Roles of High-Voltage–Activated Calcium Channel Subtypes in a Vertebrate Spinal Locomotor Network

A. BÜSCHGES, M. A. WIKSTRÖM, S. GRILLNER, AND A. EL MANIRA

Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institutet, S-17177 Stockholm, Sweden

Received 10 June 2000; accepted in final form 17 August 2000

INTRODUCTION

The basic locomotor pattern generated by spinal networks results from specific synaptic interactions between different neuronal types and their intrinsic membrane properties. These are subject to extrinsic and intrinsic modulatory inputs, thus allowing the locomotor network to operate over a wide range of frequencies and patterns (Grillner et al. 1998; Rossignol and Dubuc 1994; Sillar et al. 1997). To gain insight into the function and modulation of networks underlying behavior, it is necessary to characterize individual ion channels in the different neurons and their role in generating the motor pattern. Voltage-gated calcium channels play a major role in controlling neuronal and network activity by contributing to neurotransmitter release and ion channel activation and inactivation (Hille 1992). They are also modulated by various transmitters and modulators (Dolphin 1996; Hille 1994). Different low-voltage–activated (LVA) and high-voltage–activated (HVA) calcium channels have been identified in vertebrate neurons (Fox et al. 1987; Llinás et al. 1992; Mintz et al. 1992; Nowycky et al. 1985; Pearson et al. 1995; Randall and Tsien 1995). HVA calcium channels have been subdivided into L-, N-, and P/Q-type based on their pharmacology and molecular sequence (see Birnbaumer et al. 1994; Snutch and Reiner 1992; Tsien et al. 1991). Most studies have concentrated on investigating the role of the different calcium channels in synaptic transmission and the activation of different cellular processes. In the Xenopus embryo spinal cord, blockade of N-type calcium channels disrupts the locomotor pattern (Wall and Dale 1994). In spinal motoneurons of the turtle, membrane oscillations elicited by N-methyl-D-aspartate (NMDA) and muscarinic receptor activation have been shown to depend on calcium influx through L-type calcium channels (Guerin and Hounsgaard 1998, 1999).

The in vitro lamprey spinal cord preparation offers an advantageous experimental model system in which the contributions of specific ion channels and their modulation to motor pattern generation can be analyzed. The spinal neuronal circuitry generating swimming has been characterized (Buchanan 1982; Buchanan and Grillner 1987) and can be activated by application of NMDA (Grillner et al. 1981). Recently, whole cell patch-clamp recordings from isolated motoneurons, interneurons, and sensory neurons from the lamprey spinal cord have shown that their HVA current is mainly mediated by calcium influx through N-type channels and to a lesser extent through L- and P/Q-type channels (El Manira and Bussières 1997). At the soma, N- and P/Q-, but not L-type, calcium channels are coupled to activation of calcium-dependent potassium (KCa) channels underlying the late afterhyperpolarization (AHP) following the action potential (Wikström and El Manira 1998), which acts as a burst terminating factor during locomotor activity (El Manira et al. 1994). N- and P/Q-type calcium channels also mediate synaptic transmission from descending reticulospinal axons (Krieger et al. 1999). These studies have thus shown that the firing properties of neurons and reticulospinal transmission are primarily controlled by calcium influx through N-type channels. It is not known, however, whether these calcium channels also play a role in controlling synaptic transmission from excitatory and inhibitory network interneurons, or how the changes observed on the...
cellular and synaptic mechanisms after blockade of the different calcium subtypes affect the activity of the spinal locomotor network. In the present study, we have investigated the role of HVA calcium channel subtypes in the spinal network generating the swimming motor pattern. We show that synaptic transmission from excitatory and inhibitory interneurons is mediated primarily by N-type calcium channels, and that blockade of N- and L-type channels affects the locomotor activity.

