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Differential inhibition of cardiac and neuronal Na^+ channels by the selective serotonin-norepinephrine reuptake inhibitors duloxetine and venlafaxine

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ABSTRACT

Duloxetine and venlafaxine are selective serotonin-norepinephrine-reuptake-inhibitors used as antidepressants and co-analgesics. While venlafaxine rather than duloxetine induce cardiovascular side-effects, neither of the substances are regarded cardiotoxic. Inhibition of cardiac Na^+-channels can be associated with cardiotoxicity, and duloxetine was demonstrated to block neuronal Na^+-channels. The aim of this study was to investigate if the non-life threatening cardiotoxicities of duloxetine and venlafaxine correlate with a weak inhibition of cardiac Na^+-channels.

Effects of duloxetine, venlafaxine and amitriptyline were examined on endogenous Na^+-channels in neuroblastoma ND7/23 cells and on the α-subunits Nav1.5, Nav1.7 and Nav1.8 with whole-cell patch clamp recordings.

Tonic block of the cardiac Na^+-channel Nav1.5 and rat-cardiomyocytes (CM) revealed a higher potency for duloxetine (Nav 1.5 IC_{50} 14 ± 1 μM, CM IC_{50} 27 ± 3 μM) as compared to venlafaxine (Nav 1.5 IC_{50} 671 ± 26 μM, CM IC_{50} 452 ± 34 μM). Duloxetine was as potent as the cardiotoxic antidepressant amitriptyline (IC_{50} 13 ± 1 μM). While venlafaxine almost failed to induce use-dependent block on Nav1.5 and cardiomyocytes, low concentrations of duloxetine (1, 10 μM) induced prominent use-dependent block similar to amitriptyline. Duloxetine, but not venlafaxine stabilized fast and slow inactivation and delayed recovery from inactivation. Duloxetine induced an unselective inhibition of neuronal Na^+-channels (IC_{50} ND7/23 23 ± 1 μM, Nav1.7 19 ± 2 μM, Nav1.8 29 ± 2).

Duloxetine, but not venlafaxine inhibits cardiac Na^+-channels with a potency similar to amitriptyline. These data indicate that an inhibition of Na^+-channels does not predict a clinically relevant cardiotoxicity.

1. Introduction

Serotonin-norepinephrine reuptake inhibitors (SNRI) are a relatively new class of antidepressants commonly applied for treatment of depression disorders as well as for chronic neuropathic pain. Apart from their antidepressant and analgesic properties, several antidepressants induce relevant cardiovascular side effects, *e.g.* tachycardia, hypertension or hypotension and widening of the QT interval (Carney et al., 2002). While both venlafaxine and duloxetine are regarded relatively safe in this aspect (Taylor et al., 2013), several reports document relevant cardiac side effects for venlafaxine. While few cases of cardiac side were also reported for duloxetine (Stevens, 2008), it seems to be more or less avoid of relevant cardiovascular side effects even at extremely high dosages following intoxication (Chappell et al., 2013; Derby et al., 2007; Wernicke et al., 2007). Part of the difficulty in evaluating the potential influence of antidepressants on cardiovascular events is that depression and cardiovascular disease seem to be comorbid many patients. Furthermore, depression in early life is a risk factor for the subsequent development of ischemic heart disease. Nevertheless, the molecular mechanisms mediating cardiovascular side effects of antidepressants are still not fully understood. Cardiac voltage-gated Na^+ channels are commonly described targets for antidepressants and several other classes of therapeutics known to induce cardiotoxicity (Pancrazio et al., 1998; Poulin et al., 2014). Among

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the nine functional α-subunit Na^+ channel isoforms (Nav1.1 – Nav1.9), seven isoforms are considered "neuronal" and are thus mainly expressed in the peripheral or central nervous system (Catterall et al., 2003). In sensory neurons, the subunits Nav1.7 and Nav1.8 are considered to play important roles for neuropathic pain (Catterall et al., 2003). The cardiac subunit Nav1.5 is essential for the propagation of action potentials in the working myocard, and dysfunction of this subunit due to hereditary mutations can cause cardiac diseases such as the long-QT syndrome. Therefore, Nav1.5 is an important target for pro- and antiarrhythmic substances. As far as we are concerned, it is not known if duloxetine or venlafaxine functionally interact with Nav1.5.

As chronic pain is also frequently associated with depression, some antidepressants are recommended as first or second choice therapeutics for treatment of neuropathic pain (Finnerup et al., 2015; O'Connor and Dworkin, 2009). While it is not yet clear how antidepressants like SNRIs reduce neuropathic pain, duloxetine was recently demonstrated to potently block the sensory neuronal Na^+ channel Nav1.7 by interacting with the conserved local-anesthetic binding site (Wang et al., 2010). Neuronal Na^+ channels like Nav1.7 and Nav1.8 are important targets for analgesic drugs, and the analgesic effects of several other co-analgesics used for treatment of neuropathic pain may be due to inhibition of neuronal Na^+ channels (Lampert et al., 2010). Thus, it is possible that the analgesic property of duloxetine is due to an inhibition of Na^+ channels as well.

In the present study we asked if the low but differential cardiotoxic profiles of duloxetine and venlafaxine is explained by a lack of inhibition of Nav1.5, or by a differential inhibition of neuronal and cardiac Na^+ channels.

