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Local-anesthetic like inhibition of the cardiac sodium channel Nav1.5 α-subunit by 5-HT_3 receptor antagonists

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ABSTRACT

5-hydroxytryptamine 3 receptor (5-HT_3 receptor) antagonists are administered for prevention and therapy of nausea and vomiting. Although regarded as safe therapeutics, they can also provoke arrhythmias by prolonging the QRS interval. However, the mechanisms mediating this cardiotoxicity are poorly understood. Here we investigated effects of 5-HT_3 receptor antagonists on the cardiac Na^+ channel Nav1.5.

We explored the interaction of dolasetron, tropisetron, granisetron and ondansetron on the human α-subunit Nav1.5 heterologously expressed in HEK293 cells. Sodium currents were explored by means of whole-cell patch clamp recordings. All four substances inhibited the Nav1.5 in a concentration and state-dependent manner. Dolasetron displayed the lowest blocking efficacy, and tropisetron was the most potent blocker with a half maximum blocking concentration of 18 μM for tonic block of inactivated channels. Tropisetron was also the most potent use-dependent inhibitor, and it also induced a strong open -channel block. Both tonic and use-dependent block by tropisetron were abbreviated on the local-anesthetic insensitive mutant Nav1.5-F1760A. Co-administration of tropisetron and the local anesthetic bupivacaine or the hypnotic propofol augmented inhibition of Nav1.5. Our data demonstrate that 5-HT_3 receptor antagonists induce a local-anesthetic like inhibition of Nav1.5, and that they display different blocking efficacies. Reports on a relevant cardiotoxicity of dolasetron as opposed to other 5-HT_3 receptor antagonists do not seem to correlate with a block of Nav1.5. As inhibition of Nav1.5 was enhanced by propofol and bupivacaine however, it is possible that a combined administration of Na^+ channel blockers and 5-HT_3 receptor antagonists can provoke arrhythmias.

1. Introduction

The 5-HT_3 receptor is predominantly expressed in the chemoreceptor trigger zone and at vagal afferents in the gastrointestinal tract. 5-HT_3 receptor antagonists are commonly used for treatment and prevention of chemotherapy induced and postoperative nausea and vomiting. Although these agents show an adequate safety profile, changes in electrocardiographic parameters have been described for some 5-HT_3 receptor antagonists (Goodin and Cunningham, 2002). These effects include a prolongation of the QT- and PR-intervals, which can lead to torsade de pointes tachycardia and other potentially life threatening arrhythmias (Benedict et al., 1996; Boike et al., 1997; Keefe, 2002). It is therefore advised to administer 5-HT_3 receptor antagonists to patients with known cardiac diseases and risk of QT-prolongation with great care. The 5-HT_3 receptor antagonist dolasetron can induce a substantial prolongation of the QT-interval following intravenous application (Goodin and Cunningham, 2002). As a result of clinical study promoted by FDA, the intravenous application of dolasetron was even prohibited and is thus not longer available. While further reports have been published which describe relevant cardiovascular changes associated with 5-HT_3 receptor antagonists (Baguley et al., 1997; Benedict et al., 1996; Boike et al., 1997; Chan et al., 2006; Keefe, 2002), and more recent clinical trial found that the perioperative application of ondansetron and dolasetron is not associated with a significant prolongation of the QT interval (Obal et al., 2014). There are several possible explanations for these discrepancies between studies. Quraishi and coworkers reported that the ability of 5-HT_3 receptor antagonists to modify

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the QT interval appears to be associated with single nucleotide polymorphism (re10494366) of the gene encoding neuronal nitric oxidase (Quraishi et al., 2011). On the other hand, some 5-HT_3 receptor antagonists were reported to modify cardiac ion channels known to regulate the QT interval, including the Na^+ channel Nav1.5, Kv11.1-human Ether-à-go-go Related Gene (hERG) – channels and slow delayed rectifier potassium channels (Kuryshev et al., 2000). Furthermore, the 5-HT_3 receptor antagonist ondansetron was demonstrated to inhibit neuronal sodium channels and to induce topical local anesthesia in rats (Ye et al., 1997). Thus, it is possible that different 5-HT_3 receptor antagonists have different pharmacological properties in respect to their abilities to modify ion channels other than 5-HT_3 receptor.

By generating the upstroke of the action potential in the working myocard, the cardiac voltage gated Na^+ channel Nav1.5 is an important determinant for cardiac repolarization. This notion is supported by the fact that hereditary mutations of Nav1.5 are associated with ECG abnormalities and arrhythmias such as the autosomal dominant long QT syndrome as well as the Brugada syndrome (Baroudi and Chahine, 2000). The functional properties of many of these Nav1.5 channel mutations include reduced amplitudes of the Na^+ currents and increased non-inactivating persistent Na^+ currents (Deschenes et al., 2000; Maury et al., 2013). It was also demonstrated that both volatile (OuYang and Hemmings, 2007) and intravenous (Stoetzer et al., 2016) anesthetics also inhibit Nav1.5 channels at concentrations reasonably close to plasma levels being reached during sedation with these agents. Most of these substances seem to inhibit Nav1.5 and other sodium channels isoforms by interacting with the conserved residues referred as “the local anesthetic binding site” (Nau and Wang, 2004). Considering the clinical situation when two or several substances with this property are simultaneously administered, it seems plausible that they can induce additive effects of Na^+ channels in vivo and thus induce relevant effects critical concentrations are reached.