**Methods**

**Preparation, electrophysiology, data storage, and evaluation**

Experiments were performed on 20- to 40-cm-long adult sea lampreys (*Petromyzon marinus*, *n* = 25) and adult river lampreys (*Lampetra fluviatilis*, *n* = 30) in the anadromous stage. We did not detect any difference between the two species with respect to the effect of calcium channel blockers on the locomotor rhythm, and the results of these experiments were therefore pooled. Animals were anesthetized by immersion in a solution containing tricaine methane sulfonate (MS222, 100 mg/ml). A 6- to 15-segment-long piece of the spinal cord was dissected from the rostral half of the animal together with the notochord and mounted in a silicone elastomer (Sylgard)–lined experimental chamber continuously perfused with a cooled (8–10°C) physiological solution with the following composition (in mM): 138 NaCl, 2.1 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 4 glucose, 0.5 glutamine, and 2 HEPES. In some experiments the spinal cord was isolated from the notochord. The meninx primativa was removed from the cord using very fine forceps to allow for intracellular recordings.

Fictive locomotion was induced by adding NMDA (100–150 μM) to the physiological saline, and ventral root activity was recorded with suction electrodes. After reaching a steady-state frequency, the locomotor rhythm induced by NMDA remained stable for more than 24 h (see Parker et al. 1998). The effect of calcium channel agonists and antagonists on the frequency and stability of the locomotor activity was studied by bath applying the different drugs on the entire spinal cord (Figs. 3–8). In all the experiments of this study, the different calcium channel blockers were applied at least 30 min after locomotor activity had reached a steady-state frequency.

The effect of calcium channel blockers on the amplitude of locomotor drive oscillations was analyzed using a split-bath configuration (Dale 1986; Matsushima and Grillner 1992). In these experiments, the recording chamber was partitioned into two pools by a petroleum jelly (Vaseline) barrier. Fictive swimming was induced in the rostral pool while the contralateral ventral root was silent. The cycle period of the locomotor pattern was measured between the onset of subsequent ventral root bursts. The instantaneous frequency was calculated as the inverse of the cycle period. The coefficient of variation was defined as the standard deviation of cycle period/mean cycle period. The burst proportion was defined as the ratio of burst duration and cycle duration. “N” values in the text represent the number of experiments or neurons; “n” values represent the sample size averaged for a given experiment. Mean values of locomotor activity (cycle period, frequency, burst duration) were calculated from sequences of 120 s and averaged over 180–350 cycles. The results are expressed as means ± SD. Means were compared using the paired Student’s *t*-test from standard statistical software programs (Plotit, Graphpad). Mean values were considered to be significantly different with *P* < 0.05.

**Pharmacology**

To determine whether individual HVA calcium channel subtypes play a role in rhythm generation of the spinal locomotor network, we bath applied the following HVA calcium channel antagonists: 1–3 μM ω-conotoxin-GVIA (ω-CgTx; Peptide Institute), 0.2 μM ω-agatoxin-IVA (ω-Aga; Peptide Institute), and 10 μM nimodipine (RBI). The effect of the L-type channel agonist BayK8644 (2–4 μM, BayK, RBI) was also tested on the frequency of the locomotor rhythm. Stock solutions of nimodipine (10 mM) and BayK (10 mM) were prepared in ethanol. Control experiments revealed that the ethanol amount used to dilute nimodipine and BayK did not affect cellular, synaptic properties or electrical coupling between neurons (Krieger et al. 1999; Wikström and El Manira 1998; Wikström et al. 1998). Stock solutions of ω-CgTx (100 μM) and ω-Aga (100 μM) were prepared in distilled water and stored at −20°C. To prevent unspecific binding of the toxins to the tubes of the perfusion system, 0.5 mg/ml bovine-albumin (Sigma) was added to the physiological solution.

**Results**

*N*-type HVA Ca²⁺ channels mediate excitatory and inhibitory synaptic transmission in the spinal cord

To test whether calcium influx through N-type channels is involved in mediating synaptic transmission from propriospinal interneurons, we performed split-bath experiments (see Methods). Fictive locomotion was initiated in the rostral pool by NMDA, while locomotor-driven excitatory and inhibitory synaptic inputs were recorded in neurons in the caudal pool in

**Downloaded from** http://jn.physiology.org/ by 10.220.33.1 on November 29, 2017.
The participation of N-type calcium channels to inhibitory synaptic transmission was also examined. Crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) were stimulated extracellularly (see METHODS) (Alford and Grillner 1991), while IPSPs were recorded caudally in the contralateral neurons in the presence of AP5 (100 μM) and CNQX (10 μM). Within 60 min after the start of the perfusion with ω-CgTx (1 μM), the amplitude of the IPSPs decreased by 63.0 ± 7.0% (N = 5; 3 MNs and 2 unidentified; P < 0.001; Fig. 2, A and B). These results show that synaptic transmission from inhibitory propriospinal interneurons is dependent to a large extent on calcium influx through N-type channels.

