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Differential effects of lacosamide, phenytoin and topiramate on peripheral nerve excitability: An *ex vivo* electrophysiological study

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ABSTRACT

Background: Antiepileptic drugs (AEDs) are mainly used to control cortical hyperexcitability. Some of them (*e.g.* phenytoin (PHT) and topiramate (TPM)) have also effects on the peripheral nervous system (PNS). Lacosamide (LCM) is a novel AED that stabilizes hyperexcitable neuronal membranes by selectively enhancing the slow inactivation of voltage-gated sodium channels (VGSCs). Although the mechanism of action of LCM is fairly well understood, there are no *in vitro* data available regarding any possible PNS effects of LCM.
Objective: To investigate, *in vitro*, the effects of LCM on peripheral nerve excitability in comparison with PHT and TPM, two AEDs that act, in part, by stabilizing the fast inactivation state of VGSCs.
Methods: Experiments were conducted on the isolated sciatic nerve of the adult rat using standard electrophysiological methods. The effects of LCM on the amplitude and latency of the evoked compound action potential (CAP) during a 48 h period of drug exposure were recorded and compared with the effects of PHT and TPM.
Results: LCM produced inhibitory effects on CAP at concentrations significantly higher than the therapeutic levels (>25 μg/ml). At these concentrations (62.57–125.15 μg/ml), an acute and immediate increment of the latency and decrement of the amplitude of the CAP were observed. In contrast to LCM, PHT caused an acute decrement in the amplitude as well as an increment in the latency of the CAP even at subtherapeutic levels (5 μg/ml). With regard to TPM, the amplitude of the CAP was not affected at the supratherapeutic concentrations but at the therapeutic concentration of 33.94 μg/ml a reduced decrement of the CAP amplitude compared to the controls was observed.
Conclusions: LCM, PHT and TPM exert differential effects on peripheral nerve excitability. PHT inhibited the sciatic nerve CAP even at subtherapeutic levels whereas LCM was safe within the therapeutic concentration range. TPM did not affect the CAP amplitude even at high supratherapeutic concentrations whereas in the therapeutic range a neuroprotective effect was observed. Possible underlying mechanisms and the clinical implications of these findings are discussed.

1. Introduction

AEDs have various targets of action such as ion channels, pre- and postsynaptic receptors and intracellular signaling pathways (Kohling, 2002; Lason et al., 2011; Rogawski and Loscher, 2004), through which they exert therapeutic effects in a wide spectrum of neurological and psychiatric disorders. Their main indication is the control of epileptic seizures but they are also of clinical importance in the management of non-epileptic conditions such as migraine, neurodegenerative diseases, neuropathic pain and psychiatric disorders (Johannessen Landmark, 2008; Mantegazza et al., 2010).

There are several reports indicating a possible effect of AEDs on the peripheral nervous system (PNS). PHT was the first AED that

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has been investigated for possible neurotoxic effects on PNS function (Boylu et al., 2010; Danner, 1982; Ramirez et al., 1986; Shorvon and Reynolds, 1982; So and Penry, 1981; Swift et al., 1981; Taylor et al., 1985; Toth and Kotecha, 2004; Yoshikawa et al., 1999). Human, as well as experimental studies with PHT report a decrement in nerve conduction velocity and amplitude of the evoked muscle action potentials (Raya et al., 1992). TPM has also been reported to affect peripheral nerves. For instance, a recent study revealed that migraine patients treated with TPM showed a decrease in the strength duration time constant (SDTC) values reflecting a reduction in peripheral nerve excitability (Erdogan et al., 2012) whereas nerve conduction parameters remained unaltered, in line with previous studies (Boylu et al., 2010; Freeman et al., 2007).

LCM is a novel AED indicated as monotherapy or as adjunctive treatment of partial onset seizures with or without secondary generalization. It acts, in part, by selectively enhancing the slow inactivation state of voltage-gated sodium channels resulting in stabilization of hyperexcitable neuronal membranes and inhibition of repetitive neuronal firing (Errington et al., 2008; Niespodziany et al., 2013; Patsalos and Bourgeois, 2010). Currently, there are no data available regarding any possible electrophysiological effects of LCM on the PNS.

The aim of the present study is to investigate, *in vitro*, the effects of LCM in comparison to PHT and TPM on the excitability of the isolated sciatic nerve of the rat. We hypothesized that although these three AEDs exert their therapeutic activity, at least in part, by modifying the inactivation properties of voltage-gated sodium (Na_v) channels, critical differences in their exact mechanism of action may ultimately result in differential PNS effects.

