From the Department of Neuroscience
Karolinska Institutet, Stockholm, Sweden

K^+ CHANNELS AND THE REGULATION OF SYNAPTIC TRANSMISSION

Evanthia Nanou

Karolinska Institutet

Stockholm 2009

Published by Karolinska Institutet
Printed by Larserics Digital Print AB

ISBN 978-91-7409-486-2

In the memory of my father

ABSTRACT

Neuronal excitability is highly regulated by K^+ channels that are activated by a voltage change across the plasma membrane or by a rise in intracellular Na^+ or Ca^{2+} concentration. The main focus of this thesis has been to characterize the Na^+-activated K^+ (K_{Na}) and the Ca^{2+}-activated K^+ (K_{Ca}) channels and to determine their role in regulating synaptic transmission in the lamprey spinal cord. Lamprey spinal cord neurons express both K_{Na} and K_{Ca} channels with diverse functional roles and sources of Na^+ and Ca^{2+} necessary for their respective activation.

There are three routes for Na^+ influx to activate K_{Na} in the lamprey spinal cord. The first is through voltage-gated Na^+ channels that activate a transient K_{Na}, which is sensitive to TTX and contributes to the early repolarization phase of the action potential. The second route for Na^+ entry is through leak channels that activate a sustained K_{Na} current which is blocked by Na^+ substitution with Li^+, NMDG or choline, but is not affected by TTX. This current appears to underlie the Ca^{2+}-independent component of afterhyperpolarization induced by repetitive firing. The last route of Na^+ entry is through AMPA receptors that activate a K_{Na} current which is abolished by Li^+ and quinidine. This K_{Na} current has properties similar to the cloned Slack channels, in that it is not modulated by increased intracellular Cl^- concentration or ATP.

The role of the AMPA-induced K_{Na} was examined in excitatory synaptic transmission. K_{Na} channels interact with AMPA receptors in the soma-dendritic region and control the decay time constant of the AMPA-mediated current as well as the amplitude of the synaptic potential. Thus, the coupling between K_{Na} channels and AMPA acts as an inherent negative feedback mechanism that depresses the magnitude of excitatory synaptic responses.

These AMPA-mediated K_{Na} channels are modulated by activation of metabotropic glutamate receptor 1 (mGluR1). These channels are negatively regulated by activation of mGluR1 which involves PKC. However when intracellular Ca^{2+} is chelated, mGluR1 positively regulates AMPA-induced K_{Na} channels in a PKC-independent manner.

K_{Ca} channels are also present in the lamprey spinal cord and are activated by Ca^{2+} influx via synaptically activated NMDA receptors in the soma-dendritic region. K_{Ca} channels are located in close proximity to NMDA receptors and control the decay time constant of the EPSC and the amplitude of the corresponding EPSP. Additionally, K_{Ca} channels are also found presynaptically where they are activated by Ca^{2+} influx through voltage-gated channels. They shape the action potential waveform by determining the duration of the action potential. Thus, K_{Ca} channels act both pre- and post-synaptically to limit the extent of excitatory synaptic transmission.

In conclusion, different types of both K_{Na} and K_{Ca} channels are activated by distinct sources of Na^+ and Ca^{2+} in the lamprey spinal cord. Activation of these channels controls the magnitude of the excitatory response and may regulate the locomotor motor pattern.

Key words: K_{Na}, K_{Ca}, NMDA, AMPA, mGluR1, spinal cord, lamprey, synaptic transmission.

LIST OF PUBLICATIONS

I. Dietmar Hess***, Evanthia Nanou**[*] and Abdeljabbar El Manira. (2007) Characterization of Na^+-Activated K^+ Currents in Larval Lamprey Spinal Cord Neurons. J Neurophysiol. 97: 3484-3493. *Equal contribution.

II. **Evanthia Nanou** and Abdeljabbar El Manira. (2007) A postsynaptic negative feedback mediated by coupling between AMPA receptors and Na^+-activated K^+ channels in spinal cord neurons. Eur J Neurosci. 25: 445–450.

III. **Evanthia Nanou**, Alexandros Kyriakatos, Arin Bhattacharjee, Leonard K. Kaczmarek, Gustavo Paratcha and Abdeljabbar El Manira. (2008) Na^+-mediated coupling between AMPA receptors and K_{Na} channels shapes synaptic transmission. Proc Natl Acad Sci U S A. 105: 20941-20946.

IV. **Evanthia Nanou** and Abdeljabbar El Manira. Modulation of AMPA-induced K_{Na} current by mGluR1. Manuscript.

V. **Evanthia Nanou** and Abdeljabbar El Manira. Role of pre- and post-synaptic K_{Ca} channels in determining the strength of synaptic transmission. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

1-EBIO	1-Ethyl-2-benzimidazolinone
4-AP	4-aminopyridine
5-HT	5-hydroxytryptamine
AchR	acetylcholine receptor
AHP	afterhyperpolarization
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BAPTA	1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid
BK channel	big conductance Ca^{2+}-activated K^+ channel
C-terminus	carboxyl-terminus
CNS	central nervous system
CPG	central pattern generator
CTZ	cyclothiazide
DHPG	(S)-3,5-Dihydroxyphenylglycine
DL-TBOA	DL-threo-β-benzyloxyaspartate
DLR	diencephalic locomotor region
EDTA	ethylenediaminetetraacetic acid
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
GF109203X	2-[1-(3-Dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide
IK channel	intermediate conductance Ca^{2+}-activated K^+ channel
K_{Na} channel	Na^+-activated K^+ channel
K_{Ca} channel	Ca^{2+}-activated K^+ channel
Li^+	lithium
LTP	long term potentiation
mGluR	metabotropic glutamate receptor
MLR	mesopontine locomotor region
MS-222	tricaine methane sulfonate
NMDA	N-methyl-D-aspartate
NMDG	N-methyl-D-glucamine
PDBu	Phorbol 12,13-Dibutyrate
PDZ domain	Post synaptic density protein (PSD95)/ Drosophila disc large tumor suppressor (DlgA)/Zonula occludens-1 protein (zo-1)
PKC	protein kinase C
PSD-95	postsynaptic density-95
RCK	regulator of K^+ conductance
SK channel	small conductance Ca^{2+}-activated K^+ channel
Slack	sequence like a calcium-activated K^+ channel
Slick	sequence like an intermediate conductance K^+ channel
TEA	Tetraethylammonium
TRH receptor	thyrotropin-releasing hormone receptor
TTX	Tetrodotoxin
UCL1684	ditrifluoroacetate hydrate
UCL 1848	trifluoroacetate salt

INTRODUCTION

CENTRAL PATTERN GENERATORS UNDERLYING RHYTHMIC MOVEMENTS

Vital to their survival, animals are equipped with locomotor skills allowing them to efficiently move in complex environments. Locomotor movements are generated by neuronal networks that produce coordinated patterns and activate motoneurons in the absence of sensory feedback. Such networks linked to motor behaviors in both invertebrates and vertebrates are called central pattern generators (CPGs).

CPGs underlie rhythmic movements such as respiration, locomotion or mastication as well as protective reflexes such as swallowing or coughing. Models of CPGs involve accessible networks in which the rhythmic pattern can be related to a defined motor behavior and in which the cellular and synaptic mechanisms responsible for the motor output can be studied. These include the stomatogastric nervous system (Nusbaum and Beenhakker, 2002; Marder and Bucher, 2007), the leech heartbeat (Kristan et al., 2005), locomotion (Grillner, 2003, 2006; Kiehn, 2006; Fetcho et al., 2008; Roberts et al., 2008), respiration (Feldman and Del Negro, 2006) and mastication (Lund and Kolta, 2006).

Identification of individual neurons and their synaptic connections is a required first step towards understanding CPGs. Eventually, the characterization of the different types of ion channels and synaptic processing, together with the description of their role in shaping cellular, synaptic and network activity is needed to understand how the entire CPG operates.

The main focus of this thesis has been to characterize how specific ion channels shape neuronal activity and synaptic transmission in the spinal locomotor network.

THE LOCOMOTOR CPG

Locomotion is initiated from supraspinal command centers in the diencephalon (DLR) and mesencephalon (MLR) that ultimately activate descending reticulospinal neurons and provide the necessary excitatory drive to activate the spinal locomotor CPG (Grillner, 2003; Dubuc et al., 2008). Under experimental conditions, however, the descending excitatory input can be substituted by bath application of glutamate receptor agonists that induce locomotor activity on the spinal cord with features similar to those in intact animals.

One general feature of the vertebrate locomotor network is the interaction of ipsilateral excitation and reciprocal inhibition (Grillner, 2003; Kiehn, 2006). The excitatory drive in the spinal CPG is mediated by ipsilateral glutamatergic interneurons while inhibitory glycinergic interneurons ensure the left-right alternation of the motor pattern. In the lamprey and in *Xenopus* tadpole, the connectivity of the locomotor network has been characterized in some detail. The excitatory interneurons excite other excitatory interneurons as well as ipsilateral motoneurons and glycinergic inhibitory neurons. The latter project to the contralateral side and mediate reciprocal inhibition. In the intact spinal cord, stretch receptors, also referred to as edge cells, sense the lateral movements and provide feedback to the locomotor CPG. There are two types of stretch receptors. One activates neurons on the ipsilateral side and the other inhibits neurons on the contralateral side.

The knowledge available about the organization of the locomotor CPG in the lamprey makes this model system suitable to link the activity of identified ionic currents and receptors within the known cellular circuitry, to specific features of neuronal and synaptic processing and subsequently to the generation of motor behavior.

MEMBRANE PROPERTIES AND IONIC CURRENTS IN THE LAMPREY SPINAL CORD

Characterization of ion channels and receptors as well as identification of the membrane properties of neurons in the lamprey spinal cord critically contributes towards a deeper understanding on how the locomotor neural circuit operates. The cell intrinsic properties of the constituent neurons determine their firing properties and these in turn shape the motor output of the locomotor rhythmic behavior.

One prominent property of lamprey spinal cord neurons is the slow after-hyperpolarization (sAHP), originally thought to only be mediated by activation of SK channels (Wallen et al., 1989; Grillner, 2003). However, recent evidence indicates that a part of the sAHP is due to activation of K_{Na} channels (Wallen et al., 2007). The apamin-sensitive K_{Ca} channels underlie a large part of the sAHP and mediate the spike frequency adaptation. These channels are mainly activated by Ca^{2+} influx via N-type channels ($Cav_{2.2}$) and to a lesser extent via P/Q-type channels ($Cav_{2.1}$), (Wikstrom and El Manira, 1998). Lamprey spinal cord neurons also possess L-type ($Cav_{1.1}$- $Cav_{1.4}$) and low-voltage activated Ca^{2+} channels (T-type, $Cav_{3.1}$-$Cav_{3.3}$) that are not linked to activation of K_{Ca} channels (Wikstrom and El Manira, 1998). The T-type Ca^{2+} channels mediate the post-inhibitory rebound and can contribute to initiating a locomotor burst on one side of the spinal cord upon termination of the inhibition from the contralateral side (Matsushima et al., 1993; Tegner et al., 1997).