**L- and P/Q-type channels do not participate in mediating synaptic transmission from propriospinal neurons**

To test whether calcium influx through L- and P/Q-type channels mediates excitatory synaptic transmission from propriospinal interneurons, locomotor-driven excitatory synaptic inputs were recorded in neurons in the caudal pool in the presence of strychnine. Blockade of L- or P/Q-type channels by nimodipine (10 μM; Fig. 3A; N = 3; 1 MN, 2 gray matter neurons) or ω-Aga (0.2 μM; Fig. 3B; N = 3; 2 MNs, 1 gray matter neuron) did not affect the amplitude of locomotor-driven excitation. The effect of blockade of these channels was also tested on inhibitory synaptic transmission. IPSPs were elicited in neurons by extracellular stimulation of crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) in the presence of AP5 (100 μM) and CNQX (10 μM). Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs, which were blocked by cadmium (200 μM; N = 5; 3 MNs and 2 gray matter neurons; Fig. 3C). These results indicate that L- and P/Q-type calcium channels do not mediate the excitation of motoneurons in the presence of strychnine. Blockade of L- or P/Q-type channels by nimodipine (10 μM; Fig. 3A; N = 3; 1 MN, 2 gray matter neurons) or ω-Aga (0.2 μM; Fig. 3B; N = 3; 2 MNs, 1 gray matter neuron) did not affect the amplitude of locomotor-driven excitation. The effect of blockade of these channels was also tested on inhibitory synaptic transmission. IPSPs were elicited in neurons by extracellular stimulation of crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) in the presence of AP5 (100 μM) and CNQX (10 μM). Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs, which were blocked by cadmium (200 μM; N = 5; 3 MNs and 2 gray matter neurons; Fig. 3C). These results indicate that L- and P/Q-type calcium channels do not mediate the excitation of motoneurons in the presence of strychnine. Blockade of L- or P/Q-type channels by nimodipine (10 μM; Fig. 3A; N = 3; 1 MN, 2 gray matter neurons) or ω-Aga (0.2 μM; Fig. 3B; N = 3; 2 MNs, 1 gray matter neuron) did not affect the amplitude of locomotor-driven excitation. The effect of blockade of these channels was also tested on inhibitory synaptic transmission. IPSPs were elicited in neurons by extracellular stimulation of crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) in the presence of AP5 (100 μM) and CNQX (10 μM). Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs, which were blocked by cadmium (200 μM; N = 5; 3 MNs and 2 gray matter neurons; Fig. 3C). These results indicate that L- and P/Q-type calcium channels do not mediate the excitation of motoneurons in the presence of strychnine. Blockade of L- or P/Q-type channels by nimodipine (10 μM; Fig. 3A; N = 3; 1 MN, 2 gray matter neurons) or ω-Aga (0.2 μM; Fig. 3B; N = 3; 2 MNs, 1 gray matter neuron) did not affect the amplitude of locomotor-driven excitation. The effect of blockade of these channels was also tested on inhibitory synaptic transmission. IPSPs were elicited in neurons by extracellular stimulation of crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) in the presence of AP5 (100 μM) and CNQX (10 μM). Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs, which were blocked by cadmium (200 μM; N = 5; 3 MNs and 2 gray matter neurons; Fig. 3C). These results indicate that L- and P/Q-type calcium channels do not mediate the excitation of motoneurons in the presence of strychnine. Blockade of L- or P/Q-type channels by nimodipine (10 μM; Fig. 3A; N = 3; 1 MN, 2 gray matter neurons) or ω-Aga (0.2 μM; Fig. 3B; N = 3; 2 MNs, 1 gray matter neuron) did not affect the amplitude of locomotor-driven excitation. The effect of blockade of these channels was also tested on inhibitory synaptic transmission. IPSPs were elicited in neurons by extracellular stimulation of crossed caudally projecting inhibitory interneurons (Buchanan 1982; Ohta et al. 1991) in the presence of AP5 (100 μM) and CNQX (10 μM). Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs, which were blocked by cadmium (200 μM; N = 5; 3 MNs and 2 gray matter neurons; Fig. 3C). These results indicate that L- and P/Q-type calcium channels do not mediate

