the genesis and maintenance of neuropathic pain. Following peripheral nerve injury, both peripheral and central mechanisms are triggered. For example, peripheral hyperexcitability is associated with up-regulation of specific voltage-gated sodium channels in primary afferents, neuroma formation at the site of nerve injury and with abnormal ectopic firing in both myelinated A and unmyelinated C fibers (De vor, 2006). Increased release of endogenous substances such as prostaglandins, bradykinin and cytokines has also been related to peripheral sensitization. It has been reported that up to 10% of all genes expressed in the DRG are up- or down-regulated in animal models of neuropathy (De vor, 2006). With regard to central mechanisms, sprouting of large diameter myelinated A-fibers of damaged axons from deeper laminae (III – IV) into superficial laminae (I – II) has been implicated in the genesis of neuropathic pain (Woolf et al., 1992; Woolf et al., 1995). However, the importance of this phenomenon in the abnormal functional circuit in neuropathic pain has been questioned (Bao et al., 2002). Spinal release of substance P (SP) from primary C-fibers has also been described as a key mechanism in central sensitization, although Aβ fibers might contribute to trigger and maintain this hypersensitive state (Noguchi et al., 1995). Sensitization of the dorsal horn neurons, enhanced by wind-up and long-term potentiation phenomena, leads to augmented responses to afferent stimuli and enlarged receptive fields (Davies and Lodge, 1987; Liu and Sandkuhler, 1995). In consequence, following peripheral nerve injury profound pathophysiological changes are triggered in the peripheral nervous system (PNS) and CNS resulting in activation of
pain mechanisms that account for a state of hyperexcitability (for review see Zimmermann, 2001). A loss of inhibition caused by changes in the physiological activity of spinal interneurons and in the descending pathways may also contribute to the hyperexcitability of the dorsal horn neurons.

It has been argued that the central hypersensitivity in neuropathic pain may be attributed mainly to the failed inhibition of pain afferents rather than to excitatory events (Dickenson, 1996). These alterations participate in the development of tactile allodynia and hyperalgesia. There is much evidence that the mechanisms related to these two forms of sensory dysfunction, often coexisting in neuropathic pain, are different. Tactile allodynia is mediated by low-threshold, large diameter, myelinated A\(\beta\) fibers and punctate hyperalgesia appears to be transmitted via A\(\delta\) fibers (Ziegler et al., 1999), while thermal hyperalgesia (heat) is believed to be transmitted by high-threshold, thin unmyelinated C fibers (for review see Ossipov et al., 2000b). Experimental dorsal column lesions or lidocaine injections in the nucleus gracilis in hypersensitive rats block tactile “allodynia” without affecting thermal hyperalgesia, providing further evidence that A\(\beta\) fibers transmit afferent inputs to supraspinal structures through the dorsal columns (Saade et al., 2006; Sun et al., 2001; Willis and Westlund, 1997). Thus, the dorsal columns are not only involved in the transmission of light touch and vibration but this pathway is also highly involved in abnormal pain transmission projecting to supraspinal relays (i.e. nucleus cuneatus and gracilis). It appears that non-noxious stimuli following peripheral nerve injury are transmitted through the dorsal columns to the sensory thalamus, resulting in an hyperexcitability of VPL nucleus that contribute to central sensitization clinically expressed as tactile hypersensitivity. In addition, data from rats subjected to mononeuropathy further support that the dorsal column-thalamic pathway is involved, together with the STT, in ascending nociceptive transmission (Miki et al., 2000).

The persistence of tactile allodynia and hyperalgesia appears to depend on the descending facilitatory pathways, originating in the RVM (“ON-cells”) and contained mainly in the dorso-lateral funiculus (DLF) (Ossipov et al., 2000a; Porreca et al., 2002; Sung et al., 1998). It has been argued that the mechanisms involved in the genesis of neuropathic pain, in contrast to its maintenance, are not dependant on facilitatory influence descending from the RVM (Burgess et al., 2002; Heinricher et al., 2003). In a recent paper by Saadé et al. (2006) it has been demonstrated that a chronic lesion of dorsal columns and/or DLF only temporarily abolish the signs of neuropathy in nerve lesioned rats. On the basis of these
observations they have questioned the importance of a spinal-brainstem-
spinal loop, constituted by an ascending component in the dorsal columns
and a descending component via the DLF ipsilateral to the hypersensitive
paw, in the maintenance of neuropathic signs (“allodynia”) (El-Khoury et
al., 2002; Saade and Jabbur, 2008).

In conclusion, pain caused by lesion of a peripheral nerve depends on
a variety of pathophysiological mechanisms, some with a firm scientific
base and some merely hypothetic. There are many changes that take place
both in the PNS and the CNS, but only part of these induced changes may
be directly related to the generation and maintenance of pain. Many other
processes are simply epiphenomena, a consequence of degeneration,
altered metabolism, cell survival, stress, etc.

Pain related neurotransmitters in the spinal dorsal horn
Glutamate is the major excitatory neurotransmitter released by both large-
and small-diameter primary afferent fibers in the dorsal horn (Li et al.,
1999; Pan and Pan, 2004; Yoshimura and Jessell, 1990). The C- and Aδ
fibers also contain neuropeptides such as SP, cholecystokinin (CCK) and
calcitonin gene-related peptide (CGRP) (Willis, 2001) (for review see
Hökfelt et al., 2000).

Sensory projection neurons located in the superficial dorsal horn, in
particular in the substantia gelatinosa (lamina II), receive excitatory and
inhibitory inputs from primary afferents, interneurons and from the
descending control system. These projection neurons are excited by
 glutaminergic inputs, which in turn are modulated by other systems such as
the noradrenergic, serotonergic, GABAergic/glycinergic and the
cholinergic (Iwamoto and Marion, 1993a; Li and Zhuo, 2001; Zhuo and

In this section, the role of the inhibitory systems that have been found
to be directly related to the mode of action of SCS and that are in the focus
of interest in this thesis will be discussed more in detail.

The GABAergic system and pain
Gamma-aminobutyric acid (GABA) is the principal inhibitory
neurotransmitter in the dorsal horn and up to 30 - 45% of neurons in the
superficial laminae express GABA immunoreactivity (Todd and Sullivan,
1990). This neurotransmitter acts on two receptor subtypes, a ionotropic
GABA\textsubscript{\textalpha} receptor and the G\textsubscript{i} protein-coupled GABA\textsubscript{\textbeta} receptor (for details
see Chebib and Johnston, 1999), both found presynaptically on Aδ and C-
fibers afferents and postsynaptically (for review see Dickenson et al., 1997). When the GABAₐ receptor is activated, an increased inward Cl⁻ current occurs and the activation of GABAₐ diminishes the opening of voltage-sensitive Ca⁺⁺ channels; in any case, receptor activation results in hyperpolarization of the neuron. The GABAergic system in the spinal cord, apparently acting in a tonic way, modulates the processing of responses to painful stimuli (Dickenson et al., 1997; Yaksh, 1989). The dysfunction of this system plays a key role in the genesis and development of neuropathic pain, inducing a disinhibition of pain afferents. Thus, neuronal hyperexcitability, presumably giving rise to the abnormal sign of hypersensitivity may, at least partially, be due to deficient GABAergic inhibition (Daemen et al., 2008; Dickenson et al., 1997; Meyerson and Linderoth, 2000). There is evidence that the release of GABA in the dorsal horn of rats, following partial nerve injury, is significantly reduced and likewise the GAD enzyme, involved in the synthesis of GABA, is also decreased (Castro-Lopes et al., 1993; Cui et al., 1997b; Moore et al., 2002; Ultenius et al., 2006). The role of the GABAₐ receptor in processing of noxious and innocuous stimuli can be further exemplified by the fact that baclofen, a GABAₐ receptor agonist, significantly reduces Aβ, Aδ and C-fiber evoked responses of the neurons in the dorsal horn (Sokal and Chapman, 2003).

Finally, it is important to realize that the GABAergic system in the dorsal horn interacts with a variety of neurotransmitter systems, such as the cholinergic. Muscarinic receptors, expressed on both axon terminals and somatodendritic sites in GABAergic interneurons, produce an increased activity of these inhibitory interneurons inducing GABA release (Baba et al., 1998). Furthermore, both serotonergic and noradrenergic descending systems activate these GABAergic interneurons resulting in the release of GABA in the dorsal horn (Baba et al., 2000; Kawamata et al., 2003). The presynaptic inhibition of the primary afferents reduces the release of excitatory amino acids such as glutamate and aspartate, as well as peptides such as SP and CGRP from the terminals (Furst, 1999; Malcangio and Bowery, 1996).

**Mechanisms of action of baclofen, gabapentin and pregabalin for pain**

Baclofen is a GABAₐ receptor agonist that has been used intrathecally for spasticity since the mid-1980s and more recently also in pain management (for review see Slonimski et al., 2004). In brief, this drug acts on GABAₐ receptors located mainly in laminae I-III in the dorsal horn, increasing
potassium conductance through a G-protein, which in turn produces hyperpolarization of second order projecting neurons. In addition, baclofen may activate presynaptic GABA-B receptors resulting in a decreased release of glutamate and SP from primary afferents in the dorsal horn (Marvizon et al., 1999; Teoh et al., 1996). Even though there are some studies showing that spinal baclofen may produce antinociception (Hammond and Drower, 1984; Wilson and Yaksh, 1978), clinically, it appears to be effective only for neuropathic pain (Hwang and Yaksh, 1997; von Heijne et al., 2001).

Gabapentin and pregabalin were originally developed to be used in the treatment of epilepsy. Similarly to other antiepileptic drugs, these drugs were later recognized as effective compounds in the treatment of neuropathic pain (Dickenson and Ghandehari, 2007; Dougherty and Rhoney, 2001). Despite the fact that gabapentin and pregabalin are structurally related to GABA, they do not act as GABA receptor agonists (Jensen et al., 2002). The mode of action of these drugs is complex and several different mechanisms have been proposed, although a selective inhibition of voltage-gated calcium channels containing αδ-1 subunit appears to predominate their pharmacodynamic profile (Gee et al., 1996; Sutton et al., 2002). It has been shown that the calcium channel αδ-1 subunit appears to be up-regulated in both the DRG and the spinal cord following peripheral nerve injury (Luo et al., 2002). The inhibition of calcium currents in neuronal terminals leads to a decreased release of excitatory neurotransmitters and attenuation of postsynaptic excitability (for review see Sills, 2006). Gabapentin has proven to relieve symptoms such as burning pain, allodynia and shooting pain (Backonja and Glanzman, 2003). Several clinical trials give support that per oral gabapentin should be the first choice of treatment in neuropathic pain, particularly postherpetic neuralgia, diabetic neuropathy, phantom limb pain and chronic pain related to spinal cord injury (Dworkin et al., 2003; Tremont-Lukats et al., 2000).

Acetylcholine

Acetylcholine (ACh) is the only low-molecular-weight neurotransmitter that is not an amino acid or derived from one. The biosynthesis of ACh has only one step in its path, which is catalyzed by choline acetyltransferase, transported and stored in vesicles and finally enzymatically degraded by acetylcholinesterase in the synaptic cleft (for review see Lefkowitz et al., 1995). The inhibition of this enzyme by drugs, such as neostigmine, diminishes the removal from the cleft and consequently, increases the amount of ACh available for binding to muscarinic and nicotinic receptors.
This neurotransmitter is widely spread in the CNS and the most important cholinergic pathways include the ACh released by spinal motor neurons, which activates nicotinic receptors at the neuromuscular junctions. In the autonomic nervous system, ACh is the key neurotransmitter present in all preganglionic neurons as well as in the parasympathetic postganglionic neurons.

The cholinergic muscarinic and nicotinic receptors

In 1914, Sir Henry Dale noted that esters of choline, Amanita muscaria and Nicotiana tabacum, elicited responses similar to those of muscarine and nicotine, respectively. He introduced the idea that ACh had a dual response in the autonomic nervous system, termed muscarine and nicotine action (Dale, 1914). This remains the basis for the classification of the cholinergic receptors in two principal groups: muscarinic and nicotinic receptors. Consequently, the muscarinic receptor was defined as receptors that could be activated by muscarine and blocked by atropine, and the nicotinic receptor activated and blocked by nicotine and tubocurarine, respectively.

It was not until 1980 that pharmacologically different muscarinic receptor subtypes were recognized (Hammer et al., 1980). To date, five muscarinic receptor subtypes (M₁ - M₅) have been identified by molecular biology techniques (Caulfield, 1993; Hulme et al., 1990; Wess, 1996), while only four receptor subtypes have been pharmacologically characterized (Waelbroeck et al., 1990). The odd-numbered subtypes (M₁ - M₃ - M₅) are selectively linked to the Gq protein, which activates the inositol triphosphate formation as a second messenger and eventually excites the cell. On the other hand, the even-numbered subtypes (M₂ – M₄) are preferentially coupled to the pertussis toxin-sensitive Gi protein, which in turn blocks cAMP generation and consequently inhibits the cell (Caulfield, 1993).