2. Methods

2.1. Experimental setup

Animals received proper care in compliance with the "Guidelines for the Care and Use of Laboratory Animals" published by US National Institutes of Health (NIH publication No. 85-23, revised 1996) and the "Principles of laboratory animal care" published by the Greek Government (160/1991) based on EU regulations (86/609). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Directorate of Veterinary Services of Prefecture of Thessaloniki (License No. 144284/866/09.06.2011).

Wistar rats of either sex, 12–15-weeks old and weighing between 300 and 400 g were used. The sciatic nerves were dissected from the spinal cord to the knee. It should be noted that a number of experimental animal studies have suggested that the neurotoxicity of many structurally diverse chemicals is mediated by distal axon degeneration or have identified nerve terminals as primary sites of action causing synaptic dysfunction by unknown mechanisms (LoPachin, 2005, 2000; LoPachin and Barber, 2006). However, we refrained from using more distal parts of the sciatic nerve for two reasons. First, it would be technically more challenging due to the fine diameter of the peripheral branches and secondly it would introduce significant "inter-subject" variability which might act as a confounder.

The nerve was mounted across a three-chambered recording bath. This extracellular recording chamber was first employed by Schneider et al. (1991), in order to record CAPs propagating through a segment of rabbit peripheral nerve. In our study, the three-chambered recording bath is a modified version (described in Andreou et al., 2007) and has been used in a variety of neurotoxicological studies (*e.g.* Kagiava et al., 2008; Moschou et al., 2008; Zafeiridou et al., 2006). Each chamber was filled with oxygenated physiological solution of the following composition (in mM): 136 NaCl, 11 glucose, 4.7 KCl, 2.4 $CaCl_2$, 1.1 $MgCl_2$, 1 $NaHCO_3$, 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), (pH 7.2). All experiments were performed at a constant temperature of 26 ± 0.5 °C, which was controlled by a cooling–heating system with water circulating under the three chambers. The advantage of this nerve-preparation is the stable recording of CAPs for prolonged periods of time (>24 h).

2.2. Electrophysiological evaluation

The nerve was electrically stimulated with supramaximal stimuli presented at a frequency of 1 Hz (pulse amplitude: 2.0–3.0 V, duration: 0.01 ms) using an electrode connected to a constant voltage stimulator (Digitimer, England, UK) to evoke the CAP. The duration of the total stimulation series period was 48 h.

The evoked CAP was recorded in the recording chamber and digitally stored. Changes in the latency (time from stimulus delivery to the initial CAP deflection) and the amplitude of the CAP over time were measured in order to assess the effects of AEDs. A 1 h equilibration of the nerve in normal saline always preceded drug exposure. Thereafter, the electrophysiological recordings were performed during a 48 h incubation of the nerves in a medium containing the AED under investigation. We chose to perform long-term recordings, in contrast to the majority of previously reported studies, because theoretically they are more relevant to the chronic administration of AEDs in clinical settings.

The pharmacological agents, which were initially diluted in DMSO (dimethyl sulfoxide) (<1%), were added in the saline of the middle-perfusion chamber. The parts of the nerve in the recording and stimulating chamber remained all the time exposed to normal saline. LCM, PHT and TPM were investigated at various concentrations that were selected in reference to their therapeutic levels.

2.3. Data analysis

The alterations in the values of the latency and the amplitude of the CAP during exposure to AEDs were expressed as percentage changes relative to the mean latency and the mean amplitude prior of nerve exposure to the pharmacological agents. These variables were summarized using standard descriptive statistics and normality of data distribution was evaluated by the Shapiro–Wilk test, since the size of each group was less or equal to 50.

A two-way repeated ANOVA (independent variables: Time – Group, dependent: CAP %) was used in order to examine whether there is statistically significant difference between all pairs of substances. The assumption of sphericity was checked using Machly's test, and the Bonferroni method was used to perform all the pairwise comparisons following a significant overall test result. A $p < 0.05$ was considered significant. Descriptive analyses and ANOVA's were performed using IBM SPSS Statistics 21 software (IBM Corporation, USA).

The percentage changes of the amplitude in a time–response curve were further used to estimate the half falling time (IT_{50}), the time required for the CAP to reach 50% of the value before the exposure to the AED under investigation (value at $t = 0$). The relative potency of the AEDs was described by the half maximal inhibitory concentration (IC_{50}), the concentration that decreases the IT_{50} to 50% of the control IT_{50}. Where possible, the NOEC (no observed effect concentration) was estimated. The NOEC represents the concentration of the drug, which has the minimum effect on the physiological properties of the axons of the nerve. All the above analysis was performed using the r-package (R version 3.2.1).