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Voltage gated ion channels blockade is the underlying mechanism of BIMU8 induced cardiotoxicity

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ABSTRACT

BIMU8 is a 5-$HT4_a$ receptor agonist and used as an experimental drug to counteract opioid induced respiratory depression. In preliminary experiments serious disturbances in ECG were observed in anesthetized rabbits which prompted us to explore the underlying cause of BIMU8 induced abnormal changes in ECG recordings. Electrophysiological experiments were performed on HEK-293 cells expressing hERG, $Ca_V1.2$ and $Na_V1.5$ ion channels. In whole-cell recordings BIMU8 effectively blocked these three channels, with IC_{50} values of 0.06 ± 0.05, 1.46 ± 0.26 and 4.66 ± 0.58 μM for hERG, $Na_V1.5$ and $Ca_V1.2$, respectively. Additionally it also produced a hyperpolarizing shift of 3.27 mV in half maximal activation and 12.87 mV in fast inactivation of $Na_V1.5$ channel. These experimental findings indicate that BIMU8 is a potent blocker of hERG, $Na_V1.5$ and $Ca_V1.2$ cardiac ion channels thus revealing its proarrhythmic potential.

1. Introduction

Opioid analgesics (μ-opioid agonists) are widely used as a preferred drug for the management of postoperative pain, different chronic pain syndromes and as a part of anesthetic procedures (Boom et al., 2012). A serious limitation in their use is an unwanted side effect of opioid induced respiratory depression (OIRD), which may lead to permanent neurological injury and death due to hypoventilation and hypoxemia (Dahan, 2007). About 0.3–17% of post-operative patients develop symptoms of respiratory depression while being treated by opioid analgesics for acute pain (Cashman and Dolin, 2004). The demographic trends like ageing, obesity and chronic use of opioids for both medical and nonmedical purposes will make OIRD more prevalent in the future (Overdyk, 2010). Not only in humans but also in veterinary medicine is OIRD a cause for concern. This holds especially true in large wild mammals, as rhinoceros, where highly potent opioids such as etorphine are frequently the only available option to induce anesthesia when immobilization is needed for management purposes in free ranging animals. The drug of choice for the management of opioid induced severe respiratory depression is naloxone, but besides reversing the respiratory depression it also antagonizes analgesia, thus, compromising the pain management during crisis (Boom et al., 2012). Several strategies are being investigated to counteract unwanted side effects of opioid analgesics without affecting their analgesic or sedative efficacy. One promising approach towards amelioration of OIRD has been the use of (5-HT) serotonin receptor subtype 5-HT_{4a} agonists. 5-HT_{4a} is a member of alternatively spliced 5-HT_{4a-h} G-protein coupled serotonin receptors, which are widely distributed in brain, gastrointestinal tract and heart (Filip and Bader, 2009; Langlois and Fischmeister, 2003). Multiple labelling experiments by confocal microscopy and reverse transcriptase PCR of single cells have identified overlapping expression patterns of 5-HT_{4a} and μ-opioid receptors on respiratory motor neurons in the pre-Boetzinger complex, a region that modulates respiratory movements (Manzke et al., 2003). Opioids like morphine and fentanyl bind to μ-opioid receptors and synthesize $G\alpha_i$-guanosine triphosphate complex which decreases the cyclic adenosine monophosphate (cAMP) levels, hence, decreasing neurotransmitter release and causing alteration in membrane currents resulting in respiratory depression (Boom et al., 2012). Contrarily, 5-HT_{4a}-receptor agonists counteract respiratory depression by increasing cAMP levels without affecting analgesia (Manzke et al., 2003).