The muscarinic receptors are distributed in both the peripheral and central nervous systems and they are involved in many physiological functions e.g. cardiac function, glandular secretion, smooth muscle contraction, release of neurotransmitters, body temperature, sleep, motor control, attention, learning and memory processes and REM (rapid eye movement) sleep. Furthermore, there is strong evidence that muscarinic receptors play an important role in pain modulation, since muscarinic agonists have an antinociceptive effect (Abelson and Hoglund, 2002; Eisenach, 1999; Hood et al., 1997; Iwamoto and Marion, 1993b; Yaksh et al., 1985). The muscarinic receptors in the spinal cord are mainly present in the superficial laminae in both rats and humans (Barber et al., 1984;
Nicotinic receptors belong to the family of ligand-gated ion channels and their activation increases cellular permeability to Na⁺ and Ca²⁺ followed by a rapid depolarization. The architecture of this receptor consists of a transmembrane protein with five subunits, arranged in a manner that circumscribes an internal ionic channel.

Distinct subtypes of nicotinic receptors can be found at the neuromuscular junction and the ganglia and both types can be activated by ACh and the alkaloid nicotine (for review see Taylor, 1995). There is a large diversity of nicotinic receptors subdivided according to their subunits, which are classified into α and β subtypes. There are eight subtypes of α and four subtypes of β in the mammalian nervous system. The density of nicotinic receptors in the dorsal horn appears to be significantly lower than that of the muscarinic receptors (Gillberg et al., 1988; Khan et al., 1994).

The cholinergic system and pain

The role of cholinergic mechanisms in pain processing has attracted much less interest than for example that of the GABAergic system. Nonetheless, the spinal cholinergic system, including both muscarinic and nicotinic receptors, has since long been known to be involved in pain modulation (for review see Eisenach, 1999; Flores, 2000).

There is robust evidence that the i.t. administration of muscarinic agonists or acetylcholinesterase inhibitors results in potent analgesia in animals and humans (Duttaroy et al., 2002; Hood et al., 1997; Iwamoto and Marion, 1993b; Naguib and Yaksh, 1994). Due to the high toxicity of cholinergic agonists, only the cholinesterase inhibitor neostigmine has been tested in humans producing analgesia in acute postoperative and chronic pain (Eisenach, 1999). The strategy to explore muscarinic-mediated analgesia by inducing the release of endogenous ACh rather than using muscarinic receptor agonists has been investigated by several groups (Eisenach et al., 1996a; Eisenach et al., 1989; Hood et al., 1995; Hood et al., 1996; Roelants, 2006; Siddall et al., 2000).

The cholinergic system is highly integrated with other systems in the spinal cord. For example, the activation of the serotoninergic receptors
subtypes 5-HT$_{1A}$ and 5-HT$_{2A}$ by spinal serotonin administration produces analgesia and induces release of ACh (Kommalage and Hoglund, 2005). Moreover, the antiallodynic effects of i.t. administered 5-HT$_2$ receptor agonists may be mediated by spinal release of ACh (Obata et al., 2003). The activation of presynaptic muscarinic receptors results in analgesia and this effect is associated with a decreased release of glutamate from primary afferents (Li et al., 2002). Muscarinic receptor activation located in GABAergic interneurons potentiates the GABAergic/glycinergic inhibitory tone in the dorsal horn, and consequently also decreases the glutamate release (Baba et al., 1998; Chen and Pan, 2003; Li et al., 2002).

Although the role of spinal muscarinic analgesia is now firmly established, disagreement remains with regard on the muscarinic receptor subtypes involved. For example, some authors suggested, based on behavioural studies, that M$_1$ and M$_2$ receptors were implicated in analgesia (Iwamoto and Marion, 1993b), but others found that M$_1$ and M$_3$ receptors were principally involved (Naguib and Yaksh, 1997). However, radioligand binding studies fail to prove the existence of M$_1$ in the spinal cord (Höglund and Baghdoyan, 1997). Some argue in favour of both M$_2$ and M$_4$ receptors as being responsible for mediating muscarinic analgesia (Duttaroy et al., 2002; Gomez et al., 1999), although a distinction between these two receptor subtypes has to be mentioned. Even though the M$_2$ receptor seems to be the most predominant one in the dorsal horn (Yung and Lo, 1997), the smaller amount of M$_4$ is still claimed to be functionally significant and important for muscarinic analgesia (Ellis et al., 1999; Höglund and Baghdoyan, 1997; Mulugeta et al., 2003). Besides, there is some evidence that the M$_2$, M$_3$ and M$_4$ subtypes increase the GABAergic tone and may be responsible for the inhibition of the glutamate release from interneurons in the dorsal horn, while only the M$_2$ subtype appears to play an important role in the decrease of glutamate release from primary afferents (Zhang et al., 2006). It is worth noting that muscarinic agonists induce analgesia by GABA release, which acts particularly on GABA$_B$ receptors located in glutaminergic primary afferents. Consequently, the muscarinic-mediated analgesia may be attenuated by blocking GABA$_B$ receptors (Chen and Pan, 2003).

The clinical use of muscarinic agonists has been curtailed by the fact that the doses required to produce analgesia are almost equivalent to those that cause severe side effects (Gillberg et al., 1990; Hartvig et al., 1989). Therefore, the strategy of enhancing the release of ACh by other drugs appears to be a more useful approach (Eisenach, 1999).
The analgesic properties of nicotine were demonstrated already in 1932 by findings in a cat model of visceral pain (Davis et al., 1932). Since this substance acts on both neuronal and muscular nicotinic acetylcholine receptors, it produces not only antinociception but also severe side effects such as hypertension, seizures and neuromuscular paralysis. Nicotinic receptors situated in both the sympathetic and parasympathetic ganglia as well as in the adrenal medulla are responsible for the autonomic effects of nicotinic receptor agonists. This is why some compounds with nicotinic agonist properties such as epibatidine, an alkaloid isolated from the skin of the Ecuadorian frogs, often are toxic and therefore unsuitable for clinical usage (Badio and Daly, 1994; Qian et al., 1993).

The noradrenergic system and $\alpha_2$ adrenoreceptors in the dorsal horn

There are three major groups of adrenoreceptors: $\alpha_1$, $\alpha_2$ and $\beta$. Similarly, the $\alpha_2$ receptors are classified into three subtypes: $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$, all of which are $G_i$ protein coupled. Upon activation of $\alpha_2$ receptors, inhibition of adenyl cyclase is induced, in turn enhancing the $K^+$ currents and suppressing the $Ca^{++}$ currents (for review see Philipp et al., 2002). Both $\alpha_{2A}$ and $\alpha_{2C}$ adrenoreceptor subtypes have been identified in superficial and deep laminae in the dorsal horn. The $\alpha_{2A}$ receptor subtype has been found on primary nociceptive afferent fibers and interneurons in laminae II and III, while the $\alpha_{2C}$ receptor subtype is located only on the spinal interneurons (Riedl et al., 2009; Stone et al., 1998). Neither subtype has been detected on descending noradrenergic terminals. The $\alpha_{2A}$, but not $\alpha_{2B}$ or $\alpha_{2C}$, receptor subtype seems to play a key role in analgesia (Stone et al., 1998; Stone et al., 1999). Conversely, the activation of $\alpha_1$ receptors facilitates neuronal excitability and may have a pronociceptive effect (Millan, 2002).

There is robust evidence that descending noradrenergic projections from locus coeruleus suppress the excitability of projection neurons in the dorsal horn via activation of $\alpha_2$ adrenoreceptors (for review see Millan, 1997; Todd and Koerber, 2006). In addition, this receptor presynaptically modulates the release of excitatory transmitters such as glutamate and SP from primary afferents. Thus, the released noradrenaline from descending pathways activates $\alpha_2$ adrenoreceptors eliciting analgesia. Consequently, spinally administered $\alpha_2$ agonists are effective drugs for painful conditions, including neuropathic pain (Eisenach et al., 1996a; Eisenach et al., 1995; Honda et al., 2002; Yaksh et al., 1995). The $\alpha_2$ adrenoreceptor agonist most commonly used both clinically and in experimental studies has been clonidine, discussed in detail later in this chapter.
Mechanisms of action of clonidine for pain

Clonidine is an $\alpha_2$ adrenoreceptor partial agonist that has proven to provide pain relief in humans and to suppress pain-related behaviour in animals (for review see Eisenach et al., 1995). This drug was first tested epidurally in patients with chronic pain by Tamsen and Gordh in 1984 (Tamsen and Gordh, 1984). Since then, both epidural and intrathecal (i.t.) clonidine has been extensively used as an alternative or an adjuvant to opioids in cancer-related and postoperative pain with fewer adverse effects, avoiding respiratory depression caused by morphine (Anzai and Nishikawa, 1995; Eisenach et al., 1989; Gordh, 1988; Huang et al., 2007; Martin and Eisenach, 2001; Milligan et al., 2000; Vercauteren et al., 1990). There is some evidence that clonidine is more effective for neuropathic pain than for nociceptive pain (e.g. Eisenach et al., 1996a; Rauck et al., 1993).

Even though it is well established that clonidine acts on the $\alpha_2$ adrenoreceptor, the subtype of the receptor involved in the analgesic effect, has not yet been definitely identified. A pharmacodynamic experimental study has indicated that the $\alpha_{2A}$ adrenoreceptor subtype is implicated in antinociception in normal rats. However, following peripheral nerve injury, the expression of this, but not the $\alpha_{2C}$ receptor subtype, appears to be reduced (Stone et al., 1998; Stone et al., 1999). In consequence, the effect of clonidine in neuropathic pain could be related to the activation of an $\alpha_{2 \text{ NON-} A}$ receptor subtype (Duflo et al., 2002).

Despite the fact that clonidine activates $\alpha_2$ adrenoreceptors, several lines of evidence indicate that the analgesic effect of clonidine relies, at least partially, on spinal cholinergic mechanisms (Klimscha et al., 1997; Obata et al., 2005; Pan et al., 1999; Paqueron X, 2001). Many observations give support to this idea. First, clonidine may induce ACh release in the spinal cord of animals (Detweiler et al., 1993; Klimscha et al., 1997) and increase ACh concentration in the cerebrospinal fluid (CSF) of humans (De Kock et al., 1997). Second, the analgesic effect of clonidine can be reversed by muscarinic receptor antagonists (Pan et al., 1999) in nerve injury rats, but not in normal animals (Obata et al., 2005). Furthermore, there is evidence that clonidine-induced ACh release produces analgesia principally via muscarinic, and to a lesser extent nicotinic receptors, which in turn induces nitric oxide (NO) formation in the spinal cord (Pan et al., 1998; Xu et al., 2000). Finally, spinal clonidine may be potentiated by acetylcholinesterase inhibitors (i.e. neostigmine) in animals (Naguib and Yaksh, 1994) and patients (Hood et al., 1996).

As has been pointed out, spinally administered clonidine is an effective treatment for acute and chronic pain, but its use may be limited by
adverse effects such as sedation and cardiovascular depression (Martin TJ and JC., 2001).

**Gate Control Theory: a framework for spinal cord stimulation mode of action?**

In 1965, Ronald Melzack and Patrick Wall published their well known gate control theory in *Science*, in which a specific pain modulatory system was proposed (Melzack and Wall, 1965) (Figure 1). This theory stated that the substantia gelatinosa (lamina II) functions as a gate control, that the dorsal column system could be involved in the activation of certain areas of the brain that modulate the gate and that the first central transmission (T) cells (i.e. tract cells in the dorsal horn, whose axons cross and join the spinothalamic tract) activate neural mechanisms involved in pain perception. In brief, the spinal cord receives small myelinated and unmyelinated afferent fibers which hold the gate open while excitation of large fibers, usually inactive in the absence of stimuli, tend to close the gate. Then, the balance between small and large fiber activity, counteracting each other, modulates pain input by regulating the output of the T cells. Supraspinal influences on the gate were suggested, nonetheless without determining specific structures or systems responsible for this central inhibition. At that time, the theory was conceived to be valid for pain in general, without considerations of a possible difference between nociceptive and neuropathic pain.

A curious fact is that the first clinical application of the gate control theory was performed by Patrick Wall and William Sweet by stimulating their own infraorbital nerves using needle electrodes (Wall and Sweet, 1967). They observed that low-intensity stimulation induced analgesia to pin prick in the territory of this nerve.

In the pain literature, spinal cord stimulation (SCS), then called dorsal column stimulation (DCS), has often been explained as a direct spin-off of the gate control theory. However, today this theory should only be considered rather as a framework for understanding the mode of action of SCS because it cannot explain completely how SCS actually produces pain relief. Contrary to the original concept, SCS should not be regarded as a therapy for all kinds of acute and chronic pain, but specifically for chronic neuropathic and ischemic pain syndromes (Linderoth and Meyerson, 2009; Meyerson, 2003). Excitations and inhibitions produced by the release of different neurotransmitters converge into the gate and SCS may act by inducing changes in the balance of these two components, determining the
actual output from the gate. One can consider, therefore, that SCS constitutes one way of closing the gate.