Another important source of Ca^{2+} in the spinal neurons is through activation of NMDA receptors, which accounts for at least 50% of the total calcium entry (Bacskai et al., 1995; Cochilla and Alford, 1999). Activation of NMDA receptors in lamprey spinal cord neurons induces plateau potentials that persist in the presence of TTX (Wallen and Grillner, 1987). The initiation of the depolarizing phase is due to synaptic activation of NMDA receptors and T-type Ca^{2+} channels. The increased intracellular

Ca^{2+} activates K_{Ca} channels that terminate the depolarizing plateau. Blockade of K_{Ca} channels with apamin prolongs the duration of the plateau and delays its termination. The combined effect of K_{Ca} on the NMDA-induced plateau potentials and spike frequency adaptation contribute to determining the duration of the locomotor bursts and hence the frequency of the rhythm (El Manira et al., 1994).

In addition to K_{Ca} and K_{Na} channels, lamprey spinal cord neurons also possess voltage-activated K^+ channels. These channels are activated at high membrane potentials and produce an outward current with a transient and a sustained component. The transient K^+ current (I_{Kt}) is present in both motoneurons and interneurons. It displays a rapid activation, inactivation and recovery from inactivation and is specifically blocked by catechol (Hess and El Manira, 2001). The sustained K^+ current (I_{Ks}) is sensitive to low concentrations of TEA and high concentrations of 4-AP (Lamotte d'Incamps et al., 2004). The transient I_{Kt} seems to be important for sustaining repetitive firing (Hess and El Manira, 2001), optimizing the temporal fidelity of synaptic transmission and determining the frequency of the locomotor rhythm.

SODIUM-ACTIVATED POTASSIUM CHANNELS

Kameyama and colleagues (1984) first reported the existence of K^+ channels activated by intracellular Na^+ in cardiac myocytes. These channels, today referred to as Na^+-activated K^+ channels (K_{Na}), can be activated by relatively high concentrations of Na^+ and have a high unitary conductance. Several reports confirmed the existence of these channels (Bader et al., 1985; Hartung, 1985; Dryer et al., 1989; Schwindt et al., 1989), however their properties have been controversial. Most of the K_{Na} currents described were transient K^+ currents relatively insensitive to intracellular Na^+ and voltage independent (Kameyama et al., 1984; Dryer et al., 1989; Haimann et al., 1992). Dryer (1991) however showed that at least some of the reports supporting the existence of

transient K_{Na} currents coupled to Na^+ influx during action potentials were an artifact of inadequate voltage clamp. Two years later a sustained K_{Na} current was described in *Xenopus* spinal neurons that is highly sensitive to intracellular Na^+ and is voltage dependent (Dale, 1993). Yuan and colleagues (2003) established the molecular identity of K_{Na} channels by showing that they are encoded by a gene of the Slo family. This has allowed progress in understanding the biophysical properties and physiological functional role of these channels. Information available so far on K_{Na} channel genes, their localization in the CNS and their functional role as well as their modulation will be described in detail in the following sections.

K_{Na} channel genes and biophysics

Two genes, Slick (Slo 2.1) and Slack (Slo 2.2), encoding for K_{Na} have been identified in the CNS (Bhattacharjee et al., 2003; Yuan et al., 2003). Both Slick and Slack channels are activated by cytoplasmic Na^+, however the activity of Slick channels is greatly increased by intracellular Cl^- and can even be detected in the absence of Na^+. Both Slick and Slack channels have six transmembrane domains with a pore lining between the S5 and S6 subunit. Slack channels have a unitary conductance of ~ 180pS with a half-maximal activation of 41mM, while Slick channels have ~ 140pS and 180mM, respectively. The Slack channel has an N-terminus that is double in size compared to that of the Slick channel. They both contain two RCK (regulator of K^+ conductance) domains (likely Na^+ binding sites) and a highly conserved PDZ domain in their C-terminus. In addition Slick, but not Slack, channels contain an ATP binding site on the C-terminus and are inhibited in the presence of ATP. Both Slack and Slick channels lack positive charges in the S4 transmembrane domain although Slack channels were reported to show some voltage dependence.

Localization of K_{Na} in the CNS

In situ hybridization and immunohistochemical data show that both Slack and Slick channels are highly expressed in the brain in areas such as the olfactory bulb, red nucleus, facial nucleus, pontine nucleus, oculomotor nucleus, deep cerebellar nuclei, vestibular nucleus, the thalamus, amygdala and substantia nigra (Bhattacharjee and Kaczmarek, 2005). Slick, however, is also found in the hippocampal CA1, CA2, and CA3 regions, the dentate gyrus, supraoptic nucleus, hypothalamus, and cortical layers II, III, and V (Bhattacharjee and Kaczmarek, 2005). Moreover, electrophysiological data support the presence of K_{Na} channels in excitable tissues such as cardiac myocytes (Kameyama et al., 1984), brain areas (Egan et al., 1992; Bhattacharjee et al., 2003; Bhattacharjee and Kaczmarek, 2005; Bhattacharjee et al., 2005), spinal cord (Egan et al., 1992; Safronov et al., 1996) and they have also been detected in *Xenopus* oocytes (Egan et al., 1992). Finally, Slack channels colocalize with the postsynaptic density 95 (PSD-95), (Uchino et al., 2003) and glutamate receptors (Nanou et al., 2008), (see Paper III in this thesis).

Pharmacology of K_{Na} channels

Recent evidence identified the first activator of Slack channels called bithionol, a bis-phenol antiparasitic compound (Yang, 2006). Additionally, two blockers; quinidine and bepridil significantly block K_{Na} channels. Likewise, lithium (Li^+) does not activate K_{Na} channels (Dryer, 1994) and has been used in most studies to 'block' K_{Na} currents when Na^+ is replaced with Li^+ (Bhattacharjee and Kaczmarek, 2005).

Functional role of K_{Na} channels

K_{Na} channels have been shown to regulate the action potential waveform (Haimann et al., 1992; Dale, 1993; Hess et al., 2007; Huss et al., 2007), (see Paper I in this thesis). These channels can be activated by a single spike followed by an afterhyperpolarization

(AHP) mediated by a K_{Na} conductance (Cangiano et al., 2002; Franceschetti et al., 2003). Additionally, the size of a depolarizing after potential (DAP) is modulated by activation of K_{Na} channels (Liu and Stan Leung, 2004). In the case of a burst of action potentials, K_{Na} has been shown to induce an adaptation of the burst firing rate (Sanchez-Vives et al., 2000). Furthermore, reports show that K_{Na} channels are involved in the accuracy of timing of action potentials at high frequencies (Yang et al., 2007). These channels are also suggested to contribute to the resting membrane potential (Haimann et al., 1992; Dryer, 1994). In addition to the studies examining the activation of K_{Na} channels via coupling to Na^+ channels, evidence shows that these channels also interact with glutamate receptors (Liu et al., 1998; Nanou and El Manira, 2007; Nanou et al., 2008), (see Paper II, III in this thesis).

Modulation of K_{Na} channels

Recent evidence suggests that Gαq-protein coupled receptors (GqPCRs,) (M1 muscarinic receptor and mGluR1 metabotropic glutamate receptor) interact with and modulate K_{Na} channels (Santi et al., 2006). Activation of GqPCRs results in opposing modulation of the two K_{Na} channel types. Slick channels are strongly inhibited while Slack channels are strongly activated. This difference involves the activation of the protein kinase C (PKC) pathway. In addition, it has been proposed that PKC regulation of Slack/Slick heteromeric potassium channels is distinct from that of homomeric potassium channels (Chen et al., 2007). Electrophysiological data show that activation of PKC reduces homomeric Slick currents by ~ 50%. However, PKC activation results in a 90% decrease of the induced current of Slack/Slick heteromeric channels. Likewise, activation of AchR1 (acetylcholine receptor, muscarinic subtype) and the angiotensin II receptor seems to similarly affect K_{Na} channels (Santi et al., 2006). However, another report shows that both Slack and Slick channels are inhibited by

activation of Gαq-protein coupled thyrotropin-releasing hormone (TRH) receptors (Berg et al., 2007).

CALCIUM-ACTIVATED POTASSIUM CHANNELS

Gardos (1958) first described the effect of increased intracellular Ca^{2+} concentration on K^+ channels in erythrocytes. Later the existence of the so-called Ca^{2+}-activated K^+ channels (K_{Ca}) was confirmed in molluscan neurons and in cat spinal motoneurons (Meech and Strumwasser, 1970; Krnjevic and Lisiewicz, 1972; Meech and Standen, 1975). These channels can be activated by submicromolar concentration of Ca^{2+} and have a wide range unitary conductance. The genes encoding for SK channels were identified by Kohler (1996) and three groups of K_{Ca} channels are today described (BK, IK, SK) based on their conductance (big, intermediate, small). Their source of activation as well as their functional role has been controversial. Information known so far for SK channel genes including their localization in the CNS and their functional role will be described in detail in the following sections.

SK channel genes and biophysics

Three genes encode for SK channels, SK1 (K_{Ca} 2.1), SK2 (K_{Ca} 2.2), SK3 (K_{Ca} 2.3), (Kohler et al., 1996). SK channels sensitive to cytoplasmic Ca^{2+} are activated by submicromolar Ca^{2+} concentrations with half-maximal activation ranging from ~2-14 μM in symmetrical K^+ conditions (Blatz and Magleby, 1986; Grissmer et al., 1992; Park, 1994; Kohler et al., 1996; Xia et al., 1998; Fakler and Adelman, 2008). SK channels have a unitary conductance of 9-10 pS. These channels have six transmembrane domains with a pore lining between the S5 and S6 segment. In contrast to BK channels, SK channels, are voltage independent and lack a calcium domain, but

their C-terminus is coupled to calmodulin that acts as a calcium sensor (Xia et al., 1998).

Localization of SK channels in the CNS

In situ hybridization and immunohistochemical data show that all three SK subunits are highly expressed in the CNS. Both SK1 and SK2 are expressed in many brain areas such as the neocortex, hippocampus, thalamus, and cranial nuclei (motor trigeminal, motor facial, oculomotor and trochlear), (Stocker et al., 1999; Stocker and Pedarzani, 2000; Sailer et al., 2004; Stocker, 2004). SK3, however, is expressed in the thalamus, medial habenula, brainstem, supraoptic nucleus, lateral septum, substantia nigra pars compacta, ventral tegmental area, dorsal raphae nucleus and locus coeruleus (Pedarzani et al., 2000; Stocker and Pedarzani, 2000; Tacconi et al., 2001; Wolfart et al., 2001; Sailer et al., 2004; Stocker, 2004). Although immunohistochemical data show that SK1 are only present in the dendrites of hippocampal CA1 neurons (Sailer et al., 2002; Sailer et al., 2004), localization of SK1 is found in the soma of CA1 neurons in culture (Ishii et al., 1997). In contrast, SK3 are localized presynaptically in the neuromuscular junction (Roncarati et al., 2001) and in dissociated hippocampal neurons (Obermair et al., 2003).