**FIG. 1.** Effects of blocking N-type calcium channels on synaptic drive within the locomotor network during fictive swimming. A: experiments were performed using split-bath configuration, in which N-methyl-d-aspartate (NMDA) induced fictive locomotion was recorded in a rostral ventral root while locomotor-driven synaptic inputs were recorded in a caudal contralateral motoneuron. Blockade of N-type calcium channels by ω-CgTx (35 min) markedly reduced the amplitude of the excitatory drive. B1: membrane potential excitatory inputs in the presence of strychnine during one cycle with individual excitatory postsynaptic potentials (EPSPs). B2: histogram showing the distribution of the amplitude of individual compound EPSPs in control, C1: application ω-CgTx decreased the amplitude of the locomotor-driven excitation. C2: the amplitude of individual EPSPs was also reduced as shown by the shift of the histogram toward lower values. Insets in B1 and C1 show expanded traces from the regions indicated by the black bars, and the arrowheads indicate individual EPSPs. The dots and bars in B2 and C2 represent mean ± SD.

**FIG. 2.** Block of calcium influx through N-type calcium channels reduces crossed inhibitory transmission in the spinal locomotor network. Compound inhibitory postsynaptic potentials (IPSPs) were elicited in contralateral gray matter neurons by extracellular stimulating of crossed-caudal projecting interneurons on the contralateral side of the spinal cord in the presence of ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-amino-5-phosphonopentanoic acid (AP5). A: application of 1 μM ω-CgTx decreased the amplitude of the monosynaptic IPSP. B: average traces from 30 consecutive sweeps in control and in 1 μM ω-CgTx.
FIG. 3. L- and P/Q-type channels are not involved in mediating synaptic transmission from propriospinal interneurons. 

A: locomotor drive excitatory input was recorded in a caudal neurons using split-bath configuration. Blockade of L-type channels in the caudal pool by nimodipine had no effect on the amplitude of the excitatory input.

B: the effect of blocking P/Q-type channels on excitatory synaptic drive was tested using split-bath experiments. Application of ω-Aga in the caudal pool did not affect the amplitude of the synaptic drive.

C: IPSPs were elicited by stimulation of crossed caudally projecting interneurons, and their amplitude was monitored over time. Neither nimodipine nor ω-Aga affected the amplitude of the IPSPs.
synaptic transmission from excitatory and inhibitory propriospinal interneurons.

**Blockade of N-type channels affects membrane potential oscillations and locomotor rhythm frequency**

The influence of ω-CgTx on membrane potential oscillations during fictive locomotion was analyzed in motoneurons (N = 4) and unidentified gray matter neurons (N = 2). Figure 4A shows a recording from a motoneuron that displayed membrane oscillations and fired action potentials in phase with the ipsilateral ventral root burst. A hyperpolarization of the motoneuron to −85 mV abolished the action potentials while a phasic synaptic drive was maintained (Fig. 4A). Application of ω-CgTx induced a progressive deterioration of the locomotor activity (Fig. 4, B and C), although the motoneuron was still able to produce membrane potential oscillations and fire action potentials. When the membrane potential of the motoneuron was hyperpolarized, the rhythmic oscillations were abolished and no synaptic input was observed (Fig. 4, B and C). These results indicate also that membrane potential oscillations persist in the absence of synaptic drive. In some cases, a blockade of N-type calcium channels affected both the amplitude of the oscillations and the locomotor rhythm. Figure 4D shows recordings from a gray matter neuron, which exhibited membrane potential oscillations and fired action potentials during the ipsilateral ventral root burst. Application of ω-CgTx dramatically reduced the amplitude of the oscillations and disrupted the locomotor rhythm (Fig. 4E). These results establish that N-type calcium channels play an important role in mediating synaptic transmission in the spinal cord. The fact that the oscillations in some neurons disappear on hyperpolarization suggests that they may correspond to NMDA-induced oscillations (Sigvardt et al. 1985; Wallén and Grillner 1987), which are not abolished by ω-conotoxin GVIA (see Discussion).