To summarize, the gate control concept “stands the test of time” (Dickenson, 2002) and for the understanding of the spinal mechanisms underlying the pain relieving effect of SCS it still serves as the “basic concept”.

![Diagram of the gate control theory](image)

**Fig. 1** The gate control theory is based on the interaction of four classes of neurons in the spinal dorsal horn: (a) unmyelinated nociceptive afferents - C fibers (also thin myelinated Aδ fibers) that hold the gate open, (b) large myelinated Aβ fibers that tend to close the gate, (c) inhibitory interneurons and (d) projection neurons (PN). The balance between nociceptive and non-nociceptive afferents, counteracting each other, modulates the output of the projection neurons to supraspinal levels. Modified from Basbaum and Jessell (2000).

**Present knowledge on physiological and neurochemical mechanisms of SCS**

Even though considerable research has been made in the field of SCS during the last two decades, much remains fragmentarily understood concerning the mechanisms of action of SCS. Initially, the studies conducted to explore the mode of action of SCS were performed in anesthetized normal animals (e.g. Handwerker et al., 1975; Shetter and Atkinson, 1977). In those experiments, only noxious stimuli were applied and stimulation consisted in short periods of time, less than one minute, using parameters that differ completely from those used in patients (e.g.
Chandler et al., 1993). Hence, limited clinical relevance of such investigations can be inferred. In early studies, it was shown that SCS applied in normal rats may induce the release of certain neurotransmitters such as SP, serotonin and GABA. However, these changes are not necessarily related to its pain relieving effect (Duggan and Foong, 1985; Linderoth et al., 1992; Linderoth et al., 1994). The introduction of the use of animal models of neuropathy in conjunction with the employment of stimulation parameters mimicking the clinical situation represents a new era in the study of the underlying mechanisms of SCS in neuropathic pain (Meyerson et al., 1995).

SCS may modulate many physiological processes depending on the level of the spinal cord where the stimulation is applied, inducing effects such as bronchodilatation, peripheral vasodilatation, etc (for review see Linderoth et al., 2009; Linderoth, 2006). When applied in neuropathic pain, there is convincing evidence that different neurotransmitter systems may be involved, some of which have been partially identified (for review see Linderoth and Meyerson, 2000; Meyerson and Linderoth, 2006). Convincing data provide evidence that SCS may induce GABA release in the dorsal horn of mechanically hypersensitive rats following peripheral nerve injury (Stiller et al., 1996b). In subsequent experiments it was further observed that SCS may reduce the release of the excitatory amino acids (EAA) glutamate and aspartate associated with an incremental GABA release in the dorsal horn (Cui et al., 1997b). The inhibition of EAA release by SCS was blocked by the administration of a GABA\textsubscript{B} antagonist (Cui et al., 1997a). Both the GABAergic and cholinergic systems appear to play a key role in the pain relieving effect of SCS and they will be discussed in more detail later in this chapter.

The hyperexcitability of multimodal wide-dynamic range (WDR) neurons in the dorsal horns is related to an increased release of excitatory amino acids (e.g. glutamate) and a decreased inhibition by local spinal GABAergic interneurons (Gwak et al., 2006). Previous experiments in rats demonstrated that SCS inhibits the WDR neuron hyperexcitability (Yakhnitsa et al., 1999) by inducing GABA release in the dorsal horn, with a subsequent decrease of glutamate release. Moreover, it seems that SCS also can suppress abnormal neuronal hyperexcitability by modulating C fiber-related long-term potentiation of WDR cells (Wallin et al., 2003).

The primary effect of SCS has been attributed to the antidromic activation of ascending dorsal column fibers inducing segmental neurochemical changes in the dorsal horn. However, there is increasing evidence that an orthodromic activation occurs involving supraspinal
mechanism via a loop in which the descending dorsolateral funiculus (DLF) plays a role in the attenuation of neuropathic behaviour signs by SCS (El-Khoury et al., 2002; Saade et al., 2006). Furthermore, recent investigations indicate that SCS may activate descending serotoninergic pathways that modulate spinal pain transmission via the GABAergic system (Song et al., unpublished).

Fig. 2 Schematic representation of the possible mode of action of SCS in neuropathic based on present knowledge derived predominantly from experiments performed on animal models of mononeuropathy. Both segmental and supraspinal mechanisms are represented. Possible supraspinal relays are not included because of insufficient knowledge about the organisation of a proposed supraspinal loop. Broken arrow lines represent antidromic, and full line arrows orthodromic activation in the dorsal columns, their collaterals and in primary A-afferents. The diagram does not depict a possible SCS activation of the dorsolateral funiculus (DLF). It is conceivable that numerous transmitters and modulators are involved in the modulation exerted by interneurons (represented by “X”). Descending control of second order neurons is here represented as inhibitory only, although there is evidence suggesting also a facilitatory supraspinal input. SP – substance P; EAA – excitatory amino acids (glutamate and aspartate); ACh – acetylcholine; 5-HT – serotonin. Modified from Meyerson & Linderoth (2006).
It appears that the opioid system is not involved in the effect of SCS. In fact, only a few studies address the possible role of endogenous opioids in pain relief by SCS (Hawkes et al., 1981). In one of these studies, elevation of opioids in the CSF induced by stimulation has been found (Tonelli et al., 1988). It is methodologically doubtful whether neurotransmitter found in the CSF reflects changes induced by SCS in the dorsal horn. Furthermore, the effect of SCS cannot be attenuated by coadministered naloxone (Meyerson et al., 1977). Interestingly, the dynorphin system may be activated by high frequency transcutaneous electrical nerve stimulation (TENS) (Han et al., 1991) and this cannot be effectively blocked by naloxone. No data on the involvement of dynorphin in the mode of action of SCS are available.

The mechanisms that are involved in the pain relieving effect of SCS according to the present knowledge are summarized in Figure 2.

**History and clinical application of spinal cord stimulation**

According to the gate control theory the proposed gating mechanisms implicate that SCS would be effective for both acute and chronic pain of nociceptive origin. The paradoxical fact is that SCS does not seem to interfere with normal pain responses or with nociceptive pain thresholds (e.g. Lindblom and Meyerson, 1975). Today there is a clear consensus among clinicians and pain experts that SCS is probably only effective for chronic neuropathic pain (Meyerson and Linderoth, 2000) and indirectly for ischemic pain as a result of a decreased tissue ischemia (Linderoth et al., 2009). The most common indications of SCS are lumbosacral rhizopathy, pain following peripheral nerve injury, Complex Regional Pain Syndromes (CRPS), angina pectoris and ischemic limb pain (Birknes et al., 2006).

The first clinical application of SCS for pain in patients was reported in 1967 by Norman Shealy (Shealy et al., 1967). In the first decade after its introduction, the indiscriminate application of SCS for a wide spectrum of pain diagnoses resulted in a poor long-term outcome (Ray, 1978). However, during the last two decades there has been an increased awareness that SCS is a therapy preferentially for well-selected patients with neuropathic pain, in whom pharmacological treatments had been tried in vain. The improved results reported in later studies, in which up to 80% of patients have obtained satisfactory pain relief (Kumar et al., 2008; Lee and Pilitsis, 2006), are probably due to a more stringent selection of patients and the progress in the development of multipolar electrodes and multiprogrammable neurostimulators (Linderoth and Meyerson, 2009;
A definite pain diagnosis and its chronicity, identifying neuropathic and nociceptive components - that often co-exist - are mandatory to consider a patient as a candidate for a SCS trial. A thorough pain assessment needs to include the analysis of certain pain features, related to the outcome of SCS, and they deserve to be briefly summarized. For example, patients suffering from pain in the extremities are more likely to respond than patients displaying trunk pain. Generally, if patients have constant pain they are also more likely to respond to SCS compared to if they have paroxysmal and shooting pain. Another relevant feature is the preservation of some cutaneous sensibility in the painful area indicating viability of Aβ fibers. However, in some conditions with major denervation, e.g. phantom pain, SCS can be effective if paresthesiae can be evoked in the denervated area (phantom). Pain at rest is more likely to be relieved than pain associated to load. In addition, a psychological evaluation, preferably performed by a pain-oriented psychologist or psychiatrist, is recommended because certain psychological features may influence the outcome of SCS (Burchiel et al., 1995; Dumoulin et al., 1996; Kupers et al., 1994). However, the association between some personality disorders and the SCS outcome has been questioned by others (North et al., 1996).

For SCS to be considered, it is generally accepted that the pain syndrome has to be pharmacologically resistant (cf. Hansson et al., 2009). However, this concept needs to be revised because many patients suffering from neuropathic pain often gain a partial effect with drugs although they tend to have poor compliance due to adverse effects. On the contrary, SCS has virtually no side effects, but major drawbacks are the high initial cost and the need for the staff demanding follow-up. The standardized percutaneous stimulation trial before considering a permanent implantation of a SCS system, as presently employed, has also contributed to an improved patient selection for a chronic implantation (Lazorthes et al., 1995).

The application of SCS has grown exponentially worldwide, and it is estimated that more than 18,000 new SCS systems are implanted every year (Linderoth et al., 2006). This is reflected by the increasing number of publications including some meta-analyses and several randomized controlled studies (Kemler et al., 2000; Kemler et al., 2008; Kumar et al., 2008; Kumar and Wilson, 2007; Mailis-Gagnon et al., 2004; North et al., 1995).
Intrathecal administration of drugs for pain

The blood-brain barrier precludes or slows the entrance of most drugs into the CNS. Therefore, when a drug is intended to reach the CNS rapidly (e.g. in meningitis and spinal anaesthesia), the intrathecal route of administration may be a better alternative than intravenous routes. Drugs administered to the intrathecal space in the lumbar region initially reach high concentrations in the CSF at this location but then are redistributed by rostral spread in the CSF, penetration into the nervous tissue and by uptake into the systemic circulation. Furthermore, the physiological flow of the CSF and the baricity, in conjunction with the position of the patient, determine the rostral spread of the drug. Finally, the hydrosolubility of the drug influences its elimination. Hydrophobic drugs have a more rapid uptake from the CSF and consequently their rostral distribution within the neuroaxis is less affected (Bernards, 1999).

The objective of the i.t. administration of analgesic drugs is to target the spinal cord at a defined level, and to obtain the desired therapeutic response with few or no side effects in patients with severe pain resistant to oral or parenteral therapy. There are currently three main groups of drugs that have been demonstrated to be effective for the management of chronic pain of both malignant and benign origin: opioids, local anaesthetics and non-opioids such as baclofen and clonidine (Bennett et al., 2000; Borg and Krijnen, 1996; Eisenach et al., 2000; Ghafoor et al., 2007; Hassenbusch et al., 1995; Lind et al., 2004; Penn, 2003).

The development of programmable electronic pumps for i.t. administration of drugs has shown to be useful and relatively safe. However, in non-malignant chronic pain there are no standard guidelines with regard to patient and drug selections (Krames and Olson, 1997), an important argument why a pre-implant trial with bolus or continuous i.t. administration is mandatory (Prager and Jacobs, 2001).
THESIS AIMS

The general aim of this thesis has been to contribute to the understanding of the mechanisms underlying the pain relieving effect produced by spinal cord stimulation and to explore the possibility of enhancing SCS efficacy by pharmacological adjuvants in rats and later to apply such combined therapy in patients. To achieve this, more specific aims have been defined:

1. To investigate the role of the cholinergic system in the pain relieving effect of SCS in rats with mechanical hypersensitivity following peripheral nerve injury and to identify the nicotinic or muscarinic receptor subtypes involved in the suppressive effect of SCS on the hypersensitivity (study I).

2. To explore whether the α2-adrenergic receptor agonist clonidine suppresses mechanical hypersensitivity following peripheral nerve injury in the rat. A further aim has been to investigate in rats whether subeffective doses of clonidine can potentiate the effect of SCS when stimulation *per se* has no suppressive effect (study II).

3. To explore the abilities of the anticonvulsants gabapentin and pregabalin to suppress mechanical hypersensitivity following peripheral nerve injury and to investigate the capabilities of subeffective doses of these anticonvulsants to potentiate SCS when stimulation *per se* has no suppressive effect. In addition, to correlate the results of the behavioural studies to the neuronal activity in the dorsal horn of the spinal cord in rats with peripheral nerve injury following SCS and gabapentin alone, as well as following SCS combined with gabapentin (study III).

