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Pressure effects on membrane-based functions and energy metabolism: a review

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This review will consider the effects of hydrostatic pressure on some cellular functions related to membrane-localized processes. After a general survey of experimental evidence showing the wide variety of membrane-linked mechanisms that are perturbed by changes in hydrostatic pressure, it will focus on the pressure-sensitivity of the processes involved in ionic and osmotic regulation in crabs and fish, including membrane-localized ATPases and oxidative metabolism. The results of long-term exposure (30 days) of freshwater eels *Anguilla anguilla* at 101 ATA of hydrostatic pressure clearly indicate Na^+ balance impairment at the tissue level (muscle and gill). That impairment occurs at the same time as a new state of energetic metabolism which results from adjustments of intertissue coupling of anaerobic and aerobic metabolisms. Considering its life cycle, however, *Anguilla* can reasonably be considered as 'preadapted' to pressure. Experiments conducted on the crab *Eriocheir sinensis*, which normally never encounters high levels of pressure, show that physiological processes involved in hydromineral balance control are outstandingly resistant to pressure. Disturbances in hydromineral balance and energetic metabolism are rapidly corrected and adjusted to a new state of activity.

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Membrane state and functions

It is well known that cellular and subcellular membranes consist of a bilayer of phospholipids with embedded proteins. These proteins can be receptors, ion channels, or enzymes, as in enzymatic complexes of the respiratory chain (internal membrane of the mitochondria) or ATPases enabling ion transfer. Owing to this structure, a modification of the physical state of the membrane can modify its related functions.

Membrane phospholipids are arranged in bilayers and are present in a physical form which is intermediate between a solution (sol) and a gel. This physical state is responsible for the flexibility, rigidity and viscosity of the membrane. As a gel is an ordered state (less fluid membrane), molecules are condensed and occupy a lesser volume. Thus a membrane can be between two states, disordered (fluid, sol) or ordered (rigid, gel). The sol-gel equilibrium can be modified with temperature and/or pressure following the Clausius-Clapeyron equation:

$$\frac{dT}{dP} = \frac{\Delta V \cdot T}{\Delta H}$$

where T is temperature, and ΔV and ΔH are the volume and enthalpy variations of the system during the reaction. As an increase of the temperature induces the change from the gel state to the sol state (phase transition), the dT/dP ratio is the increase in transition temperature observed when pressure P is increased. Such an equation shows a fundamental point: pressure and temperature can act in opposite directions. For example, a pressure increase (constant T) or a temperature decrease (constant P) induces a shift of the equilibrium towards the gel state with changes in enthalpy and volume (Macdonald 1984). Such changes in the membrane physical state are the results of molecular changes and their consequences for membrane functions. Thus, pressure and temperature can modify membrane quality and consequently the functioning of the included proteins, just impeding protein deformation inside the membrane for example. Moreover, considering only enzyme reactions, Johnson & Eyring (1970) have shown that kinetics can be affected by pressure and /or temperature following the equation,

$$K_p = K_0 \, e^{(-P\Delta V^{\neq}/RT)}$$

where K_0 and K_p are the rates at $P = 0$ and P respectively, T is temperature, and $\Delta V^{\neq}$ is the activation volume.

Why and how to study high pressure ?

Oceanic waters represent 70% of the earth's surface with a mean depth of about 3800 m. Consequently, 80% of the biosphere is deeper than 1000 m (i.e. 100 atmospheres pressure). These data alone justify scientific interest in pressure effects. However, although temperature effects are often studied, there are few laboratories working with pressure on whole animals, probably owing to the difficult techniques and their cost. In this review, we consider only hydrostatic pressure (pressure *per se*). In fact, when a mammal is exposed to pressure, it is really submitted to hydrostatic pressure (*HP*) but also to gas pressure (*GP*) owing to air breathing. As *HP* and *GP* can be antagonists, it is impossible to isolate the specific pressure effects. For this reason, we have chosen aquatic animals (fish, crabs) which breathe water, the density of which is very little modified by pressure, so that ventilation is not impeded (as it is the case in mammals). Especially designed experimental aquaria are used for such experiments, placed in a hyperbaric chamber equipped with a high pressure water circulation system (Sébert, Barthélémy & Simon 1990).