Pharmacology of SK channels

The bee venom toxin apamin is broadly used to block all three SK subunits (Burgess et al., 1981; Vogalis et al., 2003; Stocker, 2004), however each varies in sensitivity (Stocker, 2004). SK2 are reported as the most sensitive to apamin (IC50: 27-140 pM), (Kohler et al., 1996; Strobaek et al., 2000), SK3 have an intermediate sensitivity to apamin (IC50: 0.6-4 nM), (Ishii et al., 1997; Grunnet et al., 2001; Hosseini et al., 2001), while SK1 previously thought to be apamin-insensitive are blocked by apamin with a half-maximal activation of 0.7-12 nM, dependent on the expression system used (Shah

and Haylett, 2000; Strobaek et al., 2000). Moreover, scorpion toxins such as scyllatoxin and tapamin also block SK2 channels (Strobaek et al., 2000; Pedarzani et al., 2002). Furthermore, all three subunits are blocked by bicuculline salts ($GABA_A$ antagonists), curare, N-methy-laudosine, UCL 1684 (ditrifluoroacetate hydrate) and UCL 1848 (trifluoroacetate salt), (Johnson and Seutin, 1997; Seutin et al., 1997; Khawaled et al., 1999; Vogalis et al., 2003; Pedarzani and Stocker, 2008). Finally, recent reports show that 1-EBIO (1-methyl-2benzimidazolinone) acts as an activator for SK channels (Pedarzani et al., 2001).

Functional role of SK channels

SK channels have been shown to be activated during the after-hyperpolarization (AHP) of action potentials, (Sah and Faber, 2002; Stocker, 2004). The AHP is composed of three components: fast, medium, slow. SK channels mediate the mAHP (medium) that activates within less than 5 ms and decays in several hundreds of milliseconds (Pennefather et al., 1988; Schwindt et al., 1988; Storm, 1989). In the case of a burst of action potentials, SK channels have been shown to regulate the burst-firing rate (Cingolani et al., 2002; Hallworth et al., 2003; Womack and Khodakhah, 2003). Blockade of SK channels also induces the switch from single spike activity to rhythmic burst activity (Wolfart et al., 2001; Cingolani et al., 2002; Edgerton and Reinhart, 2003; Hallworth et al., 2003), while the opposite occurs in the presence of an SK channel enhancer (Hallworth et al., 2003). Furthermore, SK channels play a significant role in determining the instantaneous firing rate (Schwindt et al., 1988; Pedarzani et al., 2000; Wolfart et al., 2001; Edgerton and Reinhart, 2003). Contrary to these reports, recent evidence suggests that instead of SK channels, rather Kv7/KCNQ/M channels mediate the mAHP at more depolarized potentials while HCN/h channels mediate it at more hyperpolarized potentials (Storm, 1989; Gu et al., 2005; Gu et al., 2008).

Evidence shows however that SK channels regulate excitatory synaptic transmission by acting as a negative feedback loop (Faber et al., 2005; Ngo-Anh et al., 2005). Furthermore, blockade of SK channels induces long term potentiation (LTP) in synaptic transmission (Behnisch and Reymann, 1998; Stackman et al., 2002). Finally, K_{Ca} channels are involved in the termination of NMDA-pacemaker induced plateau potentials (El Manira et al., 1994). Activation of K_{Ca} channels initiates the repolarization of the NMDA-induced depolarized plateau phase leading to the Mg^{2+} blockade of NMDA receptors.

AIMS

The aim of this thesis has been to characterize the Na^+-activated K^+ (K_{Na}) and the Ca^{2+}-activated K^+ (K_{Ca}) channels, identify the source of Na^+ and Ca^{2+} necessary for their respective activation and determine their role in regulating synaptic transmission.

The specific questions addressed are:

- Do lamprey spinal neurons possess Na^+-activated K^+ channels? (Paper I)
- Can K_{Na} channels be activated by Na^+ influx through AMPA receptors? (Paper II)
- What is the role of the AMPA-activated K_{Na} channels in excitatory synaptic transmission? (Paper III)
- Are AMPA- induced K_{Na} channels subject to modulation by mGluRs? (Paper IV)
- Are K_{Ca} channels activated by Ca^{2+} influx through NMDA receptors and what role do they play in controlling the strength of excitatory synaptic transmission? (Paper V)

METHODS

Different methods and techniques were used in order to study the role of K_{Na} and K_{Ca} channels in the lamprey spinal cord. The following preparations were used in this thesis:

CELL DISSOCIATION

Two to three larval lampreys (*Petromyzon marinus*) were anesthetized with tricaine methane sulfonate (MS-222) and their spinal cords were dissected out. The spinal cords were then treated with two enzymes (collagenase and protease) diluted in the culture medium (L-15 Leibovitz) where penicillin and gentamicin were added. The tissue was subsequently washed with the culture medium and triturated through a sterilized glass pipette. The dissociated neurons were then distributed in 15-20 Petri dishes and incubated at 10–12^{o}C for 1–2 days. In some experiments motoneurons and crossing interneurons were pre-labeled with a fluorescent dye in the intact spinal cord, prior to dissociation (Hess and El Manira, 2001)

IN VITRO SPINAL CORD PREPARATION

This preparation can be used for two different recording methods: intracellular and patch clamp recordings. For intracellular recordings, transformer lampreys (*Petromyzon marinus*) were anesthetized with tricaine methane sulfonate (MS222) and the spinal cord was dissected free from the notochord. The meningeal layers were then removed with fine forceps and the cord was mounted ventral side up in a cooled (8-12^{o}C) Sylgard-lined chamber. For the patch clamp recordings a thin slice above the motorneuron and interneuron area was removed with a vibratome to allow for better access to the neurons for patch-clamp recordings.

ELECTROPHYSIOLOGY

Whole-cell recordings from spinal motoneurons and interneurons in culture were made using a patch-clamp amplifier (AxoPatch 200A, Axon Instruments, Foster City, CA). For the recording of fast currents, only small neurons with a diameter <10–15 µM were used to allow for good space-clamp conditions. The mechanosensory dorsal cells, identified by their round large cell bodies, were not recorded in these experiments. The series resistance was compensated electronically by 75–85%. The solutions were applied through a gravity-driven micro-perfusion system with the tip placed close to the recorded cell.

For the analysis of synaptic transmission, intracellular or patch clamp recordings were made using the Multiclamp 700B and Axoclamp 2B, respectively. EPSPs, EPSCs and action potentials were elicited by stimulation of the medial surface on the ventral side of the spinal cord using a glass suction electrode. Patch clamp recordings were mainly made from gray matter neurons which include motoneurons, and interneurons. For the analysis of action potentials, intracellular recordings were made from reticulospinal axons.

IMMUNOPRECIPITATION

Adult rat brains were homogenized in ice-cold Tris-HCl buffer to which sucrose, EDTA and proteinase inhibitors were added; by the use of a Potter-Elvehjem glass homogenizer. The homogenized tissue was then centrifugated and the pellets were discarded while the supernatants were centrifuged again to obtain an enriched synaptosomal pellet (P2 synaptosomal-mitochondrial fraction). Crude synaptosomal fraction from rat brain, and lamprey CNS were lysed in buffer containing Triton X-100 and octyl glucoside plus proteinase inhibitors in a glass homogenizer. Protein lysates

were clarified by centrifugation and analyzed by immunoprecipitation (IP) followed by immunoblotting (IB).

IMMUNOHISTOCHEMISTRY

The spinal cord of the lamprey (*Petromyzon marinus*) was dissected out and fixed in paraformaldehyde. The fixed cord was then incubated for 24h in sucrose. Then the cord was placed in a cryostat were 14-18 μm cryostat sections are made. The slides were then incubated with the primary antibody for 24-48 h. Subsequent wash of the slides was made in PBS (Phosphate Buffered Saline) and the secondary antibody was added for 2 hours. In the case where nissl stain was also used, the slides were washed with PBS and then they were incubated for 15 min.

RESULTS AND DISCUSSION

CHARACTERIZATION OF Na^+-ACTIVATED K^+ CURRENTS IN LARVAL LAMPREY SPINAL CORD NEURONS. (PAPER I)

In this study we examined whether lamprey spinal cord neurons possess Na^+-activated K^+ channels and determined their activation during the action potential. Dissociated spinal cord neurons were recorded in the presence of calcium channel blockers. Their membrane potential was held at -60 mV and application of a voltage step to -10 mV induced an inward Na^+ current followed by an outward K^+ current consisting of a transient and a sustained component. The transient current was linked to the Na^+ influx via voltage-activated channels underlying the action potential because it was absent in tetrodotoxin (TTX). This indicates that the transient current is mediated by K_{Na} channels. To test whether the sustained outward component involves activation of K_{Na} current, Na^+ was substituted with Li^+, NMDG (N-methyl-D-glucamine) or choline that significantly reduced the amplitude of the sustained current. This current was not blocked by TTX, excluding the possibility of its activation by Na^+ influx through voltage-gated Na^+ channels. Removal of extracellular Na^+ can result in a change in intracellular Ca^{2+} concentration or pH by affecting the Na^+-Ca^{2+} exchanger or Na^+-H^+ exchanger, respectively. To test for this, the intracellular Ca^{2+} or pH were buffered with BAPTA or HEPES, respectively. In both BAPTA and HEPES, substitution of Na^+ with choline or NMDG was still able to decrease the sustained K^+ current, ruling out an effect of the Na^+-Ca^{2+} exchanger and Na^+-H^+ exchanger. These data support an activation of a sustained K_{Na} current that seems to be activated by Na^+ influx via leak channels. The sustained K_{Na} currents appear to display a voltage dependency, however this was difficult to accurately access because at more depolarized potentials the driving force for Na^+ via leak channels should decrease, thereby limiting the amount of sodium available to activate K_{Na} channels. Cloned K_{Na}

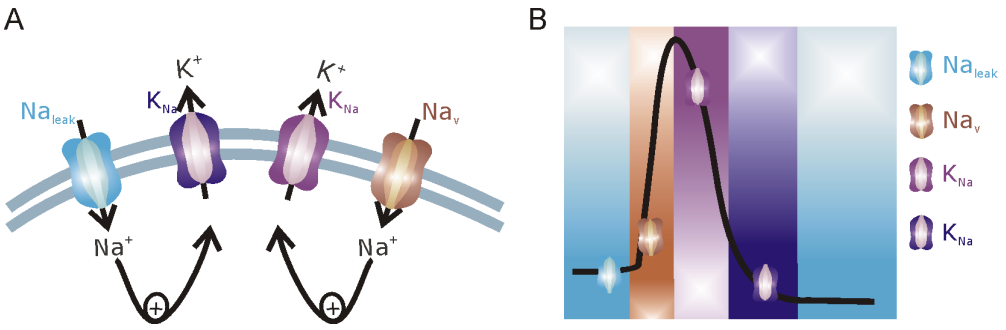

Fig. 1. Activation of K_{Na} channels by different Na^+ sources. (A) Activation of K_{Na} channels by Na^+ influx through Na^+ leak channels and Na^+ voltage-gated channels. **(B)** Schematic representation of the contribution of each current to the action potential waveform.

channels were shown to display a voltage-dependency (Bhattacharjee and Kaczmarek, 2005) despite the fact that these channels lack charges in the S4 subunit (Bhattacharjee et al., 2003; Yuan et al., 2003).

