Osmotic Behaviour of Isolated Axons of a Euryhaline and a Stenohaline Crustacean

The possibility for the cell to regulate its volume seems to be an important factor in the acclimatization of euryhaline species to media of different salinities[1-3]. However, osmometric studies of such a volume regulation process are scanty[4,5]. We have therefore undertaken a study of the osmotic properties of isolated axons of two crustaceans: the euryhaline chinese crab *Eriocheir sinensis* and the stenohaline lobster *Homarus vulgaris*.

Material and methods. Volume modifications are followed on isolated axons with a microscope provided with a camera attachment. The axons are mounted in a Rose chamber, the rubber walls of which are perforated with needles to allow perfusion with salines. Changes in salines through the perfusion system can be effected in less than 1 min. Control saline for both lobster and seawater acclimatised *Eriocheir* has the following composition: Na^+:480; K^+:10; Ca^{2+}:25; Mg^{2+}:10; Cl^-:525 mM/l; H_3BO_3:9 mM, adjusted to pH 7.6 with NaOH, is used as buffer. For experiments with axons of freshwater acclimatised *Eriocheir*, the control saline consists of a twice diluted solution of the saline described above. Hypo- and hyper-osmotic salines are made by varying the amount of NaCl in the control media. Volume changes following osmotic stresses are calculated, assuming the axon to be a radially expanding or contracting cylinder of constant length.

Results. Contrary to the situation observed with lobster axons, *E. sinensis* axons submitted to hypo-osmotic stress ($\pi_1/\pi_2 = 2.0$) show a striking volume readjustment process (Figure 1). The axons tend, indeed, to resume their initial volume after a rapid phase of swelling. Moreover, upon return to control conditions, the axons shrink to volumes smaller than the initial ones. The new volume reached in these conditions remains steady for at least 80 min. In the same way, volume regulation cannot be observed following hyper-osmotic stress ($\pi_1/\pi_2 = 0.5$).

The maximum shrinkage (osmotically inactive volume) which can be extrapolated from the results of the study of axonal volume modifications following various osmotic challenges is the same for both lobster and *E. sinensis* axons. It amounts to about 30–35% of the initial volume (Figure 2). This value is in the range of those reported in the literature for other tissues[4,6-8]. In contrast to this, the maximum swelling of *E. sinensis* axons is only 135% of the original volume. This is much lower than the values found for lobster axons preparations[9].

Discussion. Maximum swelling of *Eriocheir sinensis* axons is much lower than that of lobster axons. This difference may be due to constraints exerted by the elastic properties of the cell membrane over the swelling process[10], which would be larger in *E. sinensis* axons than in lobster ones. It is however more probably due to a volume readjustment process occurring in *E. sinensis* axons and not in lobster ones. As a matter of fact, *E. sinensis* axons submitted to hypo-osmotic stress tend to resume their initial volume after a rapid phase of swelling. Maximum swelling may thus be limited to low values by the initiation of the volume regulation process.

Cell volume regulation can only be demonstrated following hypo-osmotic stress in our experimental conditions. This phenomenon involves adjustment of the

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