Three different approaches are possible for studying pressure effects.

(a) *Using models* of membranes, tissues or isolated organs. This is interesting as a first approach to obtain basic data and

physicochemical results, but the integration in animal physiology is difficult. For example, results from such experiments show a temperature/pressure factor of about –3°C/100 atm (see Brauer, Jordan, Miller, Johnson, Dutcher & Sheeman 1985). That means that using the models to obtain the effects of a 3°C temperature decrease, it is necessary to apply 100 atmospheres pressure (101 ATA). However, for example, trout survive such a temperature decrease without any problem but are killed when exposed to 100 atm hydrostatic pressure (101 ATA).

(b) Using deep water species. This is the more common approach and over about 20 years many results have been obtained (see Somero 1991) which show that deep sea species (fish and crustaceans) have higher enzyme structural stability, and lower enzyme activities (aerobic and anaerobic pathways, ion-activated ATPases such as the Na^+–K^+.ATPase) with kinetics relatively unaffected by pressure. The general decrease observed for metabolism is related to a supposed decrease in locomotor activity (see Figure 1 and Gibbs 1997). Such experiments with deep-living species are very expensive (because they require a specially equipped vessel) and the animals which are trawled are often moribund when they arrive at the surface. Moreover, the environment at depth is complex and does not involve only high pressure: there is also a decrease in temperature, light intensity, pH, oxygen content, food availability and other factors. Consequently, the specific effects of pressure are impossible to describe.

(c) Using shallow water species exposed to high pressure. We have chosen this approach to isolate the pressure factor, using shallow-water species submitted to hydrostatic pressure in a hyperbaric chamber (see section 'Effects of hydrostatic pressure'). The method has been used with fish (yellow freshwater eel, *Anguilla anguilla*) and crustaceans (Chinese crab, *Eriocheir sinensis*). In their life cycles, eels experience high pressure during migration towards the Sargasso sea, the crab does not.

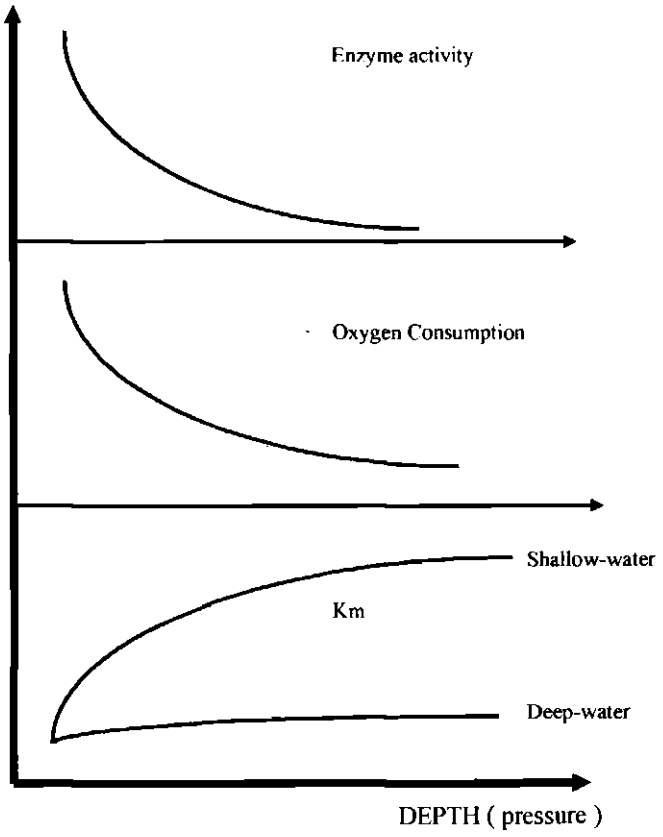

Figure 1 Schematic effects of pressure on enzyme activity, K_m in fish muscle and oxygen consumption of a fish or crustacean living at depth. The figure shows that pressure induces a decrease of the affinity of the enzyme for its substrate (K_m increase), concomitant with a decrease in metabolic activity (drop in oxygen consumption and in enzyme activity).