4. To explore and compare, based on previous results from animal and clinical studies, the possible enhancing effect of SCS by concomitant intrathecal administration of clonidine and baclofen in patients who do not obtain satisfactory pain relief with SCS alone and to evaluate the long-term outcome of baclofen as an adjuvant to SCS (studies IV and V).
MATERIALS AND METHODS

Animal studies (I-III)

Animal welfare and ethical aspects
Male Sprague-Dawley rats (B&K Universal AB, Sweden) weighing 175-400 g were used in all experiments. The animals were initially housed in groups of 4-5 rats at the local animal department for one week before the experiments started. After electrode or catheter implantation, the rats were individually housed in smaller single cages in order to prevent damage of the contacts of the electrode or the externalized catheter by other rats. The animals were exposed to a 12 h light-dark cycle and provided with low soy protein (70%) food and water ad libitum.

All experiments reported in this thesis were performed according to the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (1983) and were approved by the local ethical committee for animal research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical and evaluation procedures

Anaesthesia
The surgical procedures in studies I-III were performed under general anaesthesia delivered through an open mask system. Anaesthesia was induced by 4% Isoflurane (Forene®, Abbott, Sweden) in study I and by halothane 3% (Fluothane®, AstraZeneca, Sweden) in studies II and III and maintained with 1-2 % of the general anaesthetic in a 1:1 mixture of air and oxygen at a flow-rate of 2 l/min. A lack of response to painful stimuli indicated an adequate level of anaesthesia. No local anaesthetic was used. An automatic heating pad (CMA/150, CMA Microdialysis AB, Stockholm, Sweden) was used to keep the body temperature at 37 ± 0.5° C during surgery. In study I, postoperative analgesia after the electrode implantation was provided by i.p. 0.03 mg/kg Buprenorphin (Temgesic®, Schering-Plough, UK).
Animal models of neuropathy (I-III)

In studies I and II, a partial sciatic nerve lesion was performed according to Seltzer et al. (1990). This model of mononeuropathy is produced by tightly ligating approximately one-third to one-half of the dorsal part of the nerve with a 8-0 silk suture (Virgin silk, Ethicon/Johnson & Johnson, Belgium) at a point near the trochanter, just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. This partial traumatic nerve injury leaves a majority of the sciatic nerve fibers intact, avoiding a complete denervation of the hind paw and consequently evoked pain can be examined with von Frey filaments. The wound was sutured in layers and the animal was returned to its cage to recover. A few days after the induction of sciatic nerve injury, a portion of the rats develops signs of neuropathy in terms of hypersensitivity to mechanical and/or thermal stimuli applied to the corresponding hind paw.

In study III, two other animal models of mononeuropathy were used: a photochemical ischemic lesion in the sciatic nerve (Gazelius et al., 1996), and a chronic constriction injury (CCI) of the sciatic nerve (Bennett and Xie, 1988). To perform the ischemic lesion, a photochemically active dye (Erythrocin B, 50 mg kg−1, Aldrich-Chemie, Germany), administered i.v., which in combination with a laser beam (λ = 532 nm and P = 5 mW, Laser-Compact Company Ltd, Russia) applied directly to the nerve during 20 min, produces a platelet aggregation, inducing the formation of microthrombosis.

In the CCI model, the left sciatic nerve was loosely ligated with four adjacent 4-0 catgut sutures applied about 1 mm apart from each other.

Behavioural evaluation (I-III)

A few days after the induction of a sciatic nerve injury some of the rats develop mechanical hypersensitivity of the corresponding paw. All behavioural tests were conducted under standardized conditions with regard to noise levels, light intensity and time of day. The rat, placed in a circular observation cage with a mesh floor, was tested using von Frey nylon filaments (Marstocknervtest, Germany) in order to assess static mechanical sensitivity before, during and after i.t. drugs and SCS (Figures 3A and B). The filaments were calibrated in a range from 0.5 to 30 g, the latter selected as cut-off. Methods for assessing mechanical hypersensitivity with von Frey nylon filaments have been described in detail elsewhere (e.g. Cui et al., 1998; Kupers et al., 1998a; Chaplan et al., 1994; Kupers et al., 1998c). The filaments were applied through the mesh of the circular plastic observation cage to the mid-plantar surface of the
paw until it was gently bent. The softest filament provoking paw withdrawal at least five times out of ten applications (50% probability of withdrawal) was defined as the withdrawal threshold (WT) or degree of hypersensitivity. The assessment was started with a 4 or 5 g filament and continued in ascending or descending order of filament stiffness after a negative or positive response, respectively. Mechanical hypersensitivity was defined as 50% probability of withdrawal of a filament corresponding to 7 g or less (study I), and 8 g or less (studies II and III). As a control, the WTs were alternately compared for the same parts of the hind paw of the intact and the nerve injured paw. After establishing a baseline WT, SCS or an i.t. drug was administered (described below) and the assessment was repeated every 10 min until pre-treatment thresholds were restituted.

Fig. 3 A rat subjected to sciatic nerve injury resulting in an abnormal posture of the left hind paw (A). The setup for testing the hind paw sensibility in rats (B). The floor of the observation cage consists of a metal net permitting the access to the paws with von Frey filaments. Spinal cord stimulation may be applied while the rat is awake and freely moving in the cage.

Microdialysis (I)

Microdialysis has been extensively used in neurosciences and in pain research to monitor neurotransmitter release (Kehr et al., 1998; Kehr et al., 2001; Nelson et al., 2004; Stiller et al., 2003; Ungerstedt, 1991; Ungerstedt and Hallstrom, 1987). This technique is based on the second law of thermodynamics stating that the entropy of an isolated system will spontaneously tend to approach equilibrium. Applying this principle to microdialysis, it implies that molecules in solution diffuse through a semi-permeable membrane between two compartments, the extracellular fluid
(ECF) of the tissue and the physiological buffer solution inside the microdialysis probe. Thus, a thin semipermeable dialysis tube implanted into living tissue mimics the function of a capillary blood vessel and may detect changes in concentration of substances such as neurotransmitters in the ECF.

*In vivo* microdialysis in the spinal cord is a refined technique which has the ability to monitor the release of substances such as ACh (study I) in neuronal tissue over time. A schematic representation of the microdialysis technique for the detection of ACh is illustrated in Figure 4.

**Fig. 4** Schematic representation of the function of microdialysis when applied for analysis of extracellular acetylcholine in the spinal dorsal horn. The acetylcholine released (in light blue) in the synaptic cleft can be collected by the probe with the semipermeable membrane. It also bind to receptors, in this case muscarinic, located on the postsynaptic membrane. Neostigmine (in green) interferes with the enzymatic degradation of acetylcholine by acetylcholinesterase (in yellow) and is therefore deposited in the extracellular space from the dialysis fluid in the probe in order to enhance the acetylcholine concentration.

**Implantation of microdialysis probes (I)**

*In vivo* microdialysis was performed under general anaesthesia. The animal was mounted in a stereotaxic spinal unit and the vertebral column was
stabilized with clamps to minimize interference from breathing movements of the chest, illustrated in Figure 5.

The spinal cord was exposed via a partial laminectomy at T13, a vertebral level that corresponds to the spinal L4-L6 cord segments. The microdialysis probe (CMA 11; single cuprohane dialysis membrane, with a length of 2.0 mm and an outer diameter of 0.24 mm, molecular cut-off 6 kDa CMA Microdialysis AB, Stockholm, Sweden) was mounted on a micromanipulator (David Kopf, CA, USA), and inserted rostro-caudally in a 45° angle into the dorsal horn on the nerve injured side, to an estimated depth of 1.4 mm in the axial plane.

Fig. 5 Laboratory set-up for microdialysis of the spinal cord in rat. The pump for continuous dialysate flow through the probe is shown in the lower part of the photo and the rotating test tube holder for collection of fluid specimen to the left. Note, the clamps fixating the spinal processes adjoining the exposed spinal cord.

The perfusate consisted of a modified Ringer solution (NaCl 148 mmol/L; KCl 2.7 mmol/L; CaCl₂ 1.2 mmol/L; MgCl₂ 0.85 mmol/L) containing 10 μM of neostigmine, an acetylcholine esterase inhibitor, to prevent the degradation of ACh and thereby increasing the recovery of the
neurotransmitter (Abelson and Hoglund, 2002; Barber et al., 1984; Billard et al., 1995; Höglund et al., 2000). The microdialysis experiments consisted in collecting consecutive 30 min samples to determine the levels of ACh in three different conditions: basal levels prior to SCS, during stimulation and finally after SCS. At the end of the experiment, stimulation with 100 nM KCl in the perfusion medium was performed in order to induce a massive depolarization of all neuronal and glial cells in the vicinity of the microdialysis probe. After collection of the dialysates during consecutive 30 min periods, the samples were immediately frozen to -20°C for a subsequent High-Performance Liquid Chromatography (HPLC) analysis of ACh.

**HPLC (I)**

HPLC was applied to quantify ACh in the dialysate samples (study I). Before starting the in vivo microdialysis experiments, an in vitro recovery was analyzed for five different concentrations of ACh (50, 100, 250, 500 and 100 nM) to ensure that the recovery was stable and that the microdialysis system and HPLC could detect changes in the release of ACh throughout the experiments. Briefly, the system consists of a microbore column liquid chromatography linked to a post-column immobilized enzyme reactor and electrochemical detection on a peroxidase-redox polymer-coated electrode. The HPLC system included a micro-LC pump (LC100, ALS, Tokyo, Japan), an LC23 Degasser (ALS), a CMA/200 refrigerated microsampler (CMA Microdialysis, Sweden) equipped with a 19.5-μl loop and operating at +6°C, a BAS LC4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA) equipped with a Radial-flow electrochemical cell (BASJ, Tokyo, Japan). The detection limit for ACh was 10 fmol/20 μl. (for review see Lunte and Lunte, 1996).

**Experimental protocol (I)**

The experimental protocol of study I consists of two parts: (a) investigation of the spinal release of ACh following SCS assessed by microdialysis and HPLC; (b) influence of ACh receptor antagonists on the effect of SCS. A flow-chart of the experimental protocols is presented in Figure 6.

**Implantation of spinal electrodes (I-III)**

In studies I, II and III, rats subjected to sciatic nerve injury showing signs of mechanical hypersensitivity were implanted with a custom-made monopolar electrode system for spinal cord stimulation (e.g. Cui et al., 38
1996b; Cui et al., 1998; Linderoth et al., 1993; Meyerson et al., 1995; Stiller et al., 1996b).

Under general anaesthesia a small laminectomy was performed using a micro-drill with a diamond burr (Ø = 1-2 mm) in order to minimize the risk for puncturing the dura or damaging the spinal cord. The cathode (a solid rectangular silver plate: 3 x 1.5 x 0.25 mm) was placed in the dorsal epidural space and inserted rostrally beneath the lamina. The cathode wire was then fixed to the musculature with tight sutures. The anode (a solid silver disc of 6 mm in diameter) was implanted in the paravertebral

---

**Fig. 6** Outline of the experimental design showing schematically the two main parts of study I. First, the microdialysis group of rats was subjected to sciatic nerve lesion and the withdrawal thresholds were determined. In rats displaying tactile hypersensitivity an SCS system was implanted and the effect of SCS was assessed. This part of the study included three subgroups: rats responding to SCS (n=7), SCS non-responding rats (n=7) and a control group of normal rats (n=6). All rats were subjected to microdialysis in order to determine ACh release following SCS in the dorsal horn assessed by HPLC. Another group of hypersensitive rats after nerve injury was implanted with an SCS system and tested. In rats responding to SCS, an i.t. catheter was inserted in order to test a possible suppression of the SCS effect by different cholinergic receptor antagonists.
Subcutaneous tissue by making a small pocket in the fascia on the left side of the spine. The microcontacts, connected to the poles by silicon-insulated stainless steel wires, were tunnelled subcutaneously and sutured tightly to the neck skin. To avoid damage to the microcontact, the animal was kept in a separate cage after surgery and allowed to recover from surgery for at least 24 h before further experiments were initiated. After the electrode implantation, the condition of the rats was carefully checked. If a rat showed neurological deficits after full recovery from anaesthesia it was immediately euthanized.

Surgical preparation for microrecordings (III)

In study III, extracellular single unit activity from wide-dynamic range (WDR) neurons in the spinal dorsal horn of rats, displaying signs of peripheral neuropathy, was recorded. The effects of SCS and in combination with i.t. gabapentin on those neurons were investigated. Under general anaesthesia, microrecordings were performed with tungsten microelectrodes inserted ipsilaterally to the nerve injury in segments L3–5 medially to the dorsal root entry zone. In order to identify such neurons, activity in response to various stimuli applied to the plantar surface of the hind paws in a gradual manner (brush < press < pinch), was recorded (cf. Yakhnitsa et al., 1999). When a WDR cell was identified, non-painful mechanical stimuli, consisting of gentle paw pressure with a flat-tipped forceps, were applied for 10 s at least 60 s apart. Unitary activity was amplified and led to an analog–digital system (MacLab/4, Castle Hill, Australia) for the construction of records of neuronal responses and/or peristimulus time histograms.

