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creasing of infarction size and improvement of myocardial function [Hendgen-Cotta et al., 2008].

Nitrite-reductase activity was demonstrated *in vivo* in endothelial myoglobin of blood vessels .Produced NO activates canonical soluble guanylate cyclase/cGMP signaling pathways. It plays key role in regulation of hypoxic vasodilatation of vessels and explains about 50% of vasodilatation [Totsek et al., 2012]. In a mice having deficit for Mb nitrite- induced effects (vasodilatation and decreasing of blood pressure) were decreased or absent.

More detailed description of enzymatic properties of Mb and other heme-containing globin proteins is presented in review [Kuleva, Krasovskaya, 2015, Tsitologia v.57, №8, p.563-571].

These enzymatic properties of Mb could contribute into extreme tolerance to internal hypoxia in whales during long-term diving. When 5 species of whales with different diving time were studied and the data were compared with similar data for other species of cetaceans studied before [Helbo, Fago, 2012] it was shown that some difference between myoglobin of different species was really exists on O_2-binding , nitrite-reductase and peroxidase activities, but these variations did not correlated to average time of diving. Rather, they may reflect phylogeny and the difference in foraging behavior of cetaceans as the most significant difference was revealed between toothe and baleen whales, toothed whales having higher O_2 affinity and nitrite reductase activity. .

Average time and depth of diving strongly correlates to myoglobin concentration in muscle tissue of whales and this correlation results in high increase of total activity of myoglobin in organism *in vivo* (including capability to store O_2 and reduce NO_2 into NO). This may increase in high degree the time of diving of long-time diving animals. Therefore. diving duration is rather determined by myoglobin content in organism than myoglobin specific functional reactivity as nitrate-reductase.

It is interesting that terrestrial mammals living in high-land (for example, camelides) and adapted to chronic hypoxia have increased level of myoglobin in their skeletal muscle as compared with their relatives living in low-land (cited according to [Helbo et al, 2013]).

Thus, total nitrite-reductase activity of myoglobin in whole organism may be one of factors determining hypoxia tolerance in mammals.

H_2S INHIBITED HIPPOCAMPAL NETWORK ACTIVITY IN NEWBORN

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Hydrogen sulfide (H_2S), produced by the desulfuration of cysteine or homocysteine, functions as a signaling molecule in an array of physiological processes. In the nervous system H2S induces long-term potentiation in the hippocampus modulates neuronal excitability of subfornical organ, the nucleus of the solitary

tract and trigeminal neurons, modulates transmitter release as well as exo- and endocytosis of synaptic vesicles in motor nerve endings [Abe and Kimura, 1996, Malik and Ferguson, 2015, Chen et al., 2013; Gerasimova et al., 2015]. H_2S action is critically dependent from its concentration and cellular location: high H_2S concentrations may lead to a complete inhibition of cell respiration, mitochondrial depolarization and superoxide generation, whereas low physiological concentration exerts antioxidative effects [Wedmann et al., 2014]. It was shown that H_2S can change neuronal excitability through modulation of Na^+ channels [Kuksis and Ferguson, 2015] and different types of K^+ channels [Pan et al., 2010; Mustafina et al., 2015], Cl^- channels [Tang et al., 2010] and Ca^{2+} channels [Kukis and Ferguson, 2015]. In spite of the increasing acceptance of hydrogen sulfide as a neuromodulator and of the reported alterations of neuronal excitability, there is no report on the putative alterations of in hippocampi neurons. The aim of our research to examine the influence of H_2S on the voltage-dependent Na^+ currents and K^+ currents, since such currents are an important factor in the regulation of excitability.

