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Amitriptyline and carbamazepine utilize voltage-gated ion channel suppression to impair excitability of sensory dorsal horn neurons in thin tissue slice: An in vitro study

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ABSTRACT

Amitriptyline, carbamazepine and gabapentin are often used for the treatment of neuropathic pain. However, their analgesic action on central sensory neurons is still not fully understood. Moreover, the expression pattern of their target ion channels is poorly elucidated in the dorsal horn of the spinal cord. Thus, we performed patch-clamp investigations in visualized neurons of lamina I–III of the spinal cord. The expression of the different voltage-gated ion channels, as the targets of these drugs, was detected by RT-PCR and immunohistochemistry. Neurons of the lamina I–III express the TTX-sensitive voltage-gated Na^+ as well as voltage-gated K^+ subunits assembling the fast inactivating (A-type) currents and the delayed rectifier K^+ currents. Our pharmacological studies show that tonically-firing, adapting-firing and single spike neurons responded dose-dependently to amitriptyline and carbamazepine. The ion channel inhibition consecutively reduced the firing rate of tonically-firing and adapting-firing neurons. This study provides evidence for the distribution of voltage-gated Na^+ and K^+ subunits in lamina I–III of the spinal cord and for the action of drugs used for the treatment of neuropathic pain. Our work confirms that modulation of voltage-gated ion channels in the central nervous system contributes to the antinociceptive effects of these drugs.

1. Introduction

Spinal dorsal horn neurons are one of the key players in central sensory transmission and modulation and thus in acute pain perception as well as in the pathomechanism of chronic neuropathic pain syndromes (LaMotte, 1977; Light and Perl, 1979; Rethelyi, 1977). Furthermore, among the neurons in laminae I–III, tonically-firing neurons were reported to be nociceptive-specific neurons (Lopez-Garcia and King, 1994).

We described the pharmacological modulation of the voltage-gated ion channels of the sensory dorsal horn neurons in lamina I–III by local anaesthetics using the thin slice of the spinal cord combined with the patch-clamp method (Olschewski et al., 1998). During the last decades, the prime importance of voltage-gated ion channels in pain processing in these neurons has been demonstrated in extensive electrophysiological nociceptive studies. For action potential generation, the initial driving force is generated by sodium (Na^+) channels (Safronov et al., 1997). In lumbar dorsal horn neurons, voltage-gated potassium (K^+) channels additionally modulate the firing pattern, indicating that both Na^+ and K^+ channels are essential components governing neuronal excitability (Safronov, 1999; Hess and El Manira, 2001; Olschewski et al., 2001; Melnick et al., 2004a,b). Although voltage-gated Na^+ and K^+ channels appear to be differently expressed throughout the nervous system, our knowledge about their distribution in the lamina I–III of dorsal spinal cord is still limited (Hildebrand et al., 2011).

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To date, most of the electrophysiological studies on sensory neurons have focused only on the changes in ion channel conductance by pharmacological approaches and the impact on the excitability on the neurons is poorly investigated. Moreover, spinal dorsal horn sensory neurons in lamina I–III display different firing patterns with distinct characteristics and can be divided into tonically-firing (TFNs), adapting-firing (AFNs) and single spike neurons (SSNs). Thus a detailed analysis of changes in their action potential generation remains to be comprehensively characterized. In this regard, the mode of analgesic action of local anaesthetics in central pain pathways has already been reported (Olschewski et al., 2009; Wolff et al., 2014), however, antidepressants or anticonvulsants are poorly described. For this study we therefore selected amitriptyline, carbamazepine and gabapentin, drugs commonly used in clinical practice for pain therapy. Preclinical models have reported that amitriptyline, a potent use-dependent blocker of Na^+ channels, attenuates acute and chronic pain (Brau et al., 2001; Deffois et al., 1996; Gerner et al., 2003) as well as may reduce pain due to inhibition of the re-uptake of serotonin and its effect on nicotinic acetylcholine receptors in the central nervous system (CNS) (Sacerdote et al., 1987; Connolly et al., 1992; Gumilar et al., 2003). Carbamazepine has been shown to modulate stably expressed Nav1.7 sodium channels (Theile and Cummins, 2011). Finally, recent evidence strongly suggests that gabapentin inhibits voltage-gated calcium channels in both central and peripheral neurons and in addition, affects GABA-ergic transmission in the CNS (Stefani et al., 2001; Sutton et al., 2002; Taylor et al., 1998). Although, these drugs are attractive candidates for the treatment of neuronal hyperexcitability, studies investigating their action on excitability of CNS neurons are largely missing.