Effects of hydrostatic pressure

When these aquatic animals are exposed to *HP* = 101ATA for short periods (some hours to some days), they exhibit an increase in oxygen consumption, MO_2, during the compression period. The maximum value is obtained at the end of compression, then MO_2 progressively decreases and reaches a steady state (about 65% of the control value) after 6 to 8 days under pressure (Sébert, Péqueux, Simon & Barthélémy 1995; Sébert, Simon & Péqueux 1997). It has been previously shown that the MO_2 decrease is not due to an alteration of O_2 transport from the ambient medium to the cell, but to a decrease of oxygen use at the cell level. Therefore, it has been proposed that *HP* induces a metabolic state resembling histotoxic hypoxia that is an alteration of aerobic energy production (Sébert, Simon & Barthélémy 1993). Briefly, *HP* induces a decrease of membrane fluidity which alters substrate transfer and the functioning of the enzymes included in the phospholipid bilayer, here the enzymatic complexes of the respiratory chain. The consequences are (1) a decrease in electron flux through the respiratory chain and thus a decrease of O_2 use as electron acceptor, and (2) a decrease in energy production (ATP) by this route. This change in aerobic pathway is balanced with an activation of the anaerobic pathway. Note that all the steps of this scheme have been experimentally shown (see Sébert 1997 for review). However, the alteration in the aerobic pathway is only transient: after four weeks under pressure, nucleotide muscle content, enzyme activities, and energy charge are similar to control values (before compression). Moreover, when measured at 1 ATA, the membrane fluidity of acclimated fish is higher than in controls, which supposes its restoration during the acclimation period via a modification of membrane composition. It appears that shallow water species (eel, crab) are able to acclimate to high pressure.

The above seems to show that the adverse effects of pressure are only transient. However, some specific work on the eel shows that *HP* also induces morphological changes. This is the case in white muscle, where a shift towards smaller fibre areas is observed, together with a decrease in protein content as observed in deep-living fish (Simon, Sébert & Barthélémy 1991). Moreover, still in white muscle, it has been shown recently that *HP* not only induces changes in enzyme activities but is able to modify the metabolic design i.e a complete pathway. This is the case for the glycolytic pathway, where acclimation to *HP* induces an increase of aerobic and anaerobic fluxes, together with a decrease in the transition time from aerobic to anaerobic flux. In other words, the yellow freshwater eel (non-migrating state in freshwater) acclimated to high pressure exhibits metabolic features in

accordance with its migratory activity: increase of aerobic flux (long travel from Europe to Sargasso sea), increase of anaerobic flux together with a decrease of transition time (allows the fish to escape from predators). These changes concerning energy metabolism and metabolic design are in agreement with the hypothesis that *HP* can be considered as an ecophysiological signal to trigger physiological changes allowing eel migration. However, the same changes in metabolic design have been observed with the crab (which never experiences *HP* during its life cycle) exposed to high pressure, which means that *HP* is perhaps an ecophysiological signal for the eel but also has specific and integrated effects. This conclusion is strengthened by another example of the morphological changes induced by *HP*, concerning the gill. It has been recently shown (Dunel-Erb, Sébert, Chevalier, Simon & Barthélémy 1996) that in yellow eel acclimated to high pressure (101 ATA) there is a strong increase in the number of chloride cells, as in the gill of a sea-water fish, despite the fact that the fish is acclimated to freshwater. These results (1) confirm the fact that hydrostatic pressure can be an ecophysiological signal for the eel, and (2) suggest that modifications concerning ion transfer and osmoregulation in shallow-water species exposed to high pressure must occur.