Experiments were performed on neonatal Wistar rats (postnatal days P3–P7). The work has been carried out in accordance with EU Directive 2010/63/EU for animal experiments and all animal-use protocols were approved and Kazan Federal University on the use of laboratory animals (ethical approval by the Institutional Animal Care and Use Committee of Kazan State Medical University N9-2013). After isolation, the rat brains were placed into a cooled oxygenated artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 126; KCl 3.5; $CaCl_2$ 2.0, $MgCl_2$ 1.3, $NaHCO_3$ 25, NaH_2PO_4 1.25 and glucose 10, (pH 7.4). Horizontal slices (400 μm thick) were cut using a HM 650 V vibratome (Microm International, Germany). All recordings were made from the CA3 pyramidal cell layer under visual control by means of an Axio Examiner A1 microscope (400×, Carl Zeiss, Germany) using differential interference contrast and patch-clamp amplifier Axopatch 200B (Axon Instruments, USA). For whole-cell recordings electrodes filled with either 135 mM potassium gluconate solution contained (mM): $CaCl_2$ – 0.1, EGTA – 1, HEPES – 10, NaATP – 2 and NaGTP – 0.4 (pH 7.25), osmolarity 290 mOsm, with the pH adjusted to 7.3 with CsOH or KOH. To study Na^+ currents a voltage clamp protocol was used with depolarizing test pulses of 250 ms duration which were applied at 10 mV increments from −70 to +40 mV from a holding potential of –75 mV using cesium methylsulphate based pipette solution and TEA 2 mM in external solutions. Current–Voltage (*I–V*) relationships were constructed from measurements of peak inward currents. To estimate an inactivation profile of Na^+ currents we used an inactivation step protocol by holding neurons at -75 mV, inducing 100 ms voltage steps from –110 to –20 mV at 10 mV increments and then finally stepping the voltage to the test potential of –10 mV for 500 ms where the measurement was taken. To study K^+ currents a voltage clamp protocol was used with depolarizing test pulses of 250 ms duration which were applied at 10 mV increments from −130 to +40 mV from a holding potential of –75 mV using potassium gluconate based pipette solution and TTX (1 μM) in external solutions. Current–voltage (*I–V*) relationships were constructed from measure-

ments of current amplitudes at the end of the 250 ms test pulses. The signals were digitized using an analog-to-digital converter (Digidata 1440A, Axon Instruments, Molecular Devices, USA). PClamp10.3n (Axon Instruments, USA), and Origin 8.5 (OriginLab Corporation, USA) programs were used for acquisition and analysis of electrophysiological data. Group measurements are expressed as mean ± SEM; error bars through data points indicate SEM. The statistical significance of differences was assessed with Student's *t*-test. The level of significance was set at $p < 0.05$. For a source of H_2S was used sodium hydrosulfide (NaHS, Sigma-Aldrich, USA) and in solution this compound dissociates to give HS^- which associates with H^+ to produce H_2S. Our previous experiments indicate that from base concentration of NaHS, only 11–13% is effective as H_2S in solution when taking pH, temperature, salinity of the perfusate and evaporation of H_2S into account [Sitdikova et al., 2014]. Stock solutions of NaHS were prepared immediately before each experiment and kept hermetically sealed in a dark place. In control conditions a current peak of I_{Na} activation was observed at –30 mV with amplitudes of 1.4 nA and the voltage of half-maximal activation were –35 mV mV ($n = 8$). Bath application of 100 μM NaHS induced a rightward shift of I_{Na} activation current peaks at –10 mV without changing the amplitude of inward currents. The voltage of half-maximal activation also shifted to close 0 mV and were –11 mV ($n = 7$). Application of an inactivation step protocol revealed a rightward shift of the inactivation curve in response to 100 μM NaHS. It was shown that half-maximal inactivation shifted in average from –47 mV ($n = 8$) compared to control conditions with –29 ($n = 7$, $p<0.05$) after NaHS application. Thus NaHS did not influence the I_{Na} amplitude but induced a rightward shift of the activation curve which in turn increases the threshold of action potentials and decreases excitability. Bath application of TTX (1 μM) did not prevent NaHS-induced depolarization of the membrane potential of CA3 pyramidal neurons.

Also NaHS had effects on the K^+ channels of hippocampal neurons. So, bath application of NaHS induced the reduction of outward K^+ currents at voltages from +5 to + 40 mV ($n=3$, $p<0.05$) and these effects were prevented by preliminary application of TEA (5 mM).

So in hippocampal CA3 neurons NaHS reduced outward potassium currents which may underlie the neuron's depolarization. Also H_2S induced rightward shift of activation and inactivation of Na^+ current thus decreasing neuronal excitability and preventing network activity. In conclusion, our experiments reveal a potential neuromodulatory role of H_2S in the nervous system by direct regulation of ion channels.

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References

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