Implantation of intrathecal catheters (I-III)

Nerve injured rats presenting with consistent signs of neuropathy (i.e. decrease of the paw withdrawal threshold to innocuous tactile stimuli) were subjected to implantation of an i.t. catheter. Under general anaesthesia, a polyethylene catheter (Lynn Scott, USA) was inserted via a cannula between the L5-6 lamina and advanced 4.5cm rostrally up to the lumbar enlargement. The catheter was then fixed to the fascia, tunnelled subcutaneously and fixed to the neck skin.

The position of the catheter was confirmed physiologically, by injecting 300 μg lidocaine (Xylocain®, AstraZeneca, Sweden); a correct location corresponded to a transient flaccid paralysis of the hind limbs. Additionally, to confirm the site of the catheter tip in study I, 10 μl methylene blue dye solution was i.t. injected via the catheter to trace the
spinal cord at the L4-L6 levels immediately before the animals were euthanized.

**Drugs**

All drugs used for spinal and intravenous administration and their mechanisms of action are listed in Table 1. The doses of the drugs were chosen on the basis of previous studies (cf. Chen and Pan, 2001; Duflo et al., 2005; Filos et al., 1994; Grace et al., 1995; Honda et al., 2002; Hwang et al., 1999; Lee et al., 2002; Lee YW, 1995; Lind et al., 2004; Obata et al., 2002; Siddall et al., 2000) or adjusted according to our pilot experiments.

<table>
<thead>
<tr>
<th>Drugs and doses/concentrations</th>
<th>Mechanisms of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine (30 μg, i.t.) in rat</td>
<td>Non-selective muscarinic receptor antagonist</td>
</tr>
<tr>
<td>Pirenzepine hydrochloride (10 μg, i.t.) in rat</td>
<td>M1 muscarinic receptor antagonist</td>
</tr>
<tr>
<td>Methoctramine tetrahydrochloride (10 μg, i.t.) in rat</td>
<td>M2 muscarinic receptor antagonist</td>
</tr>
<tr>
<td>4-Diphenilacetoxy-N-methylpiperidine methiodide (4-DAMP) (10 μg, i.t.) in rat</td>
<td>M3 muscarinic receptor antagonist</td>
</tr>
<tr>
<td>Muscarinic Toxin – 3 (MT-3) (5 μg, i.t.) in rat</td>
<td>M4 muscarinic receptor antagonist</td>
</tr>
<tr>
<td>Mecamylamine (MCM) (50 μg, i.t.) in rat</td>
<td>Non-selective nicotinic receptor antagonist</td>
</tr>
<tr>
<td>Neostigmine bromide [10μM] in dialysate in rat</td>
<td>Acetylcholine esterase inhibitor</td>
</tr>
<tr>
<td>Lidocaine hydrochloride (300 μg, i.t.) in rat</td>
<td>Voltage-gated Na⁺ channels (target of binding)</td>
</tr>
<tr>
<td>Gabapentin (10 - 100 mg/kg, i.v.); (12.5 - 200 μg, i.t.) in rat</td>
<td>α₂δ subunit of voltage dependant Ca⁺⁺ channels (target of binding)</td>
</tr>
<tr>
<td>Pregabalin (5 - 50 mg/kg, i.v.); (3.5 - 60 μg, i.t.) in rat</td>
<td>α₂δ subunit of voltage dependant Ca⁺⁺ channels (target of binding)</td>
</tr>
<tr>
<td>Baclofen (25 - 75 μg, i.t.) in human</td>
<td>GABA₉ receptor agonist</td>
</tr>
<tr>
<td>Clonidine hydrochloride (1 - 20 μg, i.t.) in rat; (25 - 100 μg, i.t.) in human</td>
<td>α₂-adrenoceptor agonist</td>
</tr>
</tbody>
</table>

**Treatment procedures**

**Spinal cord stimulation (I-III)**

Prior to the SCS experiments, the rat was placed in the circular observation cage and allowed to move freely during the tests. SCS was applied for 30 min with a frequency of 50 Hz, a pulse width of 0.2 ms and a stimulation
intensity, individually set to two thirds of the intensity producing a motor response (motor threshold: MT), i.e. slight twitching of the lower trunk muscles. These parameters were chosen to mimic those used in patients and were the same as those used in earlier studies (Cui et al., 1996b; Cui et al., 1998; Linderoth et al., 1993; Meyerson et al., 1995). The resistance between the poles of the electrical circuit differed somewhat in the implanted rats and the current needed to induce the MT thus varied. Therefore, to be able to adjust the stimulation intensity, it was necessary always to determine the MT in each individual rat for each experimental session.

Intrathecal and intravenous drug administration (I-III)

In studies I, II and III, drugs for intrathecal administration were dissolved in volumes of 10 \( \mu \)l saline and pre-warmed to approx 38°C, followed by 10 \( \mu \)l of prewarmed saline to rinse the catheter. Only one drug was tested on the same day in each animal.

In study III, drugs for intravenous administration were injected in a tail vein (mean volume of 2 ml kg\(^{-1}\)) using a winged infusion set with a 27G needle (Becton Dickinson, USA).

Human studies (IV-V)

Ethical aspects

The Ethical Committee of the Karolinska University Hospital, Stockholm evaluated and approved studies IV and V. The protocol of study V was further examined and approved by the Swedish Medical Products Agency (Läkemedelsverket). A written informed consent to participate, or to abandon whenever they wished, was obtained from all included patients in these trials.

Treatment procedures

SCS and electrode implantation in patients

SCS was applied by an epidural quadripolar or octapolar electrode, most frequently implanted percutaneously, and in a few cases via a small laminectomy. In brief, basic technical aspects of the SCS implantation will be presented below (for review see Barolat, 2002; Linderoth and Meyerson, 2009; Linderoth, 2006; North and Linderoth, 2000). The percutaneous electrodes were implanted under local anaesthesia without
the need for any sedation that might affect patient participation during the
intraoperative stimulation evaluation. With the patients in the prone
position, the epidural space was approached percutaneously using loss-of-
resistance technique. A paramedian oblique approach when inserting the
touhy epidural needle was preferred.

Assisted by fluoroscopy, the electrode was guided to the intended
position until intraoperative stimulation could induce paresthesiae covering
the entire painful region (or at least 80%). This topographic overlapping is
a prerequisite to obtain pain relief. When the test stimulation reveals that
the electrode is rightly positioned, a temporary externalization of the
electrode is performed by tunnelling it about 10 – 15 cm laterally through
the skin.

It is generally accepted that patients selected for SCS treatment
undergo a stimulation trial during a period of about two weeks, but should
not be longer than four weeks due to the increased risk of infection
(Linderoth and Meyerson, 2009). During this period the electrode is
connected to an external device, which mimics the effects of an
implantable pulse generator (IPG). This stimulation trial may assess the
degree of pain relief to be obtained after a full SCS system implantation as
well as tolerability.

The evaluation of the SCS effect during the test trial is based on
records of pain intensity on a Visual Analogue Scale (VAS). The
advantage of testing SCS for 30 min periods and of avoiding continuous
stimulation is to identify the real pain suppression, with assessment also of
the poststimulation effect in order to minimize the influence of a masking
effect of the paresthesiae. Special attention was directed onto the duration
of pain suppression after termination of SCS.

In case of successful stimulation trial, resulting in at least 50% pain
reduction with an adequate duration (more than 1 – 2h) the implantation of
the permanent electrode system was recommended. This procedure was
performed under local anaesthesia and sedation in which the electrode was
connected via an extension wire to an impulse generator that can be
programmed with different stimulation programs by telemetry.

The stimulation regime varies among different centers but at the
Department of Neurosurgery at the Karolinska University Hospital SCS is
usually applied for 30 min. The stimulation frequency is 50 - 60 Hz, the
pulse width around 300 ms (range 100 - 450 ms), and the amplitude set
individually in order to produce comfortable paresthesiae.
Fig 7 Radiograph showing an octapolar spinal cord electrode implanted at the upper thoracic level in a patient (A). Monopolar electrode implanted epidurally at the level T12 in a rat. The anode is located in the chest wall (B). The monopolar electrode for SCS in rats (C).

The implantation of a plate electrode (commonly referred to as laminotomy or surgical electrode) through a small laminectomy was needed in selected cases, when frequent dislocations of a percutaneous electrode had occurred. Plate electrodes have the advantage of a more stable position, minimizing the risk of dislocations and are the only option in case previous spinal surgery has made the percutaneous approach impossible. In most of the pain centers, these electrodes are usually implanted under general anaesthesia, but this precludes intraoperative stimulation. However, the implantation can also be performed in spinal anaesthesia, which permits stimulation during surgery with induction of paresthesiae (Kumar et al., 2009; Lind et al., 2003). This latter technique was utilized for the implantation of plate electrodes in the studies IV and V.
**Lumbar punction - bolus administration of drugs**

In study V, intrathecal clonidine hydrochloride (Catapresan® 150µg/ml, Boehringer Ingelheim), baclofen (Lioresal® 0.5g/ml, Novartis Sweden) and placebo (saline) were administered in a total volume of 2 ml. Clonidine was administered in bolus doses of 25, 50 and 75µg (and in some cases 100µg) and baclofen in doses of 25, 50 and 75µg. The injections were given in a double-blind, randomized fashion.

An extra fine lumbar puncture needle (27G) was used to minimize the risk of CSF-leakage. All i.t. injections were performed in the procedure room and blood pressure, heart rate, respiratory rate and pulse oximetry were continuously monitored. During at least the first 4 hours after the injection, patients were resting in a semi-sitting position.

**Pump implantation – Surgical aspects**

The programmable pump system such as SynchroMed II (Medtronic) is lithium battery-powered and includes in general the following components: a 20 (or 40) ml pump with a programmable dispense rate; catheter(s) for intrathecal use and a programmer (for external use) designed for the clinician to interrogate and program the pump, including several infusion modes. The parameters can be easily changed by the clinician with the external programmer by means of telemetry.

The pump implantation is performed under general anaesthesia with the patient positioned in a lateral fetal position and under fluoroscopic guidance. The dura is punctured at the L2-3 or L3-4 levels with a Touhy needle. An intrathecal catheter, inserted through the needle into the subarachnoid space, is advanced rostrally to the final intended position. The catheter is then tunnelled through the subcutaneous tissue and connected to the pump subcutaneously implanted in the lower abdomen.

**Assessment of on-going pain and changes in quality of life (IV-V)**

The evaluation of the effect of SCS was assessed utilizing the VAS in which the patients rate the intensity of pain in a 10 cm long scale, using end points from no pain to the left (0) to worst imaginable pain to the right (100). Pain was assessed every 30 min during the trials with SCS and i.t. drugs.

In studies IV and V the continuing long-term effect of the combined therapy of SCS and i.t. drug administration was evaluated using a structured interview by a “disinterested third part” pain specialist nurse. The patients were interrogated on the usefulness of “drug-enhanced SCS”,...
side effects and changes in pain medication. The questionnaire also comprised global satisfaction with this therapy (study IV and V, Table 2).

Fig 8 Radiograph from a patient treated with spinal cord stimulation combined with i.t. clonidine administered via an implanted pump. The quadripolar spinal electrode is seen in the uppermost part of the vertebral column and the intrathecal catheter ends at about the same level. The pump is seen in the lower, right part and the pulse generator in the upper left corner.
RESULTS

Incidence of mechanical hypersensitivity in rats with mononeuropathy (I-III)

In studies I and II, mononeuropathy was induced by a partial sciatic nerve injury (Seltzer et al., 1990). The incidence of mechanical hypersensitivity, assessed by von Frey filaments, was approximately 55 - 60% four to ten days after nerve lesion.

In study III, two different animal models of mononeuropathy were employed. Rats subjected to the photochemically induced ischemic nerve lesion in the sciatic nerve (Gazelius et al., 1996) presented the highest incidence of mechanical hypersensitivity of the three models included in this thesis, about 80%. The few rats subjected to the CCI model (Bennett and Xie, 1988), exhibited the lowest incidence of about 50%.

In the three models there were no significant differences in threshold magnitudes in rats classified as hypersensitive and the hypersensitivity persisted for about 4 – 8 weeks in all.

Release of ACh in an animal model of mononeuropathy (I)

The in vitro recovery rate for ACh presented low variability for the five different concentrations of ACh tested and averaged 21.6%. The in vivo microdialysis in the dorsal horn showed a significantly increased release of ACh induced by SCS in rats responding to SCS, while no significant changes were observed in the ACh release in the group of non-responding animals (study I, Fig. 2).

