Pressure, the nervous system and ion channels: Are humans too complicated?

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World records for apnea diving in humans have recently been awarded by AIDA (International Association for the Development of Apnea [1]) at around 250 meters (dynamic apnea diving, men) (AIDA International). The depth records for scuba dives on gas mixtures for human divers are settled at around ~315 meters of sea water (msw). In 2006, a U.S. Navy diver submerged to ~650 m using an atmospheric diving system suit (ADS), which helps maintain atmospheric pressure.

Without hard-suit technology, technical divers can in principle manage to descend close to ~500 meters. However, this is still a long way to catch up with the depth profiles of some marine mammals – for instance, bottlenose whales (1,200–1,600 meters; [2]) or elephant seals (1,000–1,500 meters; [3]).

Some of the most obvious adaptations to pressure by marine mammals – i.e., larger oxygen stores and partial lung deflation upon dives [4] – probably do not account for their marked depth limits compared to humans, even if they could be technically transferred to human divers.

High-pressure nervous syndrome, also referred to as high-pressure neurological syndrome (HPNS), is still the most limiting condition that sets depth limits in diving humans. Open sea- and simulated chamber dives are routinely associated with symptoms of electroencephalogram (EEG) changes, motor miscoordination or disorientation at depths exceeding 200 msw (>2.1 MPa) [5].

In He-N₂-O₂ dives to 450 meters, technical divers were reported with severe sleep disturbances and EEG changes [6]. One of the typical measures for HPNS sensitivity has been the convulsion threshold in different species. Early work had shown much lower convulsion thresholds for amphibians than mammals; but also among mammals, there is some hierarchy with more intermediate thresholds in rodents as compared to primates [7].

The human brain contains about 10¹⁰ neurons, with some 10¹⁴ synapses in total. For the mouse brain, 10⁷ neurons and 8 x 10¹⁰ synapses have been counted [8]. Figures for marine mammals are sparse, but studies on dolphin brains calculate ~75% of the neuron mass of the human brain, corrected for dolphins’ larger cortical surface. This number may also be similar in cetaceans, but there are no estimates for the number of synapses in those species [9].

Yet, this is the most important point, as the complexity of the brain is not primarily given by its number of neurons but by the connections within the network. At least for terrestrial mammals it seems that the network complexity might be directly related to HPNS sensitivity of a particular brain. In fact, there is a general imbalance of network switches in the mammalian brain toward inhibitory synapses that make up the majority of synapses in the central nervous system. As a result, convulsions or seizures are usually considered as a breakdown of inhibitory synapse activity, with a relative gain of excitatory nerve cell inputs. For example, general seizures are successfully treated with benzodiazepines in the acute state – i.e., drugs that re-establish inhibitory synapse function by opening of inhibitory Cl⁻ channels [10].

All the basic properties of excitable tissues – i.e., nerves, sensory organs, heart and skeletal muscle – to receive, process and deliver electrical information are defined by their ion channel composition and function. Understanding the specific interactions of high pressure with the network components within the brain can be tracked down to decipher how high pressure specifically interferes with voltage-dependent or ligand-gated ion channels.

In the present issue of UHM, Aviner and colleagues give a thorough review on current concepts of high-pressure effects on voltage-gated ion channels with a focus on the Ca²⁺ channel family (VDCC) that is particularly important for synaptic transmission. The
integrated network output of a single neuron at its axon hillock is a result of proper presynaptic transmitter release, local post-synaptic potential generation (either excitatory or inhibitory, EPSP or IPSP) and electrotonic conduction along the soma membrane, where it constructively or destructively interferes with post-synaptic potentials from thousands of other dendrite synapses to process a compound potential that reaches the axon hillock. If the latter is supra-threshold, it is only then that a single action potential is generated and conducted to the next neuronal switch. As stated by the authors, high pressure in general profoundly depressed synaptic transmission in all synapses examined so far [11], e.g., reducing population field EPSPs to different extents.

In their earlier work, the Grossman lab was able to show that presynaptic Ca\(^{2+}\) influx through N-type Ca\(^{2+}\) channels was one of the sensitive pressure targets, and pressure strongly impaired Ca\(^{2+}\) influx into the axonal terminal boutons, which is necessary for vesicle fusion with the membrane to release the neurotransmitters for signal transduction [12]. Several studies using blockers of N-type Ca\(^{2+}\) channels or lowering extracellular Ca\(^{2+}\) mimicked or aggravated high pressure’s action on synaptic transmission and provided indirect evidence for its presynaptic site of action [13,14]. However, the situation in central synapses is complicated by the fact that they always contain a potpourri of different Ca\(^{2+}\) channels with different activation and inactivation kinetics, localizations and pressure sensitivities. Some of them are predominantly voltage-gated, while others are exclusively ligand-gated. For example, post-synaptic ion channels are usually ligand-gated in the central and peripheral nervous system and react on binding of their corresponding neurotransmitter (e.g., glutamate, GABA, acetylcholine and similar agents). Various members of the voltage-gated Ca\(^{2+}\) channel family can be found pre- and/or post-synaptically, which renders identification of pressure effects particularly difficult to discriminate between pre- or post-synaptic action or to ascribe them to one certain channel entity in an intact synapse that cannot be further simplified. The present review gives a good current overview over the VDCC family in Table 1 (Page 249).