In addition to the two K_{Na} currents, lamprey spinal cord neurons also possess high-voltage activated K^+ channels. These channels underlie a transient (Kt) and a sustained (Ks) current (Hess and El Manira, 2001). In order to determine the role of these currents in determining the shape of the action potential, we used the waveform of an action potential as a voltage command while individual K^+ currents were isolated pharmacologically. The transient K_{Na} current activaton was tightly coupled to the Na^+ current and seems to mediate the early repolarization of the action potential and the fast AHP (Fig.1A, B). Finally, our results show that basal levels of Na^+ through leak channels activate the sustained K_{Na} current (Fig.1A, B). This current also seems to be activated by an increased intracellular Na^+ induced by repetitive firing and mediates the Ca^{2+}-independent AHP (Fig.1B), (Cangiano et al., 2002; Wallen et al., 2007).

A POSTSYNAPTIC NEGATIVE FEEDBACK MEDIATED BY COUPLING BETWEEN AMPA RECEPTORS AND Na^+-ACTIVATED K^+ CHANNELS IN SPINAL CORD NEURONES. (PAPER II)

Most of the information available about K_{Na} channels relates to the activation of these channels by Na^+ influx through voltage-gated or leak Na^+ channels. A major source of Na^+ influx into neurons is ionotropic receptors. Therefore we examined whether Na^+ influx via AMPA receptors can activate K_{Na} channels. For this, whole-cell recordings were made from isolated spinal cord neurons, and application of AMPA induced an inward current followed by an outward current upon termination of the AMPA application. The outward current was associated with an increased conductance and reversed close to the calculated reversal potential for K^+, indicating that it corresponds to a K^+ current. Substitution of Na^+ with Li^+ abolished the AMPA-induced K^+ current and markedly slowed the decay time constant of the inward current (Fig.2A, B, C). The AMPA-induced K_{Na} current was completely blocked by quinidine, which has been shown to block K_{Na} in an expression system (Bhattacharjee et al., 2003). These results show that Na^+ influx via AMPA receptors is able to activate K_{Na} channels that control the decay time constant of the inward AMPA current.

The two cloned K_{Na} channels display different sensitivity to increased intracellular Cl^- and ATP (Bhattacharjee et al., 2003). Slick, but not Slack, channels are activated by intracellular Cl^- and inhibited by ATP (Bhattacharjee et al., 2003). In order to determine the channel type mediating the AMPA-induced K_{Na} current, we used high intracellular Cl^- concentration or ATP in the intracellular solution. The AMPA-induced K_{Na} current was neither affected by high intracellular Cl^- concentration nor by ATP, suggesting that is mediated by a Slack-like K_{Na} current. Our results suggest that the AMPA-induced K_{Na} current is similar to the cloned Slack channels (Bhattacharjee et al., 2003; Yuan, et al. 2003). However, it is possible that the expression of both Slick and Slack channels was affected by the dissociation procedure in the spinal cord due to

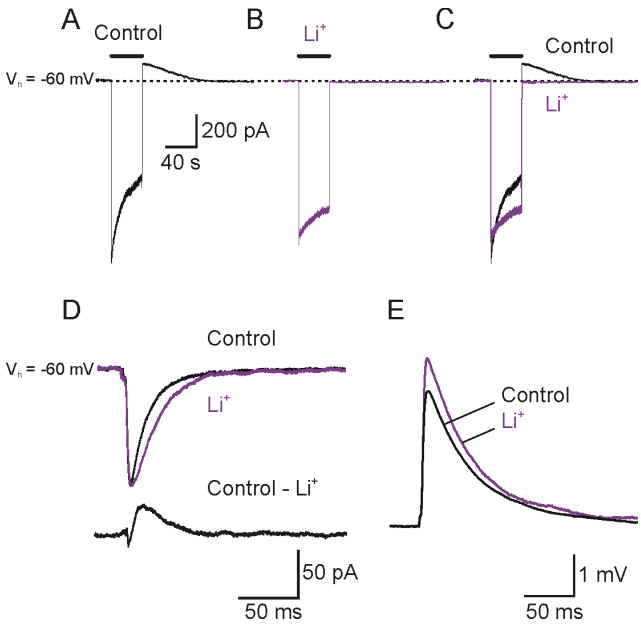

Fig. 2. AMPA-induced K_{Na} current. (A) Application of AMPA induces an inward current followed by an outward current upon termination of the AMPA application in dissociated spinal cord neurons. **(B)** Substitution of Na^+ with Li^+ abolishes the AMPA-induced K^+ current. **(C)** Superimposed current traces in control and in Li^+. **(D)** Substitution of Na^+ with Li^+ increases the decay time constant of synaptically induced AMPA EPSCs. Lower trace shows the current inhibited by Li^+. **(E)** Substitution of Na^+ with Li^+ increases the amplitude of the AMPA-mediated EPSPs.

a loss or change of neuronal processes and co- regulating proteins. Alternatively, it is also possible that the native K_{Na} channels in the lamprey spinal cord neurons represent a heteromeric assembly of Slick and Slack subunits, which may display different sensitivity to Cl^- and ATP than homomeric channels in expression systems.

K_{Na} current in this study was obtained by long application of AMPA in the order of seconds. However, synaptic activation of AMPA receptors is a fast process on the order of milliseconds. Thus, in order for K_{Na} channels to be activated, AMPA receptors and K_{Na} channels should be closely located and an increase of intracellular Na^+ concentration should occur on the order of tens of millimolar. Although our evidence thus far is indirect, the most obvious interpretation of our results is that Na^+ influx through AMPA receptors activates K_{Na} channels at the beginning of the AMPA

application and determines the decay of the AMPA induced-inward current. This is supported from the fact that Li^+ and quinidine significantly slow the decay of the AMPA-induced inward current. However, direct evidence for this requires activation of K_{Na} channels by Na^+ influx via synaptically activated AMPA receptors.

Na^+-MEDIATED COUPLING BETWEEN AMPA RECEPTORS AND K_{Na} CHANNELS SHAPES SYNAPTIC TRANSMISSION. (PAPER III)

The central question of this study was whether AMPA receptors and K_{Na} channels interact in the soma-dendritic region and how this interaction shapes synaptic transmission. In this study we used the in vitro lamprey preparation to directly examine the contribution of the K_{Na} current in regulating synaptic transmission. We first examined whether K_{Na} channels and AMPA receptors interact with each other in the synapse. For this we performed immunoprecipitation experiments, which showed that K_{Na} channels are located in close proximity with AMPA receptors in the lamprey and rat CNS.

In order to show that AMPA receptors and K_{Na} channels interact, we also used immunohistochemistry. Our data showed that neurons in the gray matter of the lamprey spinal cord express Slack-like channels and AMPA Glu2/3 receptors. Unfortunately, the antibodies tested could not be used simultaneously since they were from the same host. Imaging studies measuring Na^+ influx during repetitive stimulation in the presence or absence of Li^+ could be utilized to substantiate the interaction of these two proteins. Other studies show that Na^+ transients induced by repetitive stimulation of ionotropic glutamate receptors in the hippocampus (Rose and Konnerth, 2001) can increase the intracellular Na^+ concentration up to ~45 mM in dendrites.

We then examined whether an increase in the intracellular Na^+ concentration via synaptic activation of AMPA receptors results in activation of K_{Na}. For this, whole-

cell recordings were made from spinal cord neurons and EPSPs and EPSCs were recorded from the same neurons. Replacement of Na^+ with Li^+ increased the amplitude of the EPSPs and it increased the decay time constant of EPSCs (Fig. 2D, E). The current blocked in Li^+ was outward and reversed close to the calculated K^+ membrane potential, indicating that it is mediated by activation of K_{Na} channels. This was further confirmed by application of the K_{Na} channel activator bithionol, which decreased the amplitude of the EPSPs and decreased the decay time constant of the EPSCs. Together these results show that K_{Na} channels interact with AMPA receptors acting as a negative feedback loop to decrease the magnitude of excitatory synaptic response.

The induced changes in the amplitude of the EPSPs and decay time constant of the EPSCs by Li^+ may be due to an effect on the desensitization of the AMPA receptors (Karkanias and Papke, 1999) or on glutamate transporters (Chuhma and Ohmori, 2002). These possibilities were ruled out because Li^+ could still affect the EPSPs and EPSCs in the presence of the AMPA desensitization inhibitor cyclothiazide (CTZ) or the glutamate transporter inhibitor DL-threo-β-benzyloxyaspartate (DL-TBOA). Moreover, chelating calcium with BAPTA did not block the Li^+ effect, suggesting against a Na^+-Ca^{2+} exchanger contribution to the induced changes by Li^+. Similarly, the time course of mEPSCs was not altered in Li^+ suggesting that the AMPA receptor current *per se* remained unaffected.

MODULATION OF AMPA-INDUCED K_{Na} CURRENT BY mGluR1. (PAPER IV)

K_{Na} channels have been shown to be modulated by G-protein coupled receptors in expression systems (Santi et al., 2006; Berg et al., 2007). One important modulatory receptor in the lamprey spinal cord is the metabotropic glutamate receptor (mGluR) which has a profound effect on synaptic transmission and network activity. Thus we

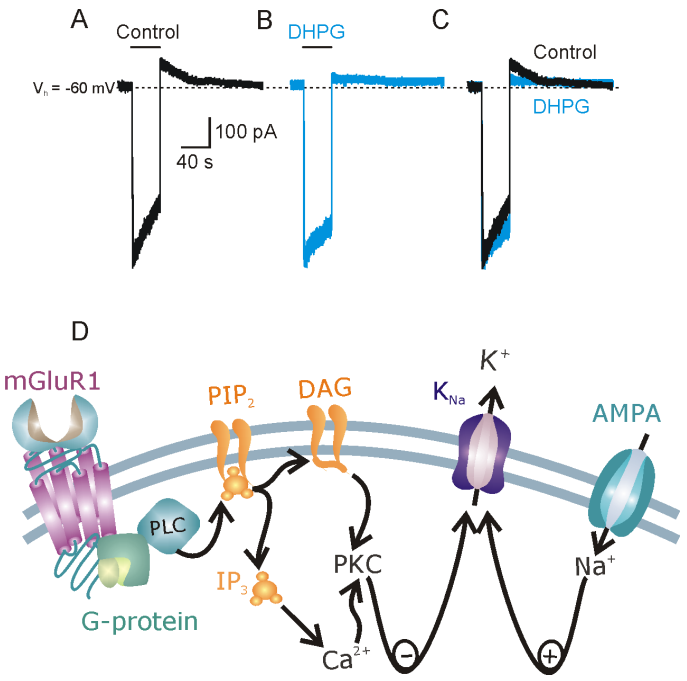

Fig. 3. Modulation of AMPA-induced K_{Na} current by mGluR1 **(A)** Application of AMPA induces a K_{Na} current in dissociated spinal cord neurons. **(B)** Application of DHPG decreases the amplitude of the AMPA-induced K_{Na} current. **(C)** Superimposed current traces in control and in DHPG. **(D)** Signaling pathway underlying the mGluR1-mediated modulation of K_{Na} channels.

examined whether AMPA-induced K_{Na} currents are modulated by activation of mGluRs in lamprey spinal neurons. Our results showed that application of (S)-3,5-Dihydroxyphenylglycine (DHPG), a group I mGluRs agonist, blocks the activated K_{Na} current. However, when intracellular Ca^{2+} is chelated by 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), application of DHPG enhances the AMPA-induced K_{Na} current. Both effects of DHPG on the K_{Na} current are blocked by the specific mGluR1 antagonist 7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt). Because the signaling mechanism activated by mGluR1 leads to activation of protein kinase C (PKC), we tested if it is involved in mediating the modulation of the K_{Na} current by mGluR1. Activation of PKC with Phorbol 12,13-Dibutyrate (PDBu), significantly inhibits the AMPA-induced K_{Na} current mimicking the effect of mGluR1 activation. On the other hand, inhibition of

PKC with 2-[1-(3-Dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X) only blocked the effect of mGluR1-mediated decrease in the amplitude of the AMPA-induced K_{Na} current, while it did not affect the enhancement of the current. Together these results suggest that AMPA-induced K_{Na} current is differentially modulated by the mGluR1 and the sign of the modulation is dependent on intracellular Ca^{2+} concentration and activation of PKC.