In all experiments (N = 16) bath application of the antagonist ω-CgTx (1–2 μM) reliably disrupted the pattern of the locomotor rhythm. The effect was gradual and preceded by a decrease in the burst frequency (Fig. 5, A and B). Application of ω-CgTx initially decreased the frequency of the locomotor rhythm and always affected the stability of the motor pattern which was subsequently disrupted (Figs. 5, A–D, and 6). The onset of the disruption of the locomotor rhythm was variable

**FIG. 4.** ω-CgTx depresses synaptic drive to spinal neurons and affects the stability of the locomotor rhythm. A: intracellular recording from a motoneuron, which exhibited membrane potential oscillations and fired action potentials. Hyperpolarization of the motoneuron abolished the action potentials and revealed phasic synaptic drive. B: application of ω-CgTx (35 min) disrupted the locomotor activity and reduced the amplitude of the synaptic drive when the motoneuron was hyperpolarized. C: prolonged application of the toxin (60 min) abolished the activity in the ventral root and depressed synaptic potential seen in the motoneuron at a hyperpolarized membrane potential. D: intracellular recording from a gray matter neuron showing phasic membrane potential oscillations in phase with the ipsilateral ventral root bursts. E: application of 2 μM ω-CgTx (25 min) reduced the frequency of the locomotor rhythm and abolished the membrane potential oscillations in the recorded neuron.

**FIG. 5.** Effects of blocking N-type calcium channels on fictive swimming. A: sample recordings from a segmental ventral root activity in an individual preparation under control conditions, 30 and 60 min after start of perfusion with 2 μM ω-CgTx. B and C: plot of the frequency of fictive swimming and burst duration versus time (mean ± SD). D: plot of the change in locomotor frequency (given as % of control) 45 min after the start of perfusion of ω-CgTx in 14 different experiments (***P < 0.001).
between preparations and ranged between 15 min and >45 min. In four experiments displaying a similar time course, \( \omega \)-CgTx significantly \( (P < 0.001) \) decreased the burst frequency (Fig. 5B), while the burst duration (Fig. 5C) and the coefficient of variation (Fig. 5D) were significantly \( (P < 0.001) \) increased. The change in the locomotor burst frequency was evaluated in experiments \( (N = 14) \) in which the rhythm was not disrupted after 45 min of perfusion of \( \omega \)-CgTx. The burst frequency significantly decreased to 79.9 ± 11.2% of control \( (N = 14; P < 0.001; \text{Fig. 5E}) \).

The distribution histograms (Fig. 6) show that the cycle duration increased in the presence of \( \omega \)-CgTx (compare control, 20, 30, and 45 min; \( n = 172 \) in each histogram) and became more variable with increasing deterioration of the swimming rhythm (Fig. 6A). After prolonged application of \( \omega \)-CgTx (50–60 min) the locomotor pattern was disrupted as the left and right ventral roots did not exhibit any alternation and the bursts could no longer be defined (Fig. 6B). The effect of \( \omega \)-CgTx developed and became established over time. In some experiments the first changes were detectable 10 min after the start of the perfusion, whereas in other experiments changes occurred later, e.g., more than 20 min following the start of application of \( \omega \)-CgTx (see Fig. 5D). Wash out was tried several times and was successful only in two cases (2 h wash out), when it was started before the rhythmic activity was completely disrupted (not shown).