Somewhat unexpectedly, the spontaneous basal ACh release in animals displaying signs of neuropathy after nerve lesion was markedly lower than that in normal animals (study I, Fig. 1a). No evident differences in the basal ACh levels were observed between SCS responders and SCS non-responders. The K+ induced ACh release, illustrating the total releasable amount of ACh in the region around the probe tip, was also significantly lower in rats presenting with hypersensitivity than that in the group of control rats (study I, Fig. 1b).
The role of the different subtypes of ACh receptors in the SCS effect

The role of the cholinergic system in the pain relieving effect of SCS was further investigated in behavioural studies aiming at identification of the cholinergic receptors that may be involved in the SCS effects. In order to identify the specific nicotinic and/or muscarinic receptor subtypes, selective and non-selective ACh receptor antagonists were i.t. administered in SCS responding rats before applying SCS. The effect of SCS in combination with different antagonists on mechanical hypersensitivity is illustrated in (study I, Fig 3). The effect of SCS was completely abolished by i.t. administration of atropine. On the contrary, the non-selective nicotinic receptor antagonist, MCM, did not affect the response to SCS. The selective muscarinic M₄ receptor antagonist, MT-3 produced a complete suppression of the SCS effect while the M₁ receptor antagonist pirenzepine as well as the M₂ receptor antagonist methoctamine produced only a partial attenuation. Finally, the M₁ receptor antagonist 4-DAMP as well as saline (control) failed to influence the SCS effect. The present data suggest that the pain relieving effect of SCS relies, at least partially, on the activation of the cholinergic system, in which the M₄ receptor appears to play a pivotal role.

Drug enhanced SCS in rats: clonidine, gabapentin and pregabalin

In the first part of the study II, dose-response characteristics regarding mechanical hypersensitivity and adverse effects of 1, 5, 10 and 20 μg i.t. clonidine were investigated (study II, Fig 1). Clonidine produced a dose-dependant and transient increase of the withdrawal thresholds in the nerve-injured hind paw. However, the high doses required for maximum efficacy produced adverse effects such as sedation, motor weakness and excessive urination.

In the second part of this study, SCS was applied for 30 min together with i.t. clonidine in individually determined subeffective doses (2 - 7.5 μg) in SCS non-responding rats. The results of this study demonstrated that such a low dose of clonidine can potentiate the effect of SCS with a significant and prolonged suppression of hypersensitivity in rats that previously had not responded to SCS alone (study II, Fig 2). The individually determined subeffective doses of clonidine required to potentiate SCS produced no adverse effects by themselves.
In study III, the effects of SCS and gabapentin/pregabalin were investigated in ischemically nerve lesioned rats (Gazelius model) presenting with mechanical hypersensitivity. In the first part of this study, dose-response and time-response characteristics regarding mechanical hypersensitivity of intrathecally and intravenously administered gabapentin and pregabalin were studied (study III, Fig 1). Both intravenous (i.v.) and i.t. administration of gabapentin and pregabalin produced a significant attenuation of hypersensitivity in a dose-dependent manner, but the i.t. route was more effective for both drugs. Systemic administration of high doses of gabapentin and pregabalin produced not only attenuation of mechanical hypersensitivity but also side effects, in particular sedation.

The effect of i.v. gabapentin on the CCI rats was similar to that observed in the ischemically nerve lesioned rats (cf. Kayser and Christensen, 2000).

In the second part of study III, subeffective doses of these anticonvulsant drugs were administered together with SCS, according to the same protocol as used in study II. Less than 10% of the ischemic nerve lesioned rats responded to SCS. However, the combination of SCS with a subeffective dose of either gabapentin or pregabalin (both i.v. and i.t.) markedly potentiated suppression of mechanical hypersensitivity in rats that did not respond to SCS per se (study III, Fig. 2). With the doses used to enhance the SCS no side effects were observed.

In the third part of study III, the activity of WDR neurons in the dorsal horn was recorded before and after administration of the subeffective doses of intrathecal gabapentin selected in the preceding experiments. In hypersensitive rats, a marked hyperexcitability of WDR neurons in terms of spontaneous and press-evoked discharge was observed, which was suppressed by the combination of SCS and the subeffective i.t. gabapentin dose. Neither SCS nor gabapentin alone attenuated this pathological discharge in the SCS non-responding rats (study III, Fig. 3).

These results provide evidence that SCS in combination with a low dose of clonidine, gabapentin or pregabalin, might be useful in treating nerve injury-induced pain, when SCS or pharmacotherapy have proven to be ineffective as a single mode therapy.

Baclofen and SCS in patients (IV-V)

Ten patients were included in the randomized, double-blind, placebo-controlled trial (study V), in whom the analgesic effect of baclofen alone and combined with SCS were assessed. A dose-dependant response for 25,
50 and 75 μg of this drug was observed (study V, Fig. 1). The administration of the lowest dose of baclofen alone produced no changes in pain intensity.

SCS combined with all doses of baclofen (25, 50 and 75 μg) produced a marked enhancement of the pain relief as compared to baseline and to the effect obtained by baclofen alone. In some patients, SCS together with baclofen induced a long-lasting pain relieving effect of up to 12 hours.

In the two patients implanted with both an SCS systems and a baclofen pump, a pain reduction of 32% and 82%, respectively, was reported at seven months follow-up (study V).

In study IV, the pain relieving effect of both SCS together with baclofen or baclofen alone remained unchanged in nine patients with a mean follow-up of 67 months. However, the doses were about 30% higher than those required at an earlier follow-up (average 32 months) in order to retain the same pain relieving effect. For the group of seven patients treated with SCS in combination with a baclofen pump included in studies IV (n=5) and V (n=2), the mean dose was 175 μg/24 h (range 85 – 270 μg) and for the patients treated only with baclofen in study IV (n=4) the mean dose was 212 μg/24 h (range 95 – 300 μg).

All the patients treated with SCS and i.t. baclofen (in both series) rated the ADL as improved, and six of these seven patients reported a “clear” or “substantial” improvement in ADL. Furthermore, the global satisfaction score for this treatment revealed that these patients were very satisfied.

Side effects of baclofen

The side effects of baclofen observed in the acute bolus trials were dose-related (study V, Table 3). The higher doses (50 and 75 μg) caused heaviness of the legs in almost all patients. The temporary immobilization related to the weakness of the legs gave rise to a deep venous thrombosis in one patient. This patient required anticoagulation therapy and thereby the protocol was temporarily interrupted for eight months.

Dizziness, heaviness of the feet and sedation were reported by one patient chronically treated with baclofen (study V). However, these adverse effects were dose dependant and finally after a dose reduction the undesired effects were markedly reduced in spite of somewhat less pain relief. In study IV, one female patient reported sexual dysfunction when the baclofen dose was augmented.
Clonidine and SCS in patients (V)

Administration of i.t. clonidine alone was significantly effective in pain reduction. However, this drug produced more severe side effects, such as hypotension and dyspnoea, than baclofen during the bolus trials causing two patients to abandon the study.

The effect of SCS was significantly enhanced by clonidine and some patients experienced a long-lasting effect up to 12 hours until the VAS values returned to baseline.

Even though the enhancement of the SCS effect was greater for higher doses of clonidine, a statistically significant difference was only obtained for the lowest dose (25 μg). This was due to the fact that the higher doses (50, 75 or 100 μg clonidine) were used less often because of side effects (study V, Fig. 1).

At the follow-up, the two patients with chronic clonidine and SCS have experienced some improvements in ADL and they were satisfied with the combined therapy. In these patients there was a further pain reduction of 35% and 38%, respectively, of that obtained with SCS alone before the trials.

Side effects of clonidine

Hypotension was by far the most common side effect during the bolus injections (study V, Table 3), although this was not recorded at the follow-up of these patients.

During the chronic administration of this drug, the most frequent side effects were sedation, headache and xerostomia. However, these side effects were attenuated or disappeared with a dose reduction of clonidine.

Placebo effect (V)

A clear placebo effect by i.t. saline was rarely observed in the trials. Conversely, the intrathecal injection of saline elicited even more pain in two patients, possibly the result of a frustrated expectation of obtaining pain relief.

Two patients reported a remarkably good pain reliving effect after the first injection of the trial with the lowest doses of clonidine and baclofen respectively, but this effect could not be reproduced by higher doses of the same drug. This phenomenon could be explained, at least partially, as a placebo effect probably due to the high expectations that patients may have had in the beginning of the trial.
DISCUSSION

In this chapter, the aim is to discuss the mechanisms underlying the pain relieving effect of SCS in animal models of neuropathy, the possibilities to enhance its efficacy by pharmacological adjuvants and finally the clinical application of this knowledge to explore the novel approach of drug-enhanced SCS in neuropathic pain patients. There is also a closing discussion on the clinical relevance of findings in animal studies and controversies in translational pain research.

ACh release in a nerve injury animal model

The principal aim of study I was to elucidate whether the cholinergic system is involved in the pain relieving effect of SCS. However, the finding that the basal release of ACh in the dorsal horn of hypersensitive rats after nerve lesion was significantly lower than in normal rats was somewhat unexpected, but is conceivably of relevance for the pathophysiology of neuropathic pain. In fact, a decreased ACh release after nerve injury has previously been reported in one study only (Dussor et al., 2005). Interestingly, this decrease parallels that of GABA in the dorsal horn of nerve injured rats described in an earlier study on SCS mechanisms (Stiller et al., 1996a).

The sources of ACh in the dorsal horn remain unclear. Some studies have provided evidence that ACh is released predominantly from cholinergic interneurons located in laminae III-V and to a lesser extent in laminae I and II (Barber et al., 1984; Höglund et al., 2000; Klimscha et al., 1997; Ribeiro-da-Silva and Cuello, 1990). There is some data suggesting that ACh may be released also by descending cholinergic pathways from supraspinal structures such as the medullary reticular formation (Bouaziz et al., 1996; Jones et al., 1986), and a minor portion may be derived from terminals of primary afferents (Dussor et al., 2005).

The pathophysiological changes underlying the reduced ACh release after nerve injury are not known, but degeneration of cholinergic afferents or changes in the anatomical synaptic architecture of interneurons have been hypothesized (Dussor et al., 2005). It is reasonable to assume that the decreased extracellular concentrations of ACh in the dorsal horn, reflecting a dysfunction of the cholinergic system, play a role in the development of “allodynia”. However, in order to establish the functional significance of the low basal levels of ACh in the genesis or maintenance of pain-related behaviour signs it would be necessary to correlate the present data with
basal levels of ACh in a group of nerve injured rats, but without signs of neuropathy. Such a protocol has in fact been used in earlier studies (Cui et al., 1997a; Stiller et al., 1996a).

We have demonstrated that SCS induces ACh release, which is associated to the analgesic effect of SCS. The mode of action of clonidine, alone and in combination with SCS, with regard to possible cholinergic mechanisms will be discussed later.

Methodological considerations of microdialysis

Certain methodological aspects of microdialysis should be considered. First, the diffusion of substances through the membrane of the probe is influenced by the size of the molecules. Also, the concentration of, for example, a neurotransmitter in a microdialysis sample is influenced by the perfusion rate of the probe, which is inversely related to the flow rate (Kehr, 1993). Consequently, low molecular weights and low flow-rates generate higher concentrations in the dialysates. Second, the concentration of a neurotransmitter in the ECF is not only dependant on its neuronal release, but also on its enzymatic degradation and cellular uptake (Stiller et al., 2003). In order to minimize the degradation of a neurotransmitter, enzyme inhibitors may be added to the perfusate, which augments the amount of the substances available for analysis. In study I, neostigmine was used in the buffer solution to counteract the enzymatic degradation of ACh to ensure maximal recovery rate at the subsequent HPLC analysis. Previous microdialysis studies have shown that the use of this drug does not otherwise interfere with the results (Billard et al., 1995; Höglund et al., 2000; Roth et al., 1996).

Neurotransmitter release, including ACh, may also be diminished as a result of general anaesthesia (Jansson et al., 2004), leading to a low relative recovery rate. This term is defined as the ratio between the concentration obtained for a specific substance in the collected samples and the actual concentration in the ECF. Relative recovery rates are particularly low for high-weight molecules, such as peptides, because of their difficulties to diffuse through the membrane. In order to ensure a constant relative recovery rate, an in vitro recovery assay should be performed before the actual experiments using different known concentrations of a particular substance. A linear correlation between the obtained dialysates and the outer medium is a prerequisite to enable a proper analysis of the microdialysis data.

To validate a correct position of the probe and viability of the neural tissue at the end of an in vivo experiment, a massive release of
neurotransmitters can be induced by potassium stimulation, which causes depolarization of all terminals and cells near the probe. The potassium response reflects the releasable pool of a certain neurotransmitter and does not represent a physiological synaptic release.

Microdialysis may be compared with other in vivo methods as e.g. the push-pull perfusion technique, which also allows ongoing analysis of the CNS neurochemical activity but causes more extensive tissue damage due to the higher flow rates used (Lisi et al., 2003).

For review of the microdialysis technique, see e.g. (Gustafsson et al., 1999; Lee et al., 2008; Stiller et al., 2003; Ungerstedt, 1991).