Differential modulation of the two K_{Na} channels is reported by mGluRs in the *Xenopus* expression system (Santi et al., 2006). mGluR1 inhibits Slick (Slo2.1) channels while it augments Slack (Slo2.2) ones. In addition, a chimera having Slo2.2 as a core and Slo2.1 as a tail is negatively regulated by PKC activation. However, in the HEK cell expression system both Slo2 channels seem to be inhibited by G-protein coupled receptors (Berg et al., 2007). Similarly to the *Xenopus* expression system activation of mGluR1/5 inhibits Slick channels. However, in HEK193T cells, activation of G-protein TRH receptor results in inhibition of both Slick and Slack channels. Thus the expression system used may account for the differential modulation of the K_{Na} current by G-protein coupled receptors. In our study the AMPA-induced K_{Na} current is facilitated by DHPG when BAPTA is used in the intracellular solution to chelate calcium, while it is reduced in the absence of a calcium chelator. This suggests for a secondary mechanism regulating the modulation of the K_{Na} channels by mGluRs.

To determine if the modulation by mGluR1 can account for the decrease in the AMPA-induced K_{Na} current, one should study its effect in synaptic transmission. This has been difficult to assess because mGluR1 acts on many targets both at the pre- and postsynaptic site in the lamprey spinal cord (Kyriakatos and El Manira, 2007). Activation of mGluR1 increases the amplitude of EPSPs by increasing glutamate release from presynaptic terminals. In addition, the amplitude of AMPA-mediated EPSPs is depressed by activation of the postsynaptic K_{Na} current.

ROLE OF PRE- AND POST-SYNAPTIC K_{Ca} CHANNELS IN DETERMINING THE STRENGTH OF SYNAPTIC TRANSMISSION. (PAPER V)

We showed that AMPA and K_{Na} channels interact with each other and shape synaptic transmission. The question we addressed in this part of the thesis is whether Ca^{2+} influx through NMDA receptors mediates a functional coupling between this receptor and K_{Ca} channels and how this modulates synaptic transmission. Hence, the role of K_{Ca} channels in synaptic transmission was studied in the lamprey spinal cord. In addition, we tested whether K_{Ca} channels are also located presynaptically in axon terminals.

We first examined the effect of apamin, known to block K_{Ca} channels, on the NMDA-induced current in isolated spinal cord neurons in culture. Application of apamin decreased the decay of the NMDA-induced current and blocked an outward current occurring at the end of NMDA application. These results indicate that Ca^{2+} influx via NMDA receptors activates K_{Ca} channels. We next tested if K_{Ca} channels are activated during NMDA-induced synaptic transmission. For this, apamin was tested on NMDA-mediated EPSPs and EPSCs. Blockade of K_{Ca} channels increased the decay time constant of the evoked EPSCs and increased the amplitude of the EPSPs in the same neurons (Fig.4B, C). In order to test whether NMDA receptors are located close to K_{Ca} channels we performed similar experiments in the presence of calcium chelators. The effect of apamin on the EPSCs and EPSPs was blocked by the fast Ca^{2+} chelator BAPTA, but not by the slow chelator EGTA. Together these results show that Ca^{2+} influx through NMDA receptors is sufficient to activate K_{Ca} channels.

We then examined whether K_{Ca} channels are also located in presynaptic terminals. For this we examined the effect of apamin on the duration of action potentials recorded in reticulospinal axons. Blockade of presynaptic K_{Ca} channels by

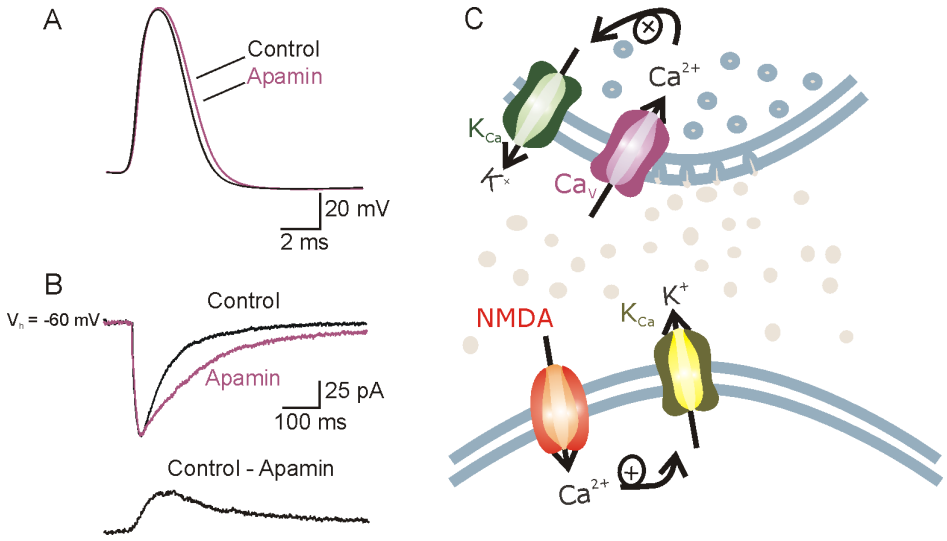

Fig. 4. Activation of K_{Ca} channels by different calcium sources at the pre- and post-synaptic sites **(A)** Blockade of K_{Ca} channels by apamin increases the duration of the action potential presynaptically. **(B)** Blockade of K_{Ca} channels by apamin increases the decay time constant of the synaptically induced NMDA EPSCs. Lower trace shows the current inhibited by apamin. **(C)** Ca^{2+} influx though Ca^{2+} voltage-gated channels activates K_{Ca} channels in the presynaptic site, while Ca^{2+} influx through NMDA receptors activates K_{Ca} channels in the postsynaptic site.

apamin slowed the repolarization of the action potential by increasing its width (Fig.4A, C). The broadening of the action potential resulted in an increase of the presynaptic Ca^{2+} influx which was also reflected by an increase in the amplitude of the AMPA-induced EPSCs in a frequency dependent manner. This increase in Ca^{+} influx and subsequent transmitter release may lead to activation of NMDA receptors postsynaptically that in turn will activate postsynaptic K_{Ca} channels. Thus, K_{Ca} channels play an important role both pre- and postsynaptically controlling the magnitude of the excitatory synaptic response.

K_{Ca} channels mediate the sAHP in the lamprey spinal cord (Hill et al., 1992; Cangiano et al., 2002; Wallen et al., 2007). Ca^{2+} entry through N-type and P/Q-type (Cav 2.1, 2.2) channels activates K_{Ca} channels in the lamprey spinal cord (Wikstrom and El Manira, 1998). A previous study in the lamprey spinal cord found no evidence

for a change in the NMDA-induced EPSP by activation of K_{Ca} channels (Cangiano et al., 2002). In that study paired intracellular recordings were made from the reticulospinal axon-motorneuron synapse. The presence of K_{Ca} channels in the dendrites makes it difficult to interpret why synaptic activation of NMDA receptors does not induce sufficient Ca^{2+} concentration to activate them. Our results now support for an NMDA-induced K_{Ca} current. One possibility is that stimulation of descending axons by a suction electrode recruits more axonal input to distal or proximal dendrites which adds to the Ca^{2+} increase in the synapse necessary to activate K_{Ca} channels. However, submicromolar concentration of Ca^{2+} is needed to activate K_{Ca} channels (Vergara et al., 1998). Conceivably, calcium imaging in the presence or absence of apamin will link the changes of Ca^{2+}-induced NMDA receptor activation to the threshold for SK channel activation. Lastly, the distribution of K_{Ca} channels in the dendrites and their co-expression with other receptors in the cell membrane remains to be determined.

CONCLUSIONS AND FUTURE PERSPECTIVES

The central finding of this work is that different types of K^+ channels control the magnitude of excitatory synaptic transmission in the lamprey spinal cord. Na^+ influx through the fast AMPA receptors activates K_{Na} channels that act as a negative feedback loop to decrease the AMPA-mediated response (Fig. 5). Likewise, Ca^{2+} influx through the slow NMDA receptors activates K_{Ca} channels in a similar manner to decrease the excitatory synaptic potentials (Fig. 5). These two processes occur at the postsynaptic site. Additionally, K_{Ca} channels are activated presynaptically by Ca^{2+} influx through voltage-gated channels and act to limit the duration of the action potential (Fig. 5). Also, a presynaptic effect of K_{Na} channels has not been excluded.

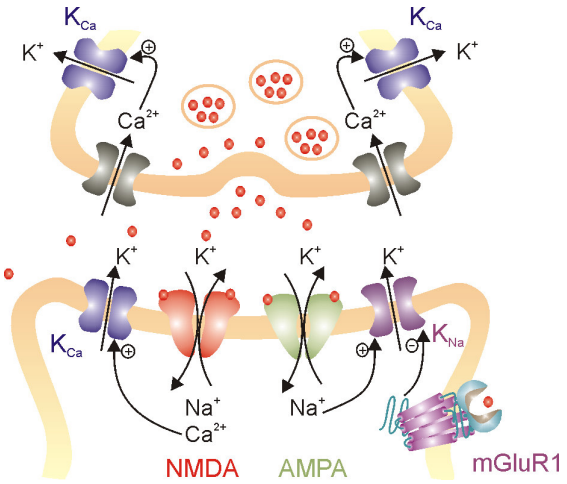

Fig. 5. Activation of K_{Na} and K_{Ca} channels at the pre- and post-synaptic sites. Ca^{2+} influx through voltage-gated channels activates K_{Ca} channels presynaptically. Postsynaptically, Na^+ influx though AMPA receptors and Ca^{2+} influx through NMDA receptors activates K_{Na} and K_{Ca} channels, respectively. Activation of mGluR1 positively modulates K_{Na} channels.

In the lamprey spinal cord, neurons fire action potentials followed by a fast and a slow afterhyperpolarization (AHP). The slow AHP (sAHP) has been shown to be mainly due to activation of apamin-sensitive SK channels (K_{Ca}) activated by Ca^{2+} influx through voltage-dependent channels in the soma-dendritic region (Hill et al., 1985; Hill et al., 1992; Cangiano et al., 2002; Wallen et al., 2007). Our results now support that SK channels contribute to the repolarization of the action potential in the

reticulospinal axons; apamin significantly increases the action potential width compared to control. Our data indicate that SK channels apart from being located close to voltage-gated Ca^{2+} channels are also located in close proximity to NMDA receptors postsynaptically. Our results are consistent with previous reports coupling NMDA receptors and SK channels. Thus, distinct localization of separate populations of SK channels will determine their coupling to different sources of Ca^{2+} leading to distinct physiological roles.