**Effects of the L-type HVA-Ca\(^{2+}\) channel antagonist nimodipine and the L-type HVA-Ca\(^{2+}\) channel agonist BayK on NMDA-induced fictive swimming**

Bath application of the L-type antagonist nimodipine at a concentration of 10 \( \mu \)M induced a modest but significant decrease in the frequency of the swimming rhythm to 92.8 ± 8.2% of control \( (N = 11; P < 0.02, \text{Fig. 7B}) \). The reduction in burst frequency occurred within 30–45 min of application of nimodipine and ranged from 3 to 15% (mean, 13.0 ± 5.0%; \( N = 8; P < 0.001; \text{Fig. 7} \)). This was obvious from plotting the change in the average normalized burst frequency (Fig. 7B). After 15–30 min of wash out, the frequency of swimming returned to control values (Fig. 7, A and B). The decrease in the swimming frequency occurred within 30–45 min of application of nimodipine and ranged from 3 to 15% (mean, 13.0 ± 5.0%; \( N = 8; P < 0.001; \text{Fig. 7} \)). This was obvious from plotting the change in the average normalized burst frequency (Fig. 7B). After 15–30 min of wash out, the frequency of swimming returned to control values (Fig. 7, A and B). The decrease in the swimming

**FIG. 6.** Time course of blocking N-type calcium channels on cycle period of “fictive swimming.” A: distribution histograms of the cycle period for 1 experiment in control, 20, 30, and 45 min following start with perfusion with \( \omega \)-CgTx. Sample sizes are \( n = 172 \) for all 4 situations. B: locomotor activity in control, on the right expanded traces from the region marked with a black bar. B2: application of 2 \( \mu \)M \( \omega \)-CgTx decreased the frequency of the locomotor rhythm and disrupted the pattern. On the right expanded traces corresponding to the part marked by the bar.

**FIG. 7.** Effects of blocking L-type calcium channels on fictive swimming. A: sample recordings of segmental ventral root activity under control conditions, 30 min after the start of perfusion with 10 \( \mu \)M nimodipine, and 15 min after the start of wash out. B: change in frequency of fictive swimming in 6 experiments (given as % of control at control, 45 min after starting the perfusion and 30 min after the start of wash out; * \( P < 0.05; ** P < 0.01 \)). C: plot of the change in average burst duration from the same experiments as in B. D: plot of the coefficient of variation.
frequency was accompanied by a significant increase in the average ventral root burst duration by 4–26% for the individual recordings after 45 min of application of nimodipine. The mean burst duration increased to $111.2 \pm 9.5\%$; $N = 10$; $P < 0.01$; Fig. 7C). No significant change was seen in the burst proportion, in the activity phases (left-right alternation) of the segmental roots in the cycle, or in the coefficient of variation (Fig. 7D), which represents a measure of the quality of swimming.

The effect of the L-type agonist Bay K (2–4 $\mu$M) was also tested on the motor pattern during NMDA-induced swimming (Fig. 8). While there was no systematic change on the swimming locomotor pattern in the first 45 min after starting perfusion (Fig. 8, A–C), a significant increase in the frequency was seen after wash out of the agonist in five of six preparations (Fig. 8, A and B). In one experiment, there was no significant change in the frequency. For a given experiment, the increase in the locomotor frequency ranged from 2 to 19% (mean, 109.0 $\pm$ 7.2% of control; $N = 6$; $P < 0.05$). The increase in burst frequency was accompanied by a significant decrease in the burst duration (Fig. 8C). No significant change was seen on the coefficient of variation (Fig. 8D).

**Effects of blocking P/Q-type HVA Ca$^{2+}$ channels on NMDA-induced fictive swimming**

The role of P/Q-type HVA Ca$^{2+}$ channels in generation of the swimming motor pattern was investigated using the specific antagonist $\omega$-agatoxin (0.2 $\mu$M; $N = 7$). Blockade of P/Q-type Ca$^{2+}$ channels had only minor effects on the frequency of the locomotor rhythm (Fig. 9, A and B). In four experiments we observed a small but significant decrease in frequency, while there was a slight, but significant increase in one and no change in the other two experiments (Fig. 9B). Overall there was no significance change in the frequency of swimming in the presence of $\omega$-Agatox (97.0 $\pm$ 6.5% compared with control, $P > 0.2$, $N = 7$; Fig. 9B). No systematic changes were detected for burst duration (not shown). The regularity of the activity was not affected by blocking P/Q-type channels as shown by the lack of a significant effect on the coefficient of variation ($N = 5$; Fig. 9C).