That pressure affects the permeability properties of cell membranes and all associated phenomena has been clearly established and is now well documented (Péqueux & Gilles 1986; Cossins & Macdonald 1989; Somero 1991). Many pressure effects which can be considered as indirect evidence of membrane property disturbances have been shown in the conduction of nerve impulses, the maintenance of nerve resting potentials, synaptic transmission, the transmembrane electrical potential difference of epithelial cells, the activities of membrane-localized enzymes such as the ion-dependent ATPases, neurotransmitter and hormone binding and signal transduction. The sensitivity of these membrane-localized functions to high pressure no doubt determines the wide variety of behavioural reactions reported in response to pressure changes. Related to this, significant evidence of pressure-induced nervous reactions is found in the descriptive reports of convulsions and paralysis which have often been selected as criteria for measuring pressure tolerance, and in the symptoms of heightened neural excitation displayed by vertebrates subjected to pressure and manifested as the High Pressure Neurological (or Nervous) Syndrome (HPNS) (tremors, convulsions, antagonism with anesthetics, uncoordinated movements). Most of this work has been conducted on whole organisms, making thorough interpretation of the pressure effects very complicated, owing to the multiplicity of the various possible sensitive levels. It is likely, in these cases, that comprehensive explanation will involve more complex interactions including processes other than membrane disturbances. Anyway, there is more direct evidence that increased pressure leads to rapid and large-scale alterations in the exchanges of ions between the organism and its environment, and between the cells and the extracellular fluids. It is interesting that the threshold pressure at which significant effects are first observed is low relative to the pressure encountered by deep-living animals. For example, Roer & Shelton (1982) have shown, in the freshwater crayfish *Procambarus clarkii*, that only 15 to 25 atm initiated an inhibition of the Na^+ uptake, while 50 or 100 atmospheres led immediately to an 80% decrease in uptake rate. Similar pressure-induced inhibition of active Na^+ uptake was also demonstrated in freshwater gammarids (Brauer, Bekman, Keyser, Nesbitt, Shvetzov, Sideley & Wright 1980; Roer, Sidelyova, Brauer & Galazii 1984). We made some complex observations on the response to pressure of isolated gills from seawater-acclimated eels *Anguilla anguilla* (Péqueux 1981). These experiments have clearly established that all ionic species, namely Na^+, K^+ and Cl^-, are not affected in the same way by pressure. Higher pressures were needed to affect Cl^- regulation processes (350 atmospheres instead of 250 atm. for Na^+), but then the magnitude of the effects was much larger than for Na^+ ions. This supports the idea that pressure acts selectively on the various transport mechanisms. That it affects all of them is substantiated by experimental evidence that a concomitant inhibition of Na^+ active extrusion processes, an increase of the passive Na^+ entry and of the K^+ permeability contribute to the pressure-induced effects observed (Péqueux & Gilles 1986). More conclusive evidence concerning the pressure sensitivity of passive movements has been obtained with human red blood cells. We have established that the net K^+ efflux from human erythrocytes, essentially considered as passive, increases slightly but almost linearly with pressure to 600–700 atm. Above that pressure range, there is a much more pronounced increase in membrane K^+ permeability (Péqueux, Gilles, Pilwat & Zimmermann 1980; Zimmermann, Pilwat, Péqueux & Gilles 1980). On human and pig erythrocytes, it has further been established that the Na^+ total efflux, as well as the 'ouabain-sensitive' Na^+ efflux which may be considered as a Na^+ pump component, are severely decreased under pressure (30–40% inhibition at 100 ATA; 95% inhibition at 1000 ATA).

These results demonstrate that, at least qualitatively, pressure affects all studied tissues in the same way. Only quantitative differences occur, either in the percentage of inhibition or in the magnitude of the pressure needed to induce inhibition.