Additionally, a part of the sAHP is mediated by a K_{Na} conductance in the lamprey spinal cord (Wallen et al., 2007). In this thesis, two types of K_{Na} currents were characterized in spinal neurons in culture. The transient K_{Na} current activated though Na^+ voltage-gated channels contributes to the early repolarization of the action potential which suggests a close proximity of these proteins. However, the sAHP may be mediated by activation of the sustained K_{Na} current activated by Na^+ influx via leak channels. The difference in the time course of these two K_{Na} currents may reflect the localization of these proteins with adjacent Na^+ sources.

In the present thesis, we show that SK channels are activated during a single NMDA-induced EPSC that control its decay time constant. Therefore, a single NMDA-mediated EPSC is necessary and sufficient to activate SK channels. However, in the case of activation of K_{Na} channels by Na^+ influx through AMPA receptors, a single EPSC results in activation of K_{Na} channels in only 50% of the cases. A train of AMPA-induced EPSCs favors an increase in the intracellular Na^+ concentration and subsequent increased activation of K_{Na} channels. Localization of the different SK channels in the lamprey spinal cord has not yet been shown, however electrophysiological data support for their presence in the dendrites. In the last paper of this thesis an additional presynaptic site of action is shown for SK channels. On the other hand, immunohistochemical and electrophysiological data show that K_{Na} channels are present

in both the soma and the dendrites of lamprey neurons. Thus, selective recruitment of either K_{Na} or K_{Ca} channels will be determined by their distribution in spinal neurons. Identification of the regions with highest channel density is needed for a detailed description of the role of K_{Na} and K_{Ca} channels in synaptic transmission.

Although, our results show potential sources of Ca^{2+} and Na^{+} that activate K_{Ca} and K_{Na} channels, respectively, direct measurement of synaptically mediated Ca^{2+} or Na^{+} transients has not yet been done. Imaging studies with the help of pharmacological tools will help to understand the functional role of K_{Na} and K_{Ca} channels in physiological conditions. Although Na^{+} transients are difficult to detect, reports show a glutamate-induced increase in Na^{+} influx during repetitive stimulation in the spines and the dendrites of the hippocampus (Rose and Konnerth, 2001)

At the network level, blockade of SK channels has been shown to increase locomotor cycle duration due to a delayed burst termination (El Manira et al., 1994). Differential changes in the burst pattern are induced during slow and fast fictive swimming. At lower burst frequencies, the locomotor pattern becomes slow and irregular, while at higher burst frequencies a limited effect is observed in the frequency and regularity. In contrast, the role of K_{Na} channels in fictive swimming has not yet been tested. The lack of specific activators and blockers makes it difficult to define their functional role. For example replacement of Na^{+} with Li^{+} not only blocks K_{Na} channels but also the Na^{+}/K^{+} -ATPase (Wallen et al., unpublished). Nevertheless, another animal model has been used to study fictive swimming, the zebrafish *Danio rerio*. The main advantage of this model is that its genome is mostly sequenced. In light of the fact that drugs are not yet discovered for a selective blockade or activation of K_{Na} channels, knockdown of individual genes will help in understanding the differential role of K_{Na} channels in a network. Similar methods can be used in the case of the apamin-non discriminatory block of SK channels; this model will help to understand

the physiological significance of K_{Ca} channels.

Several modulatory systems such as 5-hydroxytryptamine (5-HT), dopamine, gamma-aminobutyric acid (GABA) and metabotropic glutamate receptor (mGluR), intrinsic to the lamprey locomotor network, may influence the activity of K_{Ca} and K_{Na} channels. Activation of 5-HT, dopamine and GABA results in a reduction of the K_{Ca} component of the sAHP (Matsushima et al., 1993; Schotland et al., 1995; Hill et al., 2003), while its K_{Na} component remains unaffected (Wallen et al., 2007). In addition, mGluRs seem not to modulate the K_{Na} component of the sAHP. In contrast, results from this thesis show that mGluR1 negatively regulates the AMPA-induced K_{Na} current via a PKC pathway in lamprey spinal neurons in culture. Furthermore, the AMPA-induced K_{Na} current is positively regulated by mGluR1 when intracellular Ca^{2+} is chelated via a so far unknown pathway. The specificity of the above systems to modulate K_{Ca} or K_{Na} channels makes these channels ideal candidates for controlling the overall excitability of the locomotor network. For example, the blocking effect on the Ca^{2+} entry resulting in a decrease of the K_{Ca}-mediated sAHP by 5-HT, dopamine or GABA will result in a decreased burst rate activity (Wallen et al., 1989; Matsushima et al., 1993; El Manira et al., 1994; Schotland et al., 1995; Hill et al., 2003). At the same time, the K_{Na} component of the sAHP together with the positive modulation of the AMPA-induced K_{Na} current by mGluR1, due to decreased calcium entry, may result in a faster burst activity. Thus, although the modulatory effect on the locomotor network reflects co-expression of channels and receptors, the physiological significance of this co-expression in the locomotor network remains unclear and needs to be determined.

A role of K_{Na} channels in pathophysiology has been reported in mutant *C. elegans* lacking Slack channels. Animals without the Slo2 gene seemed more prone to hypoxia. Acute ischemia results in a failure of the Na^+/K^+-ATPase, which leads to a reversal of the Na^+/Ca^{2+} exchanger and activation of K_{Na} channels. Hyperpolarization

of the membrane induced by the K_{Na} current resets the membrane potential necessary for the Na^+/Ca^{2+} exchanger and this in turn may improve Ca^{2+} transport. Additionally, anti-arrhythmic drugs inhibit cardiac K_{Na} channels (Mori et al., 1996; Mori et al., 1998) suggesting for their conserved role initially reported in cardiac myocytes (Kameyama et al., 1984). In the case of K_{Ca} channels even before their cloning, a clinical study showed that the muscle of patients with myotonic muscular dystrophy; an inherited disease characterized by muscle stiffness and difficulty in relaxation of muscles after voluntary contractions; have apamin-sensitive SK channel receptors (Renaud et al., 1986). In contrast, SK channels are absent in normal human muscles or in the muscles of patients with spinal anterior horn disorders. Additionally, neuroleptics used for the treatment of schizophrenia have been reported to block the sAHP (Dinan et al., 1987). Today, SK channels are suggested to be linked to Parkinson's disease (Wang et al., 2008); cognitive impairment, pain, mood disorders and schizophrenia (Blank et al., 2004). Based on these reports, these channels undoubtedly are targets for useful drugs in medicine.

ACKNOWLEDGEMENTS

It is a pleasure to thank the many people who supported me during this thesis work.

My supervisor Abdel El Manira, thank you for your guidance, advice, encouragement, enthusiasm, patience and lots of good ideas that made science fun all these years. Thank you for being a perfectionist, for your sarcastic humor (preferably when it is directed to others ☺) and always being positive and relaxed. For taking time to listen to my endless questions and challenge my work. I hope you know what a wonderful supervisor you have been to me all these years.

My lab mates, thank you Riyadh, Jessica, Emma, Jens, Alex, Alexandros, Petronella and Sabine for your energy, strength and patience. I know that I have been a demanding one! Thank you for being you, making our lab a stimulating joyful place to do research. Thank you for all the laughter and your friendship, making the long lab hours short. Special thanks to Jens and Jessica for reading my thesis and helping with all the nice tricks that made the writing process faster.

My collaborators, Len Kaczmarek, Arin Bhattacharjee, Gustavo Paratcha and Simon Alford for sharing your expertise and inspiration with me that improved my research. Thank you Mikael Andäng, Patrik Ernfors, Thomas Perlmann, Johan Ericson, Stina Friling, Seth Malmersjo and Per Uhlén for your trust to do electrophysiology with your stem cells. Thank you for giving me the privilege and joy to work with so many different projects.

Thank you Sten Grillner, Ole Kiehn, Jeanette Hellgren Kotaleski, Tatiana Deliagina and Grigori Orlovsky for sharing your knowledge with me and the rest of the group. Special thanks to Sten for organizing all our Friday seminars and lectures making this Department an interesting, rewarding and stimulating place to do my thesis. Thank you Ron Calabrese for the nice discussions we had. For being such an inspiring researcher and teacher. Thank you Russell Hill, Peter Wallén and Brita Robertson for all your support in my research and excellent technical assistance. Thank you Russ for always taking time to read my papers and this thesis.

Thank you my dearest friends Ann-Charlotte, Anastasia, Zoltán and Matthias for your endless support and warmest encouragement, always being so kind to me, trying to take me away from my set up. Thank you for reminding me to breathe and smile, for being so stimulating and by my side.

Thank you my friends Marcus, Henrike and Andreas for bringing fresh air to me. Thank you Lorenzo, Gilli and Kim for making me want to become as competent as you. Thank you Carolina, Chus, Jeppe, Micke, Ole, Fredrik, Line, Patriq, Jesper, Ernesto, Kathy, Adolfo, Ulla, Di, Susanne, André, Richard, Hui-Min, Julia, Christoffer, Sofia, Mat, Ebba, Richardson, Kristoffer, Ariane, Karin, Kai, Martin, Stelio, Lotta, Patrik for all the good times at conferences, seminars, parties and trips. Thank you for all the good memories you created all these years.

Thank you Natasha and Orestis for being such great doctors and taking care of me, for always listening my hypochondriac complaints.

Thank you Gilberto Fisone, Anders, Emanuela, Emmanuel, Christian, Dave, Sebastian, Robert, Anita, Stefan and Staffan Cullheim for being great corridor mates.

Thank you Iris for your endless help with organizing everything, without you we would be lost! Thank you Tommy, Lasse and Anders for your efficient help with technical assistance. Thank you Elzbieta for your positive energy.

Thank you my MBL friends Julia, Ruben and Nik for making the Neurobiology course such a wonderful experience. Without you I would not have survived these exhausting challenging 9 weeks that helped me so much to improve my knowledge and research.

Thank you my family, mommy Amalia and sister Regina for always supporting the decisions I make ♥. Thank you Sonia for your endless calls checking that I am fine here in Stockholm. Thank you Froso, Maraki, Tania, Pano, Regina, Lazaro, Eftihia, Eleni, Athina, Kelly, Ms Labrini, aunts: Popi, Sofia, Magda, uncles: Dimitri, Kosta Ch., Kosta L., Mamsa Gayle for your endless support and love. Katerina, Sofia, Giouli, Alexandra, Elena, Anta, Xari, Pano, Thanasi, Giorgo for being such great friends (and remind me how bad I am ☹). Thank you Xari for help with the cover of the thesis. Thank you Stavro for supporting my decision to move to Stockholm to start my PhD. Thank you Athina (miss you so much since you left Stockholm), Ms Ntina and Mr Giorgo for all the great times I spent in your home. Thank you my aunts Evanthia and Ermioni, and my cousin Vana for challenging me and inspiring me to become who I am. Thank you for teaching me never to give up!