**DISCUSSION**

The present investigation set out to elucidate the role that individual HVA calcium channel subtypes play in the neuronal network generating locomotor activity in the lamprey by using selective agonists and antagonists. The main findings are the following: 1) synaptic transmission from excitatory and inhibitory neurons of the spinal locomotor network is mediated by calcium influx through N-type calcium channels; and 2) the overall functioning of the lamprey spinal locomotor network relies on calcium influx through N- and L-type calcium channels. In contrast, excitatory and inhibitory synaptic transmission in the spinal cord are not affected by blockade of L- and P/Q-type calcium channels. In addition, the overall network activity shows a substantial robustness against the relatively small changes in the firing properties (Wikström and El Manira 1998; Wikström et al. 1998) of spinal neurons induced by blockade of P/Q-type calcium channels.

**N-type calcium channels play an important role in the generation of the locomotor rhythm**

Our results show that synaptic transmission from excitatory and inhibitory propriospinal neurons is mediated primarily by...
calcium influx through N-type calcium channels. Similar results have been shown with regard to synaptic transmission from reticulospinal axons (Krieger et al. 1999). Blockade of N-type calcium channels reduced the amplitude of the segmental rhythmic excitatory synaptic input to motoneurons that has been suggested to correspond to activity of excitatory interneurons (Buchanan et al. 1989). Crossed inhibitory synaptic input, presumably arising from small and medium size caudally projecting (CC) interneurons, was also decreased by blockade of N-type calcium channels. These channels thus mediate synaptic transmission both from excitatory and inhibitory propriospinal neurons. The excitatory propriospinal neurons could correspond to excitatory network interneurons (EINs) (Buchanan and Grillner 1987). Dale (1986) showed that locomotor drive oscillations are arising from excitatory propriospinal interneurons with a short rostral projection (1–3 segments) and a long caudal projection (1–9 segments). The subsequently identified excitatory network interneurons (Buchanan and Grillner 1987; Buchanan et al. 1989) have been shown to project caudally up to five segments and have suggested to give rise to the locomotor drive. Although our data suggest that synaptic transmission from network interneurons relies on calcium entry through N-type channels, a direct demonstration of this requires paired intracellular recordings from identified interneurons and their targets. During fictive locomotion, a blockade of these channels induced an increase in the coefficient of variation and disrupted the motor pattern. The initial decrease of the locomotor frequency observed could therefore be due to a reduced synaptic drive resulting from the depression of synaptic transmission.

One major factor controlling the burst termination in the lamprey spinal cord is the late AHP following action potentials that is mediated through activation of apamin-sensitive $K_{Ca}$ channels (El Manira et al. 1994; Grillner et al. 1998). The $K_{Ca}$ channels underlying the late AHP are primarily activated by calcium influx through N-type channels (Wikström and El Manira 1998). A blockade of these channels decreases both the amplitude of the late AHP and spike frequency adaptation, leading to a prolonged locomotor burst and thereby a decrease in the frequency of the locomotor rhythm. It is important to note that specific blockade of $K_{Ca}$ by apamin also reduces the locomotor frequency and increases the coefficient of variation of the motor pattern (El Manira et al. 1994). There is a strong resemblance of the initial effects of inhibition of N-type calcium channels and those of inhibition of $K_{Ca}$ channels on the motor pattern. This suggests that the decrease of frequency and the increase in coefficient of variation induced by $\omega$-CgTx result, at least partly, from the decrease of the late AHP. The disruption of the rhythm and especially the loss of alternation between left and right ventral root bursts is presumably due to the depression of reciprocal inhibitory synaptic transmission. Our results thus show that N-type calcium channels are essential for the function of the spinal network generating locomotion. In the *Xenopus* embryo spinal cord, inhibition of N-type channels also disrupts the locomotor pattern by depressing synaptic transmission and increasing the threshold for firing action potentials (Wall and Dale 1994). Although the effect on synaptic and locomotor activity are similar in the lamprey and *Xenopus*, a blockade of N-type channels in the lamprey spinal cord neurons does not increase the firing threshold but rather increases the firing frequency of spinal network neurons (Wikström and El Manira 1998).