Cholinergic and GABAergic mechanisms in the effect of SCS

In spite of the extensive clinical usage of SCS, the mode of action underlying its therapeutic efficacy is only partially understood. It is conceivable that the application of electrical stimulation onto the dorsal aspect of the spinal cord induces the activation of numerous neuronal systems, however, the release of a certain neurotransmitter may not necessarily be involved in the effect produced by SCS. That was the main reason why the microdialysis experiments (study I) had to be supplemented by behavioural studies in order to confirm that the SCS effect on pain-related signs is dependent also on cholinergic mechanisms.

The dysfunction of the spinal GABAergic system has been well established in neuropathic pain (e.g. Castro-Lopes et al., 1995; Castro-Lopes et al., 1993; Dickenson et al., 1997)) and has important clinical implications. In order to determine the role of the GABAergic system in the SCS effect in animal models of neuropathy, a number of studies have been performed by our research group as described in the introduction. For instance, using microdialysis technique it was demonstrated that rats displaying mechanical hypersensitivity had significantly lower basal GABA levels in the dorsal horn than normal rats (Stiller et al., 1996a). Furthermore, it was shown that SCS produced an increased release of GABA in rats responding to SCS, whereas the release of this neurotransmitter was unaffected in the non-responding animals. The release of excitatory amino acids (i.e. glutamate and aspartate) was at the same time attenuated by SCS in the group of responders to SCS (Cui et al., 1997b). The “normalization” of the GABA levels by SCS has been associated to the attenuation of signs of neuropathy in rats displaying tactile hypersensitivity. Further studies confirmed the involvement of the GABA system when the SCS effect on “alldynic” rats was counteracted by the administration of a GABAB receptor antagonist (Cui et al., 1996a).
Moreover, the administration of a GABA$_B$ receptor agonist, baclofen, produced a clear enhancing of the SCS effect. The addition of low, per se ineffective, doses of baclofen to SCS could transform non-responding animals into “responders”. This beneficial effect of the SCS-baclofen combined therapy has been confirmed in neuropathic pain patients, although the marked potentiating effect of baclofen to SCS observed in rats could not later be confirmed in patients (Lind et al., 2004). In conclusion, all this data provides further evidence that the abnormal functionality of the GABAergic system in the dorsal horn, contributes to a state of neuronal hyperexcitability following peripheral nerve injury and also demonstrates the importance of GABAergic mechanisms behind the effect of SCS. Similarly, our results demonstrated that subeffective doses of the anticonvulsants gabapentin and pregabalin may also potentiate the effect of SCS (study III), although it is by now well established that despite these drugs being structurally related to GABA, they do not act as GABA-mimetics (Maneuf et al., 2003).

In study I, the role of the cholinergic system in the mode of action of SCS was explored. It was demonstrated that SCS induces release of ACh in the dorsal horn ipsilateral to the nerve injury side in responders to SCS, while no changes were detected in the group of non responders. The importance of the cholinergic system in the effect of SCS was substantiated in subsequent behavioural experiments by the fact that the SCS effect could be completely eliminated by i.t. atropine and a selective muscarinic M$_4$ receptor antagonist. Since only a partial attenuation of the SCS effect was produced by M$_1$ and M$_2$ receptor antagonists and no changes were observed with the administration of M$_3$ or nicotinic antagonists, it is likely that M$_4$ receptor plays a crucial role in the pain relieving effect of SCS. This conclusion has been further supported by a recent observation that a muscarinic receptor agonist, oxotremorine, may potentiate the SCS effect in SCS non-responding rats (Song et al., 2008). Interestingly, it has been reported that the effect of both low and high frequency TENS on hyperalgesia in rats is also related to the activation of spinal muscarinic receptors, but in that particular study the M$_1$ and M$_3$ receptor subtypes appeared to be more important (Radhakrishnan and Sluka, 2003).

The state of hyperexcitability in the dorsal horn, secondary to peripheral nerve injury, may be attenuated by SCS as a consequence of the modulation of several systems, such as the GABAergic and the cholinergic. These systems presumably act in concert, inducing multiple up- and downregulations and triggering a cascade, in which the release of ACh and GABA is only a part of a larger sequence of events (cf. Song et al., unpublished). Even though the segmental mechanisms in the spinal cord
seem to be pivotal for the effect of SCS, there are many indices that there may be an important involvement of supraspinal regions in the effect of SCS as well (El-Khoury et al., 2002; Saade et al., 2006).

"Drug-enhanced SCS": additive, synergistic or potentiating effects

When two treatments are administered in combination a natural question is whether the outcome is due to an additive, synergistic or potentiating effect. These terms describe the mode of interaction between drugs, or in a wider meaning of the word, treatments. An additive effect is by far the most common interaction in which the combination of two substances results in the sum of the effect of each one. In such a case, it is easy to predict the effect of the combined treatment. When two treatments are combined and the final effect is greater than the sum of each treatment given alone, the term synergism can be used instead. Finally, a potentiating effect is obtained in the case that the efficacy of one treatment can be enhanced by an ineffective one.

To determine the kind of interaction when two treatments are combined the so called isobolographic analysis is a well established and validated tool (for review on isobolographic method see e.g. Loewe, 1953; Tallarida, 2007). This method requires that a particular effect is chosen, such as 50% of the maximum effect - called ED 50 (effective dose 50%) - and a graph is constructed with the doses that produce the selected effect. This means that the isobologram is limited to a single effect level. However, the isobolographic method is difficult to apply for the analysis of a combination of SCS with drugs because no dose-response data can easily be obtained for SCS, either in rats or in patients. The hypothesis of combining SCS with intrathecal drugs to enhance or potentiate its effect in neuropathic pain patients emerged from animal experiments with the hope to transfer laboratory data to the clinical application. There are two important aspects that have to be taken into account. First, in most animal models the majority of animals subjected to nerve lesion develop signs of neuropathy, such as tactile hypersensitivity (cf. Decosterd and Woolf, 2000; Gazelius et al., 1996), while, in humans, that phenomenon is observed only in a minority of the cases after nerve injury or amputation (cf. Hansson, 2003). Second, only a portion of rats can be classified as “responders” to SCS, similar to the fact that about 60% of neuropathic pain patients obtain satisfactory analgesia (for review see Linderoth and Meyerson, 2009).

Based on these similarities, animal experiments were performed in order to enhance - or potentiate - the effect of SCS in rats not responding to
SCS. The dysfunction of the GABAergic system in the genesis and development of pain in animal models of neuropathy was the background to initiate experiments with SCS and i.t. baclofen in rats (Cui et al., 1996b). The results obtained in these experiments showed that SCS together with subeffective doses of baclofen could suppress pain-related behaviour even when these treatments by themselves individually were ineffective. That interaction could be defined as potentiation, but there were cases where the interaction was additive or synergistic. However, the pronounced suppressive effect of SCS in animals could not be reproduced in patients (Lind et al., 2004; Lind et al., 2008) and, as mentioned above, completely non-responding patients never benefited from the combined drug-SCS therapy. Moreover, only a relatively small group of patients (about 20%) benefited from the SCS-baclofen combination to motivate a permanent therapy with SCS and pump implantation. In the animal studies (studies II and III), i.t. clonidine, gabapentin or pregabalin, in doses ineffective per se, in SCS non-responders exerted a more clear potentiating effect when used concurrently.

The initiation of a trial to enhance the efficacy of SCS in patients with neuropathic pain by i.t. clonidine was again based on our animal experiments (Schechtmann et al., 2004). Interestingly, in a previous in vivo microdialysis study in the sheep it has been demonstrated that clonidine, activating α2 receptors located on interneurons, may induce ACh release (Klimscha et al., 1997). Similarly, in humans it was observed that clonidine may increase ACh concentrations in the CSF at the peak of analgesia (Eisenach et al., 1993). The analgesic effect of this drug may be enhanced by i.t. neostigmine showing an additive effect in humans (Hood et al., 1996) and a synergistic effect in animals (Detweiler et al., 1993; Naguib and Yaksh, 1994). Thus, the analgesic effect of both SCS and clonidine is related to an increased release of ACh in the spinal dorsal horn, preferentially acting on the muscarinic receptor M4. A further reason to consider clonidine as an adjuvant to SCS was that this drug, in contrast to baclofen, may be effective also for nociceptive pain (Eisenach et al., 1996b), which often coexist with neuropathic forms of pain (Baker et al., 2004; Eisenach et al., 1996a; Eisenach et al., 1995; Hassenbusch et al., 2002).

In study V, in most cases bolus injections of clonidine or baclofen produced, by themselves, a moderate reduction of the pain intensity and this effect appeared to be additive to the effect of SCS. However, in a few patients a substantial pain relief was observed only with the SCS-drug combination. In those patients the effect may be more adequately defined as synergism. It is worthy of note that, in patients, at least a partial effect of
SCS is mandatory to obtain benefits from the combined therapy. Thus, a true potentiation of SCS by i.t. drugs has not been observed in patients as it was in the case with rats.

**Intrathecal drugs: bolus vs. chronic administration**

Drugs delivered into the intrathecal space seek to target the CNS and aim to minimize high blood concentrations in the systemic circulation. Hence, in some clinical conditions i.t. administered drugs may achieve higher efficacy and lesser toxicity. However, the i.t. bolus injection of a drug leads to higher transient concentrations in the CSF than the i.t. continuous administration mode. Therefore, the mode of administration determines the pharmacodynamic profile with regard to clinical efficacy and adverse effects.

Prior to a baclofen pump implantation for spasticity, bolus tests are usually performed in order to determine the effectiveness of the drug and possible related side effects (Ochs et al., 1989; Pohl et al., 2002; Rushton, 2001). In study V, the same approach was used with tests of i.t. bolus injections of clonidine and baclofen via an extra fine lumbar puncture needle. The advantage of this technique was to avoid the implantation of an i.t. catheter connected to an external pump and, thereby reduce the risks of infections and CSF-leakage. On the other hand, the continuous administration via a catheter connected to an external pump avoids high concentrations of the drug in the CSF and mimics more closely the intended chronic SCS-drug therapy (cf. Krames, 1996). In addition, the administration of bolus injections of clonidine produced a marked hypotension in several patients, which limited the possibility to explore a hypothetical benefit of this treatment.

In study V, a severe complication was observed during the trials, i.e. deep vein thrombosis after bolus injection of baclofen in an obese patient. This complication with i.t. baclofen has been previously documented in only a few case reports for both bolus injection (Carda et al., 2008) and continuous administration via pump (Murphy, 2002). This complication observed in our study is presumably related to the temporary immobilization due to weakness of legs induced by baclofen.

In conclusion, most side effects observed during the clinical trial (study V) were attributed to the bolus administration and related to a transitory high concentration of the drug in the CSF. In contrast, continuous drug infusion can be administered in higher doses without side effects.
Pain assessment and changes in quality of life

The VAS to assess pain intensity has been extensively used (Maxwell, 1978) and is validated by The International Association of Pain (IASP) and by subcommittees at several international pain meetings (Carlsson, 1983; Dworkin et al., 2005; Jensen, 2003; Jensen and Karoly, 2001). There are other pain scales such as the verbal rating scale (VRS) and the numerical rating scale (NRS), but to date there are no guidelines for a rational choice between any of these scales (McQuay, 2005).

The use of VAS has the disadvantage that it may be interpreted in different ways by different subjects and several factors such as sex, cultural background, expectations, mood and variation in perceptions may influence the results (Lund et al., 2005).

In study V, the intrathecal injections during the trials were given in a double-blinded randomized control fashion. However, SCS could not be applied under blinded conditions since the presence of paresthesiae is always perceived by patients during stimulation. Hence, sham stimulation cannot be an alternative in this kind of trials (cf. Eddicks et al., 2007).

The effectiveness of pain treatments have classically been evaluated considering the reduction of pain intensity as the major goal (Von Korff et al., 2000). However, it is not until later years that the usefulness of a pain therapy has been assessed also by other measurements as global satisfaction score, changes of ADL and in pain medication. Such indices of health-related quality of life parameters are today mandatory in the evaluation of a pain treatment. Therefore, the pain assessment using VAS as the only measurement of the outcome of SCS treatment may be misleading (cf. Carlsson, 1983; Kemler et al., 2008).

Animal models of pain and clinical relevance

The assumption that a positive reflex withdrawal of the paw in a rat after the application of von Frey filaments is a sign of pain constitutes a paradigm in modern experimental pain research. For obvious reasons, the interpretation of pain-related behavioural signs presents several problems. For example, experimental studies in animals, such as rats or mice, can utilize a very limited spectrum of responses elicited in behavioural tests. This example illustrates that the complexity of pain experience is reduced to a sensorial experience. Other major components of pain in humans such as anxiety, anger, depression, fear characterizing the emotional distress cannot be assessed properly in these pain models.
The usefulness and the relevance of the animal models for chronic pain has been an issue of on-going debate among clinicians and basic researchers (Hansson, 2003; Mogil, 2009; Vierck et al., 2008). There is frustration over the limited success to translate decades of basic research on pain to new and more effective therapies (Hansson, 2003). Even though the relevance of these animal models may be disputed, the need for data provided by such experiments is less controversial. Hence, the experimental basic pain research is indispensable. For obvious reasons, the use of more highly evolved animals resembling humans more closely (e.g. primates) raises natural ethical concerns. However, some even argue that pain studies in animals should be abandoned and claim for more extensive testing in humans (Langley et al., 2008).