Thank you my sweet Adam for guiding me in the trip of dreams, for always being present even when miles away, for making me feel alive♥.

REFERENCES

Bacskai BJ, Wallen P, Lev-Ram V, Grillner S, Tsien RY (1995) Activity-related calcium dynamics in lamprey motoneurons as revealed by video-rate confocal microscopy. Neuron 14:19-28.

Bader CR, Bernheim L, Bertrand D (1985) Sodium-activated potassium current in cultured avian neurones. Nature 317:540-542.

Behnisch T, Reymann KG (1998) Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro. Neurosci Lett 253:91-94.

Berg AP, Sen N, Bayliss DA (2007) TrpC3/C7 and Slo2.1 are molecular targets for metabotropic glutamate receptor signaling in rat striatal cholinergic interneurons. J Neurosci 27:8845-8856.

Bhattacharjee A, Kaczmarek LK (2005) For K^+ channels, Na^+ is the new Ca^{2+}. Trends Neurosci 28:422-428.

Bhattacharjee A, von Hehn CA, Mei X, Kaczmarek LK (2005) Localization of the Na^+-activated K^+ channel Slick in the rat central nervous system. J Comp Neurol 484:80-92.

Bhattacharjee A, Joiner WJ, Wu M, Yang Y, Sigworth FJ, Kaczmarek LK (2003) Slick (Slo2.1), a rapidly-gating sodium-activated potassium channel inhibited by ATP. J Neurosci 23:11681-11691.

Blank T, Nijholt I, Kye MJ, Spiess J (2004) Small conductance Ca^{2+}-activated K^+ channels as targets of CNS drug development. Curr Drug Targets CNS Neurol Disord 3:161-167.

Blatz AL, Magleby KL (1986) Single apamin-blocked Ca^{2+}-activated K^+ channels of small conductance in cultured rat skeletal muscle. Nature 323:718-720.

Burgess GM, Claret M, Jenkinson DH (1981) Effects of quinine and apamin on the calcium-dependent potassium permeability of mammalian hepatocytes and red cells. J Physiol 317:67-90.

Cangiano L, Wallen P, Grillner S (2002) Role of apamin-sensitive K_{Ca} channels for reticulospinal synaptic transmission to motoneuron and for the afterhyperpolarization. J Neurophysiol 88:289-299.

Chen H, Yan Y, Gazula V, Yang Y, Ferreira G, Sigworth F, Salkoff L, Kaczmarek L (2007) PKC regulation of Slack/Slick heteromeric potassium channels is distinct from that of homomeric channels. In: SFN Neuroscience Meeting 2007.

Chuhma N, Ohmori H (2002) Role of Ca^{2+} in the synchronization of transmitter release at calyceal synapses in the auditory system of rat. J Neurophysiol 87:222-228.

Cingolani LA, Gymnopoulos M, Boccaccio A, Stocker M, Pedarzani P (2002) Developmental regulation of small-conductance Ca^{2+}-activated K^+ channel expression and function in rat Purkinje neurons. J Neurosci 22:4456-4467.

Cochilla AJ, Alford S (1999) NMDA receptor-mediated control of presynaptic calcium and neurotransmitter release. J Neurosci 19:193-205.

Dale N (1993) A large, sustained Na^+- and voltage-dependent K^+ current in spinal neurons of the frog embryo. J Physiol 462:349-372.

Dinan TG, Crunelli V, Kelly JS (1987) Neuroleptics decrease calcium-activated potassium conductance in hippocampal pyramidal cells. Brain Res 407:159-162.

Dryer SE (1991) Na^+-activated K^+ channels and voltage-evoked ionic currents in brain stem and parasympathetic neurones of the chick. J Physiol 435:513-532.

Dryer SE (1994) Na^+-activated K^+ channels: a new family of large-conductance ion channels. Trends Neurosci 17:155-160.

Dryer SE, Fujii JT, Martin AR (1989) A Na^+-activated K^+ current in cultured brain stem neurones from chicks. J Physiol 410:283-296.

Dubuc R, Brocard F, Antri M, Fenelon K, Gariepy JF, Smetana R, Menard A, Le Ray D, Viana Di Prisco G, Pearlstein E, Sirota MG, Derjean D, St-Pierre M, Zielinski B, Auclair F, Veilleux D (2008) Initiation of locomotion in lampreys. Brain Res Rev 57:172-182.

Edgerton JR, Reinhart PH (2003) Distinct contributions of small and large conductance Ca^{2+}-activated K^+ channels to rat Purkinje neuron function. J Physiol 548:53-69.

Egan TM, Dagan D, Kupper J, Levitan IB (1992) Na^+-activated K^+ channels are widely distributed in rat CNS and in Xenopus oocytes. Brain Res 584:319-321.

El Manira A, Tegner J, Grillner S (1994) Calcium-dependent potassium channels play a critical role for burst termination in the locomotor network in lamprey. J Neurophysiol 72:1852-1861.

Faber ES, Delaney AJ, Sah P (2005) SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. Nat Neurosci 8:635-641.

Fakler B, Adelman JP (2008) Control of K_{Ca} channels by calcium nano/microdomains. Neuron 59:873-881.

Feldman JL, Del Negro CA (2006) Looking for inspiration: new perspectives on respiratory rhythm. Nat Rev Neurosci 7:232-242.

Fetcho JR, Higashijima S, McLean DL (2008) Zebrafish and motor control over the last decade. Brain Res Rev 57:86-93.

Franceschetti S, Lavazza T, Curia G, Aracri P, Panzica F, Sancini G, Avanzini G, Magistretti J (2003) Na^+-activated K^+ current contributes to postexcitatory hyperpolarization in neocortical intrinsically bursting neurons. J Neurophysiol 89:2101-2111.

Gardos G (1958) The function of calcium in the potassium permeability of human erythrocytes. Biochim Biophys Acta 30:653-654.

Grillner S (2003) The motor infrastructure: from ion channels to neuronal networks. Nat Rev Neurosci 4:573-586.

Grillner S (2006) Biological pattern generation: the cellular and computational logic of networks in motion. Neuron 52:751-766.

Grissmer S, Lewis RS, Cahalan MD (1992) Ca^{2+}-activated K^+ channels in human leukemic T cells. J Gen Physiol 99:63-84.

Grunnet M, Jespersen T, Angelo K, Frokjaer-Jensen C, Klaerke DA, Olesen SP, Jensen BS (2001) Pharmacological modulation of SK3 channels. Neuropharmacology 40:879-887.

Gu N, Vervaeke K, Hu H, Storm JF (2005) Kv7/KCNQ/M and HCN/h, but not K_{Ca2}/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. J Physiol 566:689-715.

Gu N, Hu H, Vervaeke K, Storm JF (2008) SK K_{Ca2} channels do not control somatic excitability in CA1 pyramidal neurons but can be activated by dendritic excitatory synapses and regulate their impact. J Neurophysiol 100:2589-2604.

Haimann C, Magistretti J, Pozzi B (1992) Sodium-activated potassium current in sensory neurons: a comparison of cell-attached and cell-free single-channel activities. Pflugers Arch 422:287-294.

Hallworth NE, Wilson CJ, Bevan MD (2003) Apamin-sensitive small conductance calcium-activated potassium channels, through their selective coupling to voltage-gated calcium channels, are critical determinants of the precision, pace, and pattern of action potential generation in rat subthalamic nucleus neurons in vitro. J Neurosci 23:7525-7542.

Hartung K (1985) Potentiation of a transient outward current by Na^+ influx in crayfish neurones. Pflugers Arch 404:41-44.

Hess D, El Manira A (2001) Characterization of a high-voltage-activated IA current with a role in spike timing and locomotor pattern generation. Proc Natl Acad Sci U S A 98:5276-5281.

Hess D, Nanou E, El Manira A (2007) Characterization of Na^+-activated K^+ currents in larval lamprey spinal cord neurons. J Neurophysiol 97:3484-3493.

Hill R, Matsushima T, Schotland J, Grillner S (1992) Apamin blocks the slow AHP in lamprey and delays termination of locomotor bursts. Neuroreport 3:943-945.

Hill RH, Arhem P, Grillner S (1985) Ionic mechanisms of 3 types of functionally different neurons in the lamprey spinal cord. Brain Res 358:40-52.

Hill RH, Svensson E, Dewael Y, Grillner S (2003) 5-HT inhibits N-type but not L-type Ca^{2+} channels via 5-HT1A receptors in lamprey spinal neurons. Eur J Neurosci 18:2919-2924.

Hosseini R, Benton DC, Dunn PM, Jenkinson DH, Moss GW (2001) SK3 is an important component of K^+ channels mediating the afterhyperpolarization in cultured rat SCG neurones. J Physiol 535:323-334.
Huss M, Lansner A, Wallen P, El Manira A, Grillner S, Kotaleski JH (2007) Roles of ionic currents in lamprey CpG neurons: a modeling study. J Neurophysiol 97:2696-2711.
Ishii TM, Maylie J, Adelman JP (1997) Determinants of apamin and d-tubocurarine block in SK potassium channels. J Biol Chem 272:23195-23200.
Johnson SW, Seutin V (1997) Bicuculline methiodide potentiates NMDA-dependent burst firing in rat dopamine neurons by blocking apamin-sensitive Ca^{2+}-activated K^+ currents. Neurosci Lett 231:13-16.
Kameyama M, Kakei M, Sato R, Shibasaki T, Matsuda H, Irisawa H (1984) Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. Nature 309:354-356.
Karkanias NB, Papke RL (1999) Subtype-specific effects of lithium on glutamate receptor function. J Neurophysiol 81:1506-1512.
Khawaled R, Bruening-Wright A, Adelman JP, Maylie J (1999) Bicuculline block of small-conductance calcium-activated potassium channels. Pflugers Arch 438:314-321.
Kiehn O (2006) Locomotor circuits in the mammalian spinal cord. Annu Rev Neurosci 29:279-306.
Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J, Adelman JP (1996) Small-conductance, calcium-activated potassium channels from mammalian brain. Science 273:1709-1714.
Kristan WB, Jr., Calabrese RL, Friesen WO (2005) Neuronal control of leech behavior. Prog Neurobiol 76:279-327.
Krnjevic K, Lisiewicz A (1972) Injections of calcium ions into spinal motoneurones. J Physiol 225:363-390.
Kyriakatos A, El Manira A (2007) Long-term plasticity of the spinal locomotor circuitry mediated by endocannabinoid and nitric oxide signaling. J Neurosci 27:12664-12674.
Lamotte d'Incamps B, Hess D, El-Manira A (2004) Control of the temporal fidelity of synaptic transmission by a presynaptic high voltage-activated transient K current. Eur J Neurosci 19:3202-3210.
Liu QY, Schaffner AE, Barker JL (1998) Kainate induces an intracellular Na^+-activated current in cultured embryonic rat hippocampal neurones. J Physiol 510 (Pt 3):721-734.
Liu X, Stan Leung L (2004) Sodium-activated potassium conductance participates in the depolarizing afterpotential following a single action potential in rat hippocampal CA1 pyramidal cells. Brain Res 1023:185-192.
Lund JP, Kolta A (2006) Brainstem circuits that control mastication: do they have anything to say during speech? J Commun Disord 39:381-390.
Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu Rev Physiol 69:291-316.
Matsushima T, Tegner J, Hill RH, Grillner S (1993) $GABA_B$ receptor activation causes a depression of low- and high-voltage-activated Ca^{2+} currents, postinhibitory rebound, and postspike afterhyperpolarization in lamprey neurons. J Neurophysiol 70:2606-2619.
Meech RW, Strumwasser F (1970) Intracellular calcium injection activates potassium conductance in Aplysia nerve cells. In, p 834.
Meech RW, Standen NB (1975) Potassium activation in Helix aspersa neurones under voltage clamp: a component mediated by calcium influx. J Physiol 249:211-239.
Mori K, Saito T, Masuda Y, Nakaya H (1996) Effects of class III antiarrhythmic drugs on the Na^+-activated K^+ channels in guinea-pig ventricular cells. Br J Pharmacol 119:133-141.
Mori K, Kobayashi S, Saito T, Masuda Y, Nakaya H (1998) Inhibitory effects of class I and IV antiarrhythmic drugs on the Na^+-activated K^+ channel current in guinea pig ventricular cells. Naunyn Schmiedebergs Arch Pharmacol 358:641-648.