In the current paper, we examined the expression of Nav and Kv isoforms and the localization of channel proteins in the lamina I–III of the rat spinal cord. The second purpose of our study was to reveal the effects of amitriptyline, carbamazepine and gabapentin on these voltage-gated ion channels and to explore their functional consequences on single action potentials and on series of action potentials of intact sensory dorsal horn neurons on thin-slice preparations of rat spinal cord.

2. Materials and methods

2.1. Preparation of dorsal horn neurons

The study was performed on 200 μm transversal slices of the lumbar spinal cord (L3–6) of young rats (2–3 weeks old) of both sexes. All animals were killed by rapid decapitation according to the standards of the German and Austrian guidelines. The spinal cord was carefully removed and prepared as we established previously (Wolff et al., 2007). The experimental procedure was reported to the local Animal Care Committee (RP Giessen, Germany) and is in full accordance with national and institutional guidelines.

2.2. Chemicals and solutions for preparation and for the electrophysiological studies

Detailed description of the solutions and drugs is given in the supplement.

2.3. Study design for electrophysiology

Based on our data from studies with the same experimental model (Wolff et al., 2007), we performed a power calculation with alpha 0.05 and found a number of 10 experiments per group for a power of 80%. Therefore we aimed to get at least 10 complete sets of data for each parameter. The total number of experiments with application of a drug was set on 300 for current-clamp, on 100 for voltage-clamp in potassium channels and on 200 in sodium channels. Thus the total number of patch clamp experiments with application of a drug was 600. Only neurons with one recording under control conditions and one after superfusion with the drug were included in the analysis.

Inclusion criteria for all patch clamp experiments was the anatomical location (Safronov et al., 1997). For current clamp studies: resting membrane potential more negative than −55 mV and generation of an action potential as response to a 500 ms current pulse. The identification of the neurons (TFNs, AFNs or SSNs) was carried out based on their first response to a 500 ms current pulse prior to the detection of the singe action potentials. Tonically-firing neurones (TFNs) exhibit little spike frequency adaptation during sustained depolarization. Adapting-firing neurones (AFNs) generate a short burst of spikes at the beginning of depolarization. Single-spike neurones (SSNs) generate only one or seldom two spikes at the beginning of a depolarizing pulse. Since there is no visual tool to distinguish between tonically-firing (TFNs), adapting-firing (AFNs) and single spike (SSNs) dorsal horn neurons, all neurons were chosen blindly and retrospectively stratified into one of three groups (TFN, AFN, SSN) based on their first response. Inclusion criteria for voltage-clamp studies for investigations of potassium channels were: resting membrane potential more negative than −55 mV and generation of an action potential in response to a 1 ms current pulse. Inclusion criteria for voltage-clamp studies for investigations of sodium channels was: sodium current larger than 1 nA.

2.4. Electrophysiology

Dorsal horn neurons were identified as multipolar cells with a soma of 8–12 μm diameter located in laminae I–III of the spinal cord. The identification and differentiation of neurons and glial cells was performed based on a procedure described previously (Safronov et al., 1997). During the experiments, the cells were monitored under infrared video microscopy (Hamamatsu Photonics, Japan). Experiments in the voltage-clamp mode were carried out using ESI in order to reduce series resistance (Safronov et al., 1997). In addition, the ESI method provided a possibility to study pharmacological properties of different ion currents in identified neurons under conditions where diffusion of the blocker molecules is not impeded by the connective tissue that surrounds the neuron. Experiments in current-clamp mode were performed in whole cell mode.

2.4.1. Entire soma isolation (ESI) method

Experiments in the voltage clamp mode were carried out using ESI in order to reduce series resistance. After neuron identification in the spinal cord slices, the isolation procedure, monitored under infrared optics (Hamamatsu Photonics, Japan), was started. The ESI method was carried out as described earlier (Safronov et al., 1997). Briefly, in the whole-cell recording configuration, entire *somata* of dorsal horn neurons could easily be isolated from the slice by slow withdrawal of the recording pipette. All or nearly all of their processes is left in the slice as shown in the previous work of Safronov et al. (1997). After the neuronal recording in the slice was completed, a slight suction was applied to the recording pipette and it was gently withdrawn until the connection between the soma and the slice was broken. The suction applied was similar to the one needed to break the membrane into the formation of whole-cell configuration. The suction was released immediately after the completion of the isolation. The isolated neuron was classified as a soma if adjacent processes were not seen either during the experiment or afterwards, after the recording pipette was turned over. The isolated structure was classified as a *soma + axon* if it contained one 10–100 μm process and preserved more than 85% of the