So, how can one obtain detailed information of the pressure sensitivity and specific alterations induced by high pressures in a single class of ion channels? Recording biophysical properties of ion channels under high pressures is challenging. From the considerations above, one would have to first decide whether to prepare a “pure” system that contains only the ion channel of interest or instead use an intact preparation in which to pharmacologically dissect the contributions from different channels to overall ion flux. Then, one would have to consider how to incorporate the recording electrode setup into a pressure vessel. Penetrating microelectrode or whole-cell patch-clamp recordings are particularly sophisticated, as any mechanical disturbance during handling inside the pressure vessel can easily rupture the membrane or patch. Some of the manually very skillful work from Heinemann et al. [15] introduced the “flying patch” clamp technique, where a patch-clamp seal was formed first in saline under atmospheric conditions, and then the holder with the patch still attached was transferred to a pressure vessel.

In this configuration, activation volumes and net volume increases of ~80 A\(^3\) for acetylcholine receptor channel transitions from the agonist-free closed state to the open state could be determined from thermodynamic equations. With this technique, single-channel conductance was also found to be very pressure-resistant, making pressure act on the channel’s transition kinetics in the first place [15], a concept that has become generally accepted for other channels [16].

Finally, the nature of pressure medium needs consideration. In studies involving flying patches, hydrostatic pressure could be employed. However, when using brain slice preparations, pressurizing with water or oil is not feasible, as sealing the pressure medium against a setting that contains the tissue slice alongside, with eventually different recording and stimulation electrodes, is hardly possible. In that case, hyperbaric pressure is usually attained with compressed helium over the saline layer (e.g., [17]). However, again, we are then dealing with the “complex in situ” system that routinely measures field potentials in the extracellular space under pressure. Although all the channels remain in their natural environment, selective study of one channel class is difficult, if not impossible, if all but one VDCC class would have to be blocked pharmacologically.

One exit to this dilemma was the idea of using stable expression systems for a unique single-channel class. Xenopus oocytes are known for their reliable ability to synthesize foreign proteins when injected with exogenous mRNA. They
are very stable cells mechanically, and with a penetrating microelectrode setting it was possible to record ion currents from exogenously expressed K⁺ channels under hydrostatic (oil) pressure conditions [18].

In the present issue, Aviner and colleagues took advantage of this technique and have given preliminary results on isolated expression of neuronal VDCC in *Xenopus* oocytes and pressure effects on channel conductances. High pressure in the range of 1 MPa markedly reduced currents through the Cav3.2 channel (low-voltage threshold T-type Ca channel, TTCC). Surprisingly, high pressure (5.0 MPa) doubled maximum currents mediated by the high-voltage threshold L-type Cav1.2 VDCC. Such a result would have an immediate relevance for the high-pressure synaptic transmission on the post-synaptic input membrane of neuronal networks, where Cav1.2 VDCC can be in close proximity to Cav3.2, NMDA or AMPA (transmitter: glutamate) channels. In order to predict high-pressure effects on neuronal output spike generation within a neuronal channel ensemble, mathematical modeling and simulation of channel activity is the method of choice. Aviner and colleagues finally give results from such a model incorporating macroscopic conductances for lTCC (Cav1.2), AMPA and NMDA to show that high-pressure enhancement of lTCC alone does not increase synaptic output but crucially relies on enhanced synaptic input from glutamate receptors. This and recent evidence from the Grossman lab shows that high-pressure nervous syndrome is expected to be a result from pre- and post-synaptic modifications: Presynaptic impairment of inhibitory synaptic input is one consequence to hyperexcitability, but post-synaptic enhancement of excitatory glutamatergic transmission boosted by voltage-dependent Ca²⁺ channels is another new option.

What do these findings mean for HPNS? The pressure profile of different VDCCs is important for predicted symptoms, as channels with the highest pressure sensitivity present the exposure-limiting step on a human diver. This seems to be the TTCC with a high-pressure sensitivity around 1 MPa, where HPNS symptoms seem to start. However, symptoms are not stereotypic or 100% predictable, because the regional distribution of each VDCC plays a pivotal role. In their discussion, Aviner and colleagues finally give a summary about regional aggregation of various VDCCs. For example, the preferential localization of TTCC to the reticular thalamic region responsible for vigilance, as well as in the striatum that acts as a switch for sensory inputs to the cortex and the ARAS system (ascending reticular activating system), is compatible with observed EEG changes in human divers experiencing HPNS [6]. LTCC potentiation could eventually account for hyperexcitability in the hippocampus region that might disturb impulse or memory in affected individuals. Precise mapping of VDCC within the brain systems might eventually help to explain some of the symptoms associated with HPNS.

What makes some ion channels within the same family behave differently? This question is intuitive, given the fact that most ion channel families are highly conserved regarding amino acid sequences or tertiary structure. However, one has to bear in mind that it is not only the channel itself that regulates its function but also auxiliary subunits [19], the cytoskeleton [20] and the membrane environment. Therefore, it is still a long way to decipher all the mechanisms involved that are activated by high pressure in various parts of the brain that contribute to HPNS. In the future, new venues involving high-pressure microscopy of ion channel function using fluorescent dyes might offer more comfortable ways to study HP effects on channels within the intact preparation (21).

So, coming back to our initial thought: Not all mammals are created equal. Why do whales not experience HPNS; or do they, and we just do not know it? What makes their pressure limits exceed ours by several times? From the considerations in the beginning paragraph, it is probably not a lack of complexity of their brain, but we cannot say for sure, as available data is limited. However, past research from my group has pointed toward a possibility of pressure limits for another organ that might be of significance for diving mammals: skeletal muscle. Intriguingly, skeletal muscle function, the main locomotion organ, seemed to show a rather sharp pressure limit for reversible pressure-induced changes to muscle performance and functionality around ~20 MPa when established in skeletal muscle from terrestrial mammals (20 MPa x 20-minute exposure product, [22,23]). Interestingly, this exactly parallels diving profiles of some of the deepest-diving mammals. And probably, you are not surprised anymore: Again, it is Ca²⁺ channels involved in setting these limits.
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