Nanou E, El Manira A (2007) A postsynaptic negative feedback mediated by coupling between AMPA receptors and Na^+-activated K^+ channels in spinal cord neurones. Eur J Neurosci 25:445-450.
Nanou E, Kyriakatos A, Bhattacharjee A, Kaczmarek LK, Paratcha G, El Manira A (2008) Na^+-mediated coupling between AMPA receptors and KNa channels shapes synaptic transmission. Proc Natl Acad Sci U S A 105:20941-20946.
Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie J, Adelman JP (2005) SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines. Nat Neurosci 8:642-649.
Nusbaum MP, Beenhakker MP (2002) A small-systems approach to motor pattern generation. Nature 417:343-350.
Obermair GJ, Kaufmann WA, Knaus HG, Flucher BE (2003) The small conductance Ca^{2+}-activated K^+ channel SK3 is localized in nerve terminals of excitatory synapses of cultured mouse hippocampal neurons. Eur J Neurosci 17:721-731.
Park YB (1994) Ion selectivity and gating of small conductance Ca^{2+}-activated K^+ channels in cultured rat adrenal chromaffin cells. J Physiol 481 (Pt 3):555-570.
Pedarzani P, Stocker M (2008) Molecular and cellular basis of small--and intermediate-conductance, calcium-activated potassium channel function in the brain. Cell Mol Life Sci 65:3196-3217.
Pedarzani P, Kulik A, Muller M, Ballanyi K, Stocker M (2000) Molecular determinants of Ca^{2+}-dependent K^+ channel function in rat dorsal vagal neurones. J Physiol 527 Pt 2:283-290.
Pedarzani P, Mosbacher J, Rivard A, Cingolani LA, Oliver D, Stocker M, Adelman JP, Fakler B (2001) Control of electrical activity in central neurons by modulating the gating of small conductance Ca^{2+}-activated K^+ channels. J Biol Chem 276:9762-9769.
Pedarzani P, D'Hoedt D, Doorty KB, Wadsworth JD, Joseph JS, Jeyaseelan K, Kini RM, Gadre SV, Sapatnekar SM, Stocker M, Strong PN (2002) Tamapin, a venom peptide from the Indian red scorpion (Mesobuthus tamulus) that targets small conductance Ca^{2+}-activated K^+ channels and afterhyperpolarization currents in central neurons. J Biol Chem 277:46101-46109.
Pennefather PS, Heisler S, MacDonald JF (1988) A potassium conductance contributes to the action of somatostatin-14 to suppress ACTH secretion. Brain Res 444:346-350.
Renaud JF, Desnuelle C, Schmid-Antomarchi H, Hugues M, Serratrice G, Lazdunski M (1986) Expression of apamin receptor in muscles of patients with myotonic muscular dystrophy. Nature 319:678-680.
Roberts A, Li WC, Soffe SR, Wolf E (2008) Origin of excitatory drive to a spinal locomotor network. Brain Res Rev 57:22-28.
Roncarati R, Di Chio M, Sava A, Terstappen GC, Fumagalli G (2001) Presynaptic localization of the small conductance calcium-activated potassium channel SK3 at the neuromuscular junction. Neuroscience 104:253-262.
Rose CR, Konnerth A (2001) NMDA receptor-mediated Na^+ signals in spines and dendrites. J Neurosci 21:4207-4214.
Safronov BV, Bischoff U, Vogel W (1996) Single voltage-gated K^+ channels and their functions in small dorsal root ganglion neurones of rat. J Physiol 493 (Pt 2):393-408.
Sah P, Faber ES (2002) Channels underlying neuronal calcium-activated potassium currents. Prog Neurobiol 66:345-353.
Sailer CA, Kaufmann WA, Marksteiner J, Knaus HG (2004) Comparative immunohistochemical distribution of three small-conductance Ca^{2+}-activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain. Mol Cell Neurosci 26:458-469.
Sailer CA, Hu H, Kaufmann WA, Trieb M, Schwarzer C, Storm JF, Knaus HG (2002) Regional differences in distribution and functional expression of small-conductance Ca^{2+}-activated K^+ channels in rat brain. J Neurosci 22:9698-9707.
Sanchez-Vives MV, Nowak LG, McCormick DA (2000) Cellular mechanisms of long-lasting adaptation in visual cortical neurons in vitro. J Neurosci 20:4286-4299.

Santi CM, Ferreira G, Yang B, Gazula VR, Butler A, Wei A, Kaczmarek LK, Salkoff L (2006) Opposite regulation of Slick and Slack K^+ channels by neuromodulators. J Neurosci 26:5059-5068.

Schotland J, Shupliakov O, Wikstrom M, Brodin L, Srinivasan M, You ZB, Herrera-Marschitz M, Zhang W, Hokfelt T, Grillner S (1995) Control of lamprey locomotor neurons by colocalized monoamine transmitters. Nature 374:266-268.

Schwindt PC, Spain WJ, Crill WE (1989) Long-lasting reduction of excitability by a sodium-dependent potassium current in cat neocortical neurons. J Neurophysiol 61:233-244.

Schwindt PC, Spain WJ, Foehring RC, Stafstrom CE, Chubb MC, Crill WE (1988) Multiple potassium conductances and their functions in neurons from cat sensorimotor cortex in vitro. J Neurophysiol 59:424-449.

Seutin V, Scuvee-Moreau J, Dresse A (1997) Evidence for a non-GABAergic action of quaternary salts of bicuculline on dopaminergic neurones. Neuropharmacology 36:1653-1657.

Shah M, Haylett DG (2000) The pharmacology of hSK 1 Ca^{2+}-activated K^+ channels expressed in mammalian cell lines. British journal of pharmacology 129:627-630.

Stackman RW, Hammond RS, Linardatos E, Gerlach A, Maylie J, Adelman JP, Tzounopoulos T (2002) Small conductance Ca^{2+}-activated K^+ channels modulate synaptic plasticity and memory encoding. J Neurosci 22:10163-10171.

Stocker M (2004) Ca^{2+}-activated K^+ channels: molecular determinants and function of the SK family. Nat Rev Neurosci 5:758-770.

Stocker M, Pedarzani P (2000) Differential distribution of three Ca^{2+}-activated K^+ channel subunits, SK1, SK2, and SK3, in the adult rat central nervous system. Mol Cell Neurosci 15:476-493.

Stocker M, Krause M, Pedarzani P (1999) An apamin-sensitive Ca^{2+}-activated K^+ current in hippocampal pyramidal neurons. Proc Natl Acad Sci U S A 96:4662-4667.

Storm JF (1989) An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells. J Physiol 409:171-190.

Strobaek D, Jorgensen TD, Christophersen P, Ahring PK, Olesen SP (2000) Pharmacological characterization of small-conductance Ca^{2+}-activated K^+ channels stably expressed in HEK 293 cells. Br J Pharmacol 129:991-999.

Tacconi S, Carletti R, Bunnemann B, Plumpton C, Merlo Pich E, Terstappen GC (2001) Distribution of the messenger RNA for the small conductance calcium-activated potassium channel SK3 in the adult rat brain and correlation with immunoreactivity. Neuroscience 102:209-215.

Tegner J, Hellgren-Kotaleski J, Lansner A, Grillner S (1997) Low-voltage-activated calcium channels in the lamprey locomotor network: simulation and experiment. J Neurophysiol 77:1795-1812.

Uchino S, Wada H, Honda S, Hirasawa T, Yanai S, Nakamura Y, Ondo Y, Kohsaka S (2003) Slo2 sodium-activated K+ channels bind to the PDZ domain of PSD-95. Biochem Biophys Res Commun 310:1140-1147.

Vergara C, Latorre R, Marrion NV, Adelman JP (1998) Calcium-activated potassium channels. Curr Opin Neurobiol 8:321-329.

Vogalis F, Storm JF, Lancaster B (2003) SK channels and the varieties of slow after-hyperpolarizations in neurons. Eur J Neurosci 18:3155-3166.

Wallen P, Grillner S (1987) N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. J Neurosci 7:2745-2755.

Wallen P, Buchanan JT, Grillner S, Hill RH, Christenson J, Hokfelt T (1989) Effects of 5-hydroxytryptamine on the afterhyperpolarization, spike frequency regulation, and oscillatory membrane properties in lamprey spinal cord neurons. J Neurophysiol 61:759-768.

Wallen P, Robertson B, Cangiano L, Low P, Bhattacharjee A, Kaczmarek LK, Grillner S (2007) Sodium-dependent potassium channels of a Slack-like subtype

contribute to the slow afterhyperpolarization in lamprey spinal neurons. J Physiol 585:75-90.
Wang G, Zeng J, Ren R, Chen S (2008) Potassium channels in the basal ganglia: promising new targets for the treatment of Parkinson's disease. Front Biosci 13:3825-3838.
Wikstrom MA, El Manira A (1998) Calcium influx through N- and P/Q-type channels activates apamin-sensitive calcium-dependent potassium channels generating the late afterhyperpolarization in lamprey spinal neurons. Eur J Neurosci 10:1528-1532.
Wolfart J, Neuhoff H, Franz O, Roeper J (2001) Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. J Neurosci 21:3443-3456.
Womack MD, Khodakhah K (2003) Somatic and dendritic small-conductance calcium-activated potassium channels regulate the output of cerebellar purkinje neurons. J Neurosci 23:2600-2607.
Xia XM, Fakler B, Rivard A, Wayman G, Johnson-Pais T, Keen JE, Ishii T, Hirschberg B, Bond CT, Lutsenko S, Maylie J, Adelman JP (1998) Mechanism of calcium gating in small-conductance calcium-activated potassium channels. Nature 395:503-507.
Yang B, Desai R, Kaczmarek LK (2007) Slack and Slick K_{Na} channels regulate the accuracy of timing of auditory neurons. J Neurosci 27:2617-2627.
Yuan A, Santi CM, Wei A, Wang ZW, Pollak K, Nonet M, Kaczmarek L, Crowder CM, Salkoff L (2003) The sodium-activated potassium channel is encoded by a member of the Slo gene family. Neuron 37:765-773.