In the present study we explored the effects of the clinically used 5-HT_3 receptor antagonists dolasetron, ondansetron, granisetron and tropisetron on human Nav1.5 channels by using the whole-cell patch clamp technique. Our data reveal that the 5-HT_3 receptor antagonist tropisetron inhibits Nav1.5 channels by interacting with the local anesthetic site, and that it seems to induce an additive block of the sodium current when co-administered with anesthetic compounds.

2. Material and methods

2.1. Chemicals and solutions

Ondansetron, tropisetron, granisetron and dolasetron were purchased from Tocris Bioscience (Bristol, UK), veratridine from Biotrend (Zürich, Switzerland), propofol and bupivacaine from Sigma-Aldrich (Taufkirchen, Germany). Stock solutions of all drugs were prepared in distilled water, with exception of veratridine, propofol and bupivacaine, which were dissolved in dimethylsulfoxide (DMSO). The DMSO concentration in the final test solutions never exceeded 0.2%, which does not affect Nav1.5 channels (Schulze et al., 2014). The bath solution contained (mM): 140 NaCl, 3 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES and 15 glucose. pH was adjusted to 7.4 with tetraethylammonium hydroxide. The pipette solution contained (mM): 10 NaCl, 140 CsF, 1 EGTA, 10 HEPES and was adjusted with CsOH to pH 7.4. The osmolarity of all solutions was adjusted with glucose to 290–300 mosm/L. Borosilicate glass capillaries were used for pulling patch pipettes (GB150ETF-8P, Science Products, Hofheim, Germany). Using a horizontal micropipette puller (Sutter Instrument, Model P1000) pipettes were produced with a resistance of 1–2 MΩ after heat polishing.

2.2. Cell culture and transfection

HEK293-cells stably expressing wild type human Nav1.5 channels were used as described previously (Schulze et al., 2014). The cells were kept in cell culture flasks in low glucose DMEM (Gibco, Darmstadt, Germany) supplemented with 10% FBS (Biochrom, Berlin, Germany), 1% penicillin/streptomycin (Gibco) and 0.4% Zeocin (Gibco). The mutant channel Nav1.5-F1760A was constructed with a mutagenesis kit (Quickchange XL kit, Qiagen GmbH, Hilden) according to the instructions of the manufacturer. HEK239t cells were transfected for at least 24 h with F1760A using NanoFectin transfection kit (PAA Laboratories GmbH, Pasching, Austria). Cells were co-transfected with 0.25 µg pEGFP-N1 (Clontech, Palo Alto, CA, USA) to visualize transfection effect with UV-light. The dorsal root ganglion neuroblastoma hybridoma cell line ND7/23 (purchased from European Collection of Cell Cultures, Porton Down, United Kingdom) were cultured in Dulbecco's modified Eagle medium, supplemented with 100 U/ml penicillin/streptomycin, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (all DMEM, GIBCO-Invitrogen, Karlsruhe, Germany).

2.3. Electrophysiology and data analysis

Whole-cell patch clamp experiments were performed using an EPC9 patch clamp amplifier (HEKA electronics, Lambrecht, Germany). Test solutions were applied with a gravity driven application system with the outlet at approximately 100 µm from the respective clamped cell. Currents were recorded at room temperature. Currents were filtered at 5 kHz, acquired at 20 kHz and data were stored on the hard disk of a PC for off-line analysis. In order to minimize voltage errors, the series resistance was compensated by 60% and the capacitance artefact was reduced using the amplifier circuitry. Linear leak subtraction was performed for all experiments and is based on the resistance estimates from four hyperpolarizing pulses applied before the test pulse. Pulse and PulseFit software (HEKA Electronics, Lambrecht, Germany) were used for recording and analysing data. For further analysis, fitting of curves and production of Figs. the Microcal Origin 8.5.1 software (OriginLab Corp., Northampton, MA, USA) was used. To obtain dose response curves, residual sodium currents in the presence of a drug (with respect to the currents elicited with the same protocol in the respective control experiment) were plotted against the applied concentration of the drug [C]. The averaged data were fitted using the Hill equation, yielding the concentration for half-maximum channel blockade (IC_{50}): Hill-equation (Eq. (1)): $y = y_{max} \times \{(IC_{50}{}^n)/(IC_{50}{}^n \times C^n)\}$, with y_{max} representing the maximal amplitude, IC_{50} the concentration at which $y/y_{max}=0.5$; n is the Hill coefficient of sigmoidicity.

Current amplitudes obtained for fast and slow inactivation were normalized to the respective maximum current (which was assessed at −150 mV for fast inactivation and at −120 mV for slow inactivation) and plotted against the respective membrane potential of the pre-pulse. Boltzmann fits to the resulting current–voltage plots yielded the membrane potential at half-maximum channel availability ($h_{0.5}$): Boltzmann equation (Eq. (2)): $y=1/\{1+\exp((Epp-h_{0.5})/kh)\}$, where Epp is the membrane potential, $h_{0.5}$ is the voltage at which $y=0.5$, and kh is the slope factor. Drug effects on the recovery from fast and slow inactivation were examined by a protocol consisting of two test pulses to 0 mV applied with increasing time intervals between, respectively, 0 and 84.7 ms (fast inactivation) and 0 and 7.3 s (slow inactivation). Normalized currents for recovery of fast inactivation were fitted with a single exponential function: (Eq. (3)) $y=A1 \times \exp\{(-x/t1)+y0)\}$. The time course of the graph of slow inactivation exhibited a biphasic progression and was therefore fitted with a double exponential function giving each phase its own time constant (τ), Eq. (4): $y=A1 \times \exp(-x/\tau 1)+A2 \times \exp(-x/\tau 2)+y0$.

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