2. Materials and methods

2.1. Patch clamp technique and data acquisition

Whole-cell patch clamp recordings were conducted at room temperature. Pipettes (Science Products, Hofheim, Germany) were pulled on a DMZ-Universal Puller (Zeitz, Germany) and heat polished to give a resistance of 1.8–2.2 MΩ when filled with the pipette solution. All experiments were conducted using an EPC10 amplifier (HEKA Instruments Inc., NY, U.S.A.). Currents filtered at 5 kHz and sampled at 20–50 kHz. Data were acquired on a PC with the Patchmaster v20 × 60 software (HEKA Instruments Inc., NY, U. S.A.). The series resistance was compensated by 60–80% to minimize voltage errors and the capacitance artefacts were cancelled using the amplifier circuitry. Linear leak subtraction, based on resistance estimates from four hyperpolarizing pulses applied after the depolarization test potential, was used for all protocols except for use-dependent block at 10 Hz. The offset-potential was zeroed before the cells were patched. Test solutions were focally applied using a self-made, gravity driven application system. Only one cell per dish was used. Patch clamp data were analyzed with the Pulsefit software (HEKA Instruments Inc., NY, USA). Curve fitting and statistical analyses were performed with Origin 8.5 (Microcal Software,v Northampton, MA). Data were presented as mean ± S.E. M. or fitted value ± S.E. of the fit. To obtain IC50-values, peak current amplitudes at different drug concentrations were normalized to the value obtained in control solution. The data were fitted with the Hill equation y=ymax*IV50n/IC50n*Cn, where ymax is the maximal amplitude, IC50 the concentration at which y/ymax =0.5, and n is the Hill coefficient. To obtain the inactivation curves, peak currents evoked by a test pulse were measured, normalized and plotted against the conditioning prepulse potential. The data were fitted by the Boltzmann equation (y=1/1+exp EPP–0.5)/kh, where 0.5 is the voltage at which y=0.5 and kh is the slope factor).

2.2. Voltage protocols

Resting channels were explored by test pulses to 0 mV applied at 0.1 Hz in cells held at −120 mV and inactivated channels were induced by a 10 s long pre-pulse to −70 mV followed by a 100 ms long pulse at −120 mV allowing recovery from inactivation and finally a test pulse to 0 mV. Peak amplitudes of currents at different drug concentration were normalized with respect to the peak amplitude in control solution and plotted against the substance-concentration. Data were fitted with the Hill equation.

For use-dependent block, cells were held at −120 mV and currents were activated at 10 Hz by test pulses to 0 mV. Peak currents were normalized to the amplitude of the first pulse and plotted against the pulse number.

Fast inactivation was induced by 50 ms long pre-pulses ranging from −120 mV to 0 mV in steps of 5 mV, and the remaining fraction of available channels was examined with a 20 ms long pre-pulse to 0 mV and slow inactivation induced by 10 s pre-pulses ranging from −120 mV to −10 mV in steps of 10 mV, followed by a 100 ms pulse at −120 mV allowing recovery from fast inactivation and finally a test pulse to −10 mV.

Recovery from inactivation was explored with a two-pulse paradigm allowing estimation of recovery of both fast and slow inactivation. Cells were held at −120 mV and test pulses to 0 mV were applied, after inter-pulses with variable lengths at −120 mV and a 10 s long pre-pulse to −70 mV. The time interval between both test-pulses was varied between 0 and 7.3 s With this protocol, we obtained a time course with a biphasic course, that is, it was best fitted with a double exponential fit giving each phase its own time constants (τ1 and τ2).

2.3. Cell culture and transfection

Human embryonic kidney (HEK) 293 cells stably expressing Nav1.7 and Nav1.5 were cultured in Dulbecco's modified Eagle medium (DMEM, GIBCO-Invitrogen, Germany), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biochrom, Germany), 1% Taurin and 1% Geneticin G418 for Nav1.7 (GIBCO-Invitrogen, Germany) and 0.4% zeocin for Nav1.5 (GIBCO-Invitrogen, Darmstadt, Germany) was added to the medium. Nav1.8 expresses poorly in HEK 293 cells and was therefore investigated in ND7/23 as described previously (Leffler et al., 2007). Neuroblastoma ND7/23 cells were cultured in DMEM supplemented with 10% FBS (Biochrom, Germany), HEPES (2.5%), penicillin/streptomycin (1%, GIBCO-Invitrogen) and Taurin (1%, 0.3 M). Green fluorescent protein was co-expressing with Nav1.8 in order to visualize transfected cells. Transfected cells were used for experiments within 2 days.

Neonatal rat ventricular cardiomyocytes were isolated from 1 to 3 day old Sprague-Dawley rats by Percoll densitiy gradient centrifugation as previously described (Heineke et al., 2005). On the day after isolation, the cells were switched to Medium 199 (Sigma Aldrich, Munich, Germany) supplemented only with L-glutamine and penicilline/streptomycine (GIBCO-Invitrogen, Darmstadt, Germany).

2.4. Chemicals

Duloxetine and venlafaxine (Sigma Aldrich, Munich, Germany) were prepared as 100 mM stock in dimethyl-sulfoxid (DMSO, Sigma Aldrich, Munich, Germany) and stored at 4 °C. Test solutions for experiments were prepared directly prior to patch clamp recordings and titrated in extracellular solutions to the desired concentrations and were focally applied using a self-made, gravity driven application system. Tetrodotoxin (Alomone labs, Jerusalem, Israel) was prepared as 100 mM stock solution in water.