All experimental results described above concern experiments conducted for short periods of time under more or less elevated hydrostatic pressure. The question which arises now is whether the transport processes and osmoregulation of crabs and fish are affected when animals are submitted for more or less prolonged periods of time to hydrostatic pressure, in the aquarium specially designed and described by Sébert, Barthélémy & Simon (1990). The results of a study of the effects of long-term (30 days) exposure to 101 ATA hydrostatic pressure of freshwater eels demonstrate that the hydromineral balance of the fish is significantly disturbed upon pressure application (Sébert, Péqueux, Simon & Barthélémy 1991). It is interesting to point out that after one month under pressure, many inorganic osmotic effectors in compressed fish show levels which are not significantly different from controls, but an increase is observed in plasma osmolarity, Na^+, Cl^- and Mg^{2+} contents. In muscle and gills of the same animals, only Na^+ and Cl^- levels increase considerably under pressure, while K^+ and water remain unaffected. If some doubt still exists concerning the exact nature of the changes possibly occurring at the extracellular or at the intracellular level, evidence supports the idea of a redistribution of some inorganic ion species in the course of the induction by

pressure of a new steady state level of the hydromineral balance. It is clear that animals undergo transient disturbances that they are able to overcome as they resume blood characteristics upon prolonged pressure exposure. The idea of a pressure-induced disturbance of the mechanisms responsible for the natremia is further substantiated by results on membrane ATPase activity. Concomitant with the above reported changes in ion contents, the gill (Na^++K^+)ATPase activity has been shown to drop by up to 55% in pressure-exposed animals (Sébert, Péqueux, Simon & Barthélémy 1991). This may explain the reduced activity of the active Na^+ transport processes responsible at the gill level for both the maintenance of a low Na^+ content in the intracellular fluid and the control of the transepithelial Na^+ movements involved in the regulation of the blood sodium content. In addition, the same paper presents evidence of an alteration in the affinity of the enzyme for Na^+ ions, suggesting either an alteration of the kinetic properties of the existing protein or the *de novo* synthesis of a new enzyme molecule. Disturbances in the maintenance of the hydromineral balance have also been observed in Chinese crabs *Eriocheir sinensis* exposed for four days to 101 ATA. It should be pointed out that this animal never experiences high levels of pressure during its life cycle, while it has a comparable cyclic mode of life with a period in freshwater and a period in seawater. As in the eel, plasma osmolarity, Na^+ and Cl^- contents also exhibit a tendency to increase (15, 17 and 28% respectively), as is also the case in muscle and gill tissues. In contrast with the fish, there is moreover a tendency for the maximum activity of the gill (Na^++K^+)ATPase to increase, which could reasonably account for the Na^+ content rise observed in gills, muscle and plasma. Concomitantly, the cytochrome oxidase activity is substantially enhanced in both types of gills (up to 330% in posterior gills) reflecting a substantial increase in oxidative metabolism, hence a likely increase in mitochondrial activity. Despite these alterations, these surface animals themselves, as well as the physiological processes involved in the control of their hydromineral balance when in freshwater, remained outstandingly resistant to pressure and were able to withstand prolonged periods of exposure at 101 ATA without any apparent damage (Sébert *et al.*, 1991). The above results suggest that compensation occurs in the Chinese crab even more rapidly than in the eel. It is interesting to point out that these alterations occur at the time when a new state of energetic metabolism results from adjustments of intertissue coupling of anaerobic and aerobic metabolisms induced by pressure. These conclusions are further substantiated by measurements of adenylate energy charges in the same tissues, showing that energy charges, which are significantly lower after 3 h, have already resumed control values after 6 h and eight days under pressure.

Conclusions and perspectives

It is clear from this paper that membrane function and energy metabolism at elevated hydrostatic pressure are fundamental problems in the field of marine and diving biology.

A major impact of hydrostatic pressure is on the cell membrane and it is evident that acclimation to pressure involves physiological and also structural changes. However, experiments have clearly shown that even when significant pressure-induced disturbances occur upon short-term exposure, they are overcome more or less easily upon long-term exposure. The ability of these animals to live for a prolonged time at high pressure, despite transient alteration of their energy metabolism, their plasma and cellular hydromineral balance, results from a rapid compensation and a subsequent acclimation to the disturbing effects of pressure. The nature of all mechanisms involved is far from being clearly identified and further investigations in the field are obviously needed to provide a clear picture of the pressure sensitivity, and of the acclimation procedure of all the membrane-associated mechanisms at work in osmoregulating species.

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