BIMU8 is a benzimidazolone derivative synthesized by Boehringer Ingelheim (Italy), in an attempt to develop a novel 5-HT_3 receptor antagonist. They found unexpected prokinetic activity which was due to activation of 5-HT_4 receptors (Schiantarelli et al., 1990). Later it was shown that, BIMU8 can counteract OIRD without any effect on analgesic efficacy of opioids (Manzke et al., 2003; Wang et al., 2007). We used BIMU8 in an attempt to prove its effectiveness in counteracting OIRD in rabbits, a species exquisitely sensitive to this side effect. Unexpectedly, we observed serious disturbances in ECG recordings

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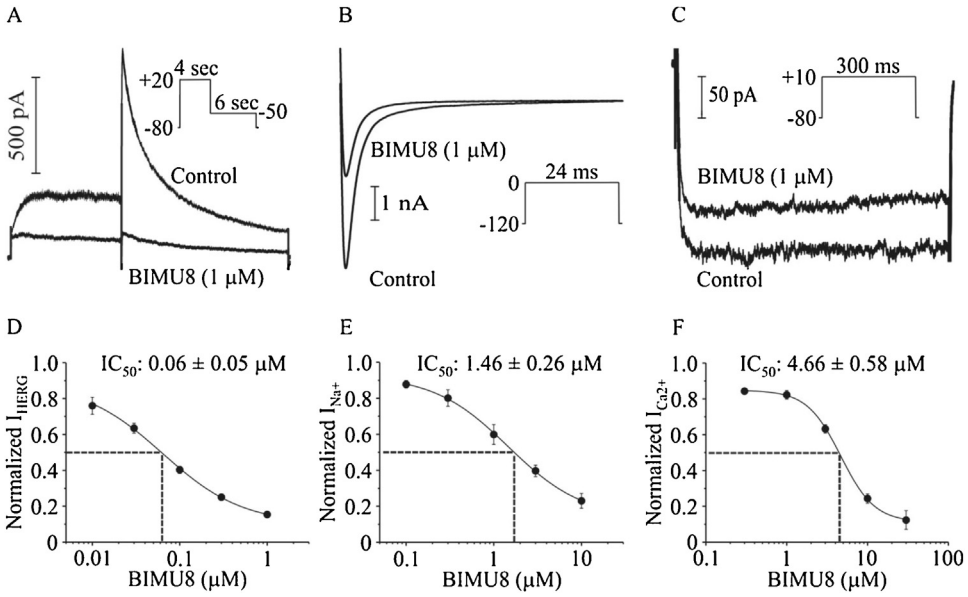

Fig. 1. Original current recordings and respective IC_{50} values. Representative current tracing showing blockade of (A) outward hERG potassium currents, (B) inward $Na_V1.5$ sodium currents, (C) inward L-type $Ca_V1.2$ calcium currents, by application of 1 μM BIMU8 to compare respective blockade for each ion channel, while inset shows applied pulse protocol. IC_{50} values of BIMU8 are indicated for (D) hERG channel, (E) $Na_V1.5$ channel, (F) $Ca_V1.2$ channel, and calculated by recording and fitting Hill equation on the respective blocked currents by application of different concentrations of BIMU8. Each drug concentration was checked on 3–5 different cells.

(unpublished data), which encouraged us to explore the mechanism underlying the cardiac effects. Previously it has been described that BIMU8 can increase L-type calcium current (I_{Ca}) indirectly in human atrial myocytes due to elevated levels of cAMP mediated by 5-HT_4 receptor activation (Ouadid et al., 1992). It also decreased voltage gated potassium current in colliculi neurons from mouse embryo by the same mechanism (Fagni et al., 1992). Because 5-HT_4 receptors are not expressed in the rabbit heart (Castro et al., 2005; Ouadid et al., 1992), the ECG changes observed after BIMU8 administration suggest a direct interaction of the drug with ion channels. Therefore, in the present study we investigated the effect of BIMU8 on cardiac HERG ($K_V11.1$), L-type calcium ($Ca_V1.2$) and sodium ($Na_V1.5$) channels. All the three described channels were expressed in HEK-293 cells and the effect of BIMU8 was assessed by whole-cell voltage clamp experiments.