The premise that rats subjected to nerve injury suffer spontaneous chronic pain is contradicted by the fact that, in general, they display normal behaviour, gain weight, show normal sociability and grooming, etc. This means that, generally, they do not exhibit aversive behavioural signs that could indicate the presence of chronic pain. However, locomotion may be affected in some of these models of neuropathy, a consequence of motor deficit. Also, a change in the hind paw posture may also be observed (Meyerson et al., 1995; Seltzer et al., 1990). It is reasonable to assume that this abnormal posture could be more related to motor axon injury and not to the presence of chronic pain. Hence, the assumption that animals subjected to nerve injury suffer from spontaneous, ongoing chronic pain cannot be inferred from these observations. On the other hand, it is conceivable that their exaggerated responses to noxious, as well as innocuous, stimuli represent a pathological form of evoked pain. Evoked withdrawal responses to tactile stimuli are not strictly indicative of pain but, since it occurs in some neuropathic pain patients, this phenomenon could be considered a sign that is associated to the experience of pain. Experimental pain research is often based on measurements such as withdrawal responses representing only the sensory discriminative aspect of pain experience. Thus, the interpretation of certain abnormal responses such as exaggerated withdrawal reflexes, caused by non-noxious stimuli, should be called hypersensitivity (mechanical or thermal) and the term allodynia should be avoided.

In the present thesis, all the animal experiments have been performed in male Sprague-Dawley rats because they are less influenced by hormonal changes associated to the reproductive cycle in female rats. However, there is robust epidemiological evidence showing that chronic pain is more common in middle-aged and in female patients. Furthermore, it has been observed that the potency for analgesic of drugs
is sex-dependent, showing a higher potency in males (Berkley, 1997; Gagliese and Melzack, 1997).

The tactile hypersensitivity exhibited in these animal models has been interpreted as equivalent to allodynia observed in patients with neuropathic pain. However, it should be recalled that none of the models of neuropathy mimics the most frequent symptom in patients, in whom continuous pain represents the major complaint, while mechanical allodynia is, in such conditions, present in only 20 – 40 % of the neuropathic pain patients (cf. Hansson, 2003; Martin et al., 2003; Otto et al., 2003). Thus, any extrapolation of data from animal models to humans with neuropathic pain has to be performed with caution (e.g. Lind, 2007).

**Translational research: From bench to bedside - from bedside to bench**

The concept of “from bench to bedside” could be interpreted in a one-way direction. On the other hand, translational research should imply a two-way approach, going from “bedside to bench” in order to address relevant clinical problems in a laboratory setting and “from bench to bedside” to use data from reliable animal models and pain measurements transferred to a clinical application (Mao, 2002). There is a gap between basic and clinical pain research, the major problem being the clinical application of data from animal experiments. In spite of convincing evidence of efficacy a pharmacological, or even a non-pharmacological, treatment when tried in animal models of pain, the same mode of therapy often proves to be inefficacious, or useless because of severe side-effects, if applied in patients. It is of major importance that basic scientists and clinicians interact in such a way that “translational research” create a link between these two different environments in order to provide a better understanding of neuropathic pain and its treatment (Mao, 2002; Meyerson and Linderoth, 2006; Vierck et al., 2008). The key points to bridge the gap between experimental and clinical research have been under debate in most of the international pain meetings for many years, although no immediate solutions are available. It is evident that experimental research hypotheses, selection of animal models and assessment criteria have to be selected more from a clinical perspective (cf. Mogil and Crager, 2004).

This thesis should be considered as an attempt to translate knowledge from basic research to a concrete clinical situation: What can we do to help chronic pain patients, treated with SCS as the “last alternative”, in cases where the pain relieving effect has faded away? Which are the alternatives? The experimental model of SCS may be used to obtain additional
knowledge of the mechanisms behind its effects, which consequently would facilitate further refinement of SCS therapy and improve clinical results. Furthermore, results from studies of SCS mechanisms may contribute to the understanding of the physiological mechanisms underlying the generation and perpetuation of chronic pain after nerve injury.

The development of SCS, as a direct consequence of the gate-control theory, is in fact an excellent example of translational research. However, the inhibition of nociceptive signals by “the gate mechanism”, which was the fundamental concept of this theory, could not be reproduced in the clinical application of SCS. The expected consequence of this theory would imply that SCS could be an effective tool also for nociceptive pain. Therefore it may be considered as a paradox that SCS is mainly effective in neuropathic pain, with the exception of ischemic pain. Also, the studies presented in this thesis represent examples of transfer of data from bench to bedside. In fact, these animal experiments have enabled the introduction of a novel treatment that may benefit some patients with neuropathic pain.
CONCLUDING REMARKS

“Drug-enhanced spinal cord stimulation” addresses a relevant clinical problem for patients who have been suffering from incapacitating neuropathic pain for a long period, in some cases for many years. Since SCS for most patients constitutes the last therapeutic alternative, a fading analgesic effect of SCS is in fact an unresolved issue.

Today, the drug-enhanced SCS therapy appears to be useful only for a limited group of well selected patients. However, we believe that the further exploration of SCS physiology will identify new receptors to target. Novel and more selective drugs will be available to enhance the analgesic effect of SCS with better compliance and fewer side effects.

Although “pain is in the brain”, as stated by Allan Basbaum in his lecture at the IASP meeting in 2002, the present thesis has provided further evidence for the usefulness of targeting pain at a spinal level by SCS, or by drug-enhanced SCS, which has proven to be a valuable treatment approach.
ACKNOWLEDGEMENTS

This thesis bears the name of only a single author; however, it is the result of considerable intellectual teamwork. Therefore, I would like to express my sincere gratitude to all the people who have contributed to this thesis. In particular, I would like to thank:

Professor Bengt Linderoth, my main supervisor both in the lab and in the clinic for introducing me to the field of pain research and functional neurosurgery, for sharing with generosity your unique knowledge on spinal cord stimulation. For your kindness and consideration in always taking care of my family and, once again, Bengt- thank you for giving me a very nice piano!

Professor emeritus Björn A. Meyerson, my co-supervisor and mentor for inviting me to the Karolinska Institutet to work in the functional neurosurgery research group, for teaching me so much about pain research and for your extraordinary guidance in the art of writing. It has been a true honour to work together with you.

Johan Wallin, my true friend, for your scientific and non-scientific - but always generous support. Thank you for introducing me into the nature of laboratory work. Johan, I am simply grateful for everything. It has also been really fun to work with you!

Göran Lind, my colleague and friend, for sharing your vast knowledge in functional neurosurgery and for taking the time to listen and give me wise advice.

Jaleh Winter, for great friendship, for sharing your long experience in neuromodulation, for many interesting discussions and for your warm dedication to our patients.

My co-authors, Jian-Guo Cui, Vadim Yakhnitsa- до завтра!, Camilla Ultenius- for valuable input and discussions and Zhiyang “Tony” Song- for interesting discussions and for being cool.

Per Almqvist, for useful and critical feedback at the end of this thesis and for the nice trip to Toronto. I hope to continue our interesting research discussions and projects!

Professor Mikael A. Svensson- for your valuable scientific and moral support in both my research and clinical work and because you simply encourage science!
Present and former members of the neurosurgical research lab: Kanar Alkass, Lisa Arvidsson, Thomas Asklund, Olof Bendel, Tjerk Bueters, Gabriel von Euler, Michael Fagerlund- for friendship and for many fun lunch talks, Caroline Gahm, Christina von Gertten, Jonas Hydman, Diayou Li, Johan Lundberg, Per Mattsson, Xia Meijer, Morten Moe, Jonathan Nordblom- for being a great friend, Marcus Ohlsson, Giselle Prunell dos Santos, Ann-Christin Sandberg-Nordqvist, André Wennersten- for friendship and sharing my obsession with excellent dark chocolate, Ulf Westerlund and Håvard Ølstorn. You all contribute to a pleasant and stimulating working atmosphere at the lab. Special thanks to Britt Meijer- for always being warm and for your friendly guidance in lab matters.

Present and former colleagues at the Department of Neurosurgery not mentioned above: Bo-Michael Bellander, Ernest Dodoo, Göran Edner- for your sharp and critical input in our clinical studies, Adrian Elmi, Petter Förander, Bengt Gustavsson, Staffan Holmin, Professor Hans von Holst, Lars Kihlström, Christer Lindqvist, Professor Bodo Lippitz, Professor Tiit Mathiesen- for inviting me to play in DuranDuran, Ilias Nikolaidis, Ingrid Ohsllson-Lindblom, Martin Olsson, Kyrre Pedersen, Inti Peredo- for your valuable support in surgery and specially in particular difficult moments - ¡gracias!, Jenny Pettersson-Segerlind, Ingvar Olafsson- for being nice and for helping me on my trip to Iceland, Tiit Rähn, Halldór Skulason, Annika Suneson, Elfar Úlfarsson- for your always wise points of view, Lars Wallstedt and Maria Zetterling.

Professor Per Hansson for sharing your outstanding knowledge on neuropathic pain, Carl-Olav Stiller, Ann-Sofie Leffler, Gerd Engholm for your valuable help being the “third disinterested part” in our clinical study (V).

Colleagues and friends at the Department of Neurology: Professor Lou Brundin, Charlotta Lind for moral support, Sofia Hylin-for being a great friend, Giuseppe Stragliotto.

Maj-Britt Andersson and the whole staff at our ward R14: Your help has been essential to perform the clinical studies. Your dedication and professionalism in caring for our patients is fantastic.

Mainy Olofsson for your expertise in secretarial matters and Göte Hammarström for excellent help in engineering and technological design.

Keith Mullett and Ali Sarem-Aslani, Medtronic Europe for genuine interest in my research and valuable feed-back.
Linda Bell for friendship, moral support and your careful proofreading of my thesis.

David Baxter for friendship and excellent help with my English writing. Special thanks to Judith Socha for generous help with graphic design. Klas Abelson for valuable help regarding microdialysis and Daniel Olsson at LIME for precise statistical input.

Colleagues and friends at the Department of Neurosurgery in Buenos Aires: Raul Frugoni for sharing with me his unique knowledge in stereotaxy and for sparking the fire to come to the Karolinska Institutet the first time, Javier Gardella, Fabián Vitolo (¡mi maestro!) who introduced me into neurosurgery, Marcelo Pomsztein, Javier Goland, Mariano Socolovsky and Fabián Piedimonte.

Astor Piazzolla, my true Maestro: for being always there, following almost every single written line in this thesis.

My great friends who during these years have become my “Swedish family” Anders, Anna, Ariel, Camila, Cilla, Daniel, Débora, Hanna, Hernan, Johan, Jonas, Lasse, Lilian, Linda, Linnea, Mara, Marina, Mikaela, Paula, Tessy, Åke and Åsa.

Special thanks to Eva Linderoth for her kindness.

My mother Diana, my sister Florencia, Juan, Margarita, von Tía Beatríz y von Albert, Alejandro y Aileen and the Teper family for endless support and love. My friends in Buenos Aires: Bielo, Gaby, Jeremías, Julius, Laura, Martin, Mariano, Mariela, Rouch and Valeria for continuing friendship and sharing our thoughts and humour from far away.

My father José, who is somewhere here today, with his nice big white moustache, smiling and enjoying this moment more than anyone in the world.

Most of all, I want to thank you María, my beloved wife, for coming with me to Sweden to fulfil this dream to write a doctoral thesis at the Karolinska. With your profound love, everlasting patience and unconditional support this thesis was written. And finally, a mis adorados hijitos Björn y Leo por ser mis bombonazos, lo que más quiero en este mundo y por hacer evidente, cada día, que son el sentido de mi vida. A ustedes quiero dedicarles esta tesis.

The studies on which this thesis is based were supported by grants from Karolinska Institutet, The Magnus Bergwall Foundation and Medtronic Europe S.A.
REFERENCES


Jones, B.E., Pare, M., Beaudet, A., 1986. Retrograde labeling of neurons in the brain stem following injections of [3H]choline into the rat spinal cord. Neuroscience 18, 901-916.


Song, Z., Ultenius, C., Meyerson, B.A., Linderoth, B., Pain relief by spinal cord stimulation involves serotonergic mechanisms. An experimental study in a rat model of mononeuropathy. (submitted)

Stewart, W., Maxwell, D.J., 2003. Distribution of and organisation of dorsal horn neuronal cell bodies that possess the muscarinic m2 acetylcholine receptor. Neuroscience 119, 121-135.


