Electrophysiological effects of 4-aminopyridine on fictive locomotor activity of the rat spinal cord in vitro

G. Taccola and A. Nistri

Neurobiology Sector and INFM Unit, International School for Advanced Studies (SISSA), Trieste, Italy

Summary

Recently the K\(^+\) channel blocker 4-aminopyridine (4-AP) has been suggested to be useful to improve motor deficits due to spinal cord lesions. There is, however, little basic research support for this action of 4-AP. In this study we have used as a model the neonatal mammalian spinal cord in vitro that generates a rhythmic activity termed fictive locomotion (induced by bath-application of NMDA + 5-HT) with phasic electrical discharges alternating between flexor and extensor motor pools and between left and right motoneurons within the same segment. When 4-AP was added in the presence of sub-threshold concentrations of NMDA + 5-HT, there was facilitation of fictive locomotion which appeared with alternating patterns on all recorded ventral roots (VR). Furthermore, in the presence of 4-AP, weak dorsal root (DR) stimuli, previously insufficient to activate locomotor patterns, generated alternating discharges from various VRs. The present data show that 4-AP could strongly facilitate the locomotor program initiated by neurochemicals or electrical stimuli, indicating that the spinal locomotor network is a very sensitive target for the action of 4-AP.

Keywords: Central pattern generator; spinal cord lesion; rhythmic patterns; oscillations.

Introduction

Fampridine-SR, a new sustained release oral tablet form of 4-AP is currently under phase III clinic trial for its therapeutic efficacy in patients with Multiple Sclerosis (MS) and chronic spinal cord injury [2]. The rationale for this approach stems from the fact that low concentrations of 4-AP are considered to block transient, voltage activated, outward K\(^+\) currents. The most striking effect seen with K\(^+\) channel blockers is an enhancement of transmitter release at many central and peripheral synapses as a consequence of increased Ca\(^{++}\) influx into presynaptic terminals.

4-AP sensitive K\(^+\) channels are also present in the internodal area of the axon membrane shielded under the myelin sheath. Traumatic injury causes apoptosis of oligodendrocytes with disruption of the myelin wrapping which then unmasks 4-AP sensitive K\(^+\) channels located in juxtaparanodal and internodal regions. The activity of such previously-hidden K\(^+\) channels results in axonal conduction failure at central and peripheral level [5]. Hence, block of voltage-dependent fast K\(^+\) channels by 4-AP has two important effects that are thought to ameliorate the central conduction deficit experienced by patients following MS or traumatic cord injury: it prolongs the duration of the action current in focally demyelinated internodes and it enhances central and peripheral synaptic transmission.

We have considered the possibility that 4-AP might improve spinal cord function by modulating and/or reactivating the operation of the specialized spinal network devoted to generate rhythmic motor patterns responsible for locomotion. Such network is named Central Pattern Generator (CPG). The spinal CPG can generate in vivo, even in the absence of external stimuli, phasic electrical discharges alternating between flexor and extensor motor pools and between left and right motoneurons within the same segment.

A very similar pattern can be produced also by superfusing the isolated mammalian spinal cord with excitatory agents like NMDA and serotonin (5HT; see [1, 3]) or by repeated stimuli applied to one DR [4]. Since the main rhythmic burst in L2 is flexor-related and the main burst in L5 is extensor-related, it is common to call the rhythmic activity locomotor-like when the L2 and L5 bursts alternate on one side of the cord and when there is segmental left-right alternation.
Because of its well defined inputs via DR fibres and motor output via VR axons, and because of its long-term stability, the isolated spinal cord of the rat represents a very advantageous in vitro model to evaluate the pharmacological action of drugs, like 4-AP, proposed for the symptomatic treatment of spinal cord injured subjects. The present study sought to clarify if 4-AP could act on the spinal CPG.

Fig. 1. 4-AP enhances alternating motor patterns during DR stimulation. Sample records are from three VRs (left, l, and right, r) whose segmental identification is abbreviated alongside the traces. All data are from the same preparation. (A) During a train of 40 strong pulses to a single DR, there is a slowly developing VR depolarization with superimposed alternating patterns. (B) When the test is repeated in the presence of 4-AP the number of alternating patterns is clearly increased. (C) The same preparation is stimulated with a train of weak DR pulses unable to induce cumulative depolarization or oscillations. (D) When the test is carried out in the presence of 4-AP, there is appearance of cumulative depolarization and alternating oscillations.