2. Materials and methods

2.1. Chemicals and synthesis of BIMU8

BIMU8 was kindly provided by Dr. Hanno Gerritsmann, Research Institute of Wild Life Ecology, University of Veterinary Medicine, Vienna. Minimum essential medium (MEM), Dulbecco's modified Eagle medium (DMEM), MEM non-essential amino acids (MEM NEAA), sodium pyruvate, geniticin, penicillin/streptomycin, L-glutamine were purchased from Gibco Thermo Fischer Scientific Incorporation. All other chemicals were purchased from Sigma-Aldrich Corporation, unless otherwise mentioned.

2.2. DNA clones, transfection and cell culture

HEK-293 cells stably expressing hERG channels were obtained from Prof. Steffen Hering, University of Vienna, Austria (Grienke et al., 2015), while HEK-293 cells stably expressing $Na_V1.5$ channels were donated by Prof. Jonathan C Makielski, University of Wisconsin, USA (Makielski et al., 2003). Stable cell lines were maintained in MEM growth medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids and 400 ng/ml geniticin until 80% confluency and sub-cultured on glass coverslips 6 h before experiments. The cDNA encoding $Ca_V1.2$ α_{1c} kindly provided by Dr. M. Grabner, University of Colorado, USA (Grabner et al., 1998), and auxiliary β_{2a} (Perez-Reyes et al., 1992) and α_2-δ_1 (Ellis et al., 1988) were transiently transfected in HEK-293 cells in 2:1:1 μg ratio by using X-tremeGene transfection reagent (Roche) according to manufacturer's protocol. For transient transfection HEK-293 cells were maintained in DMEM growth medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and experiments were performed 36–48 h after transient transfection.

2.3. Electrophysiology

Electrophysiological experiments were carried out as described previously (Iqbal et al., 2015). Briefly, glass capillaries (Harvard Apparatus Ltd.) were pulled by DMZ universal puller and the electrodes having resistance greater than 5 MΩ were discarded. Cells were placed in one ml chamber and visualized by inverted microscope (Axiovert S100TV). Whole-cell ionic currents were measured by Axopatch 200B amplifier, while data was digitized at 100 kHz by using Digidata 1440A and low pass filtered at 5 kHz. Liquid junction potentials were corrected online by Clampex and after obtaining whole-cell configuration both whole-cell capacitance and series resistance were compensated. Sodium currents were evoked by a test pulse of 24 ms to 0 mV. Activation curves were recorded by depolarizing pulses ranging from −100 to +70 mV for 24 ms, while inactivation curves were obtained by prepulses of 1 s from −120 to −30 mV in 5 or 10 mV increments followed by a 24 ms test pulse to 0 mV. The holding potential was kept constant at −140 mV throughout experiments of sodium channel and a 10 s gap was given between each sweep. The intracellular solution contained CsF 120 mM, CsCl 20 mM, HEPES 5 mM, EGTA 5 mM, while extracellular solution was composed of NaCl 20 mM, Choline chloride 120 mM, KCl 4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.75 mM and HEPES 5 mM. The pH of both solutions was maintained at 7.4 with CsOH and NaOH, respectively. Ionic currents from HEK-293 cells stably expressing hERG channels were evoked by a prepulse of 4 s at +20 mV followed by a test pulse of −50 mV for 6 s, while pulse protocol for step and tail currents included prepulses of 4 s ranging −70 to +30 in 10 mV increments followed by a test pulse of −50 mV for 6 s. The holding potential was kept at −80 mV for all hERG channel experiments. The intracellular solution contained KCl 130 mM, $MgCl_2$ 1 mM, EGTA 5 mM, HEPES 10 mM, Mg-ATP 5 mM, while the composition of extracellular solution was NaCl 143 mM, KCl 5.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, HEPES 5 mM, NaH_2PO_4 0.33 mM and glucose 16.6 mM. The pH of extracellular and intracellular solution was maintained at 7.4 with NaOH and 7.25 with KOH respectively. Ionic currents from $Ca_V1.2$ channels were evoked by a pulse of 300 ms at +10 mV. The pulse protocol for activation consisted of 300 ms pulses to potentials between −80 mV