Effects of blocking P/Q- and L-calcium channel subtypes on rhythm generation in the spinal locomotor network

P/Q-type calcium channels contribute to the late AHP by about 20% (Wikström and El Manira 1998) but do not mediate excitatory and inhibitory transmission from propriospinal neurons. A blockade of P/Q-type channels, however, reduces synaptic transmission from reticulospinal by about 20–30% (Krieger et al. 1999). P/Q-type channels do not seem to be activated during NMDA-induced membrane potential oscillations because $\omega$-Aga has no effect on these oscillations (Wikström et al. 1998). Blocking P/Q-type channels during fictive locomotion did not result in a consistent significant change in the swimming motor output. For individual preparations subtle or no changes were observed. It is possible that the degree to which cellular properties are affected by P/Q-type calcium channels is below the level that is needed to affect the network performance. This could be due to the fact that during each locomotor cycle, neurons fire few spikes and that a small decrease in the late AHP is not large enough to change the spike frequency adaptation and thereby the burst termination during fictive locomotion. Finally, it is conceivable that the effects of blocking P/Q-type channels only becomes apparent when neurons fire several action potentials, which can occur during low swimming frequencies.

Blocking L-type channels in NMDA-induced fictive locomotion induced a significant decrease in burst frequency associated with a concurrent increase in burst duration in the ventral roots. A potentiation of L-type calcium channels by BayK resulted in a slight increase in swimming frequency. Interestingly, after wash out of BayK the burst frequency increased further. This could be due to an indirect effect of increasing calcium influx through L-type channels. The effects of inhibition or potentiation of L-type calcium channels on the locomotor rhythm does not result from a decrease of synaptic transmission or spike frequency adaptation because calcium influx through these channels are involved in neither the activation of $K_{Ca}$ channels (Wikström and El Manira 1998) nor in mediating synaptic transmission from propriospinal neurons and reticulospinal axons in the lamprey spinal cord (Krieger et al. 1999). These channels are, on the other hand, involved in NMDA-induced membrane potential oscillations (Wikström et al. 1998). Inhibition of L-type calcium channels increases the duration of the plateau of NMDA-induced oscillations, and in some cases a suppression of the oscillations was observed. Agonists of these channels had the converse effect with a decrease in the plateau duration. The effects of L-type channel inhibition and potentiation on the frequency of the locomotor rhythm could therefore be mediated via modulation of NMDA-induced oscillations. Other mechanisms may also contribute to the observed changes in the locomotor rhythm frequency induced by nimodipine. An involvement of L-type channels in plateau potentials has also been shown in turtle spinal cord motoneurons (Guertin and Hounsgaard 1998, 1999). Membrane oscillations induced by both muscarinic and NMDA receptors were abolished when calcium influx through L-type channels was blocked (Guertin and Hounsgaard 1998, 1999).

In conclusion, we have addressed the importance of the
different calcium channel subtypes in the generation of locomotor pattern in the lamprey spinal cord. L-type channels that are mainly involved in NMDA-induced membrane potential oscillations are involved in controlling the frequency but not the pattern of the locomotor rhythm. N-type channels, which represent the major component of the total somatic calcium current (El Manira and Bussières 1997), control both the firing properties of neurons and excitatory and inhibitory synaptic transmission, and are thus important in the operation of the locomotor network. These channels may thus be the target of several modulatory systems acting both presynaptically to affect synaptic transmission and postsynaptically to modify the firing properties of neurons, and thus modulate the frequency and stability of the locomotor rhythm.

We thank D. Parker and P. Wallén for comments on the manuscript. We are also grateful to H. Axelglen and M. Bredmary for excellent technical assistance.

This work was supported by Swedish Medical Research Council Grants 11562 to A. El Manira and 3026 to S. Grillner and a Heisenberg Fellowship of the German Science Foundation to A. Büschges (Bu857/4-1).

Present address of A. Büschges: Zoologisches Institut der Universität Köln, Weyertal 119, 50931 Köln, Germany.

REFERENCES


Dolphin AC. Facilitation of Ca<sup>2+</sup> current in excitable cells. Trends Neurosci 19: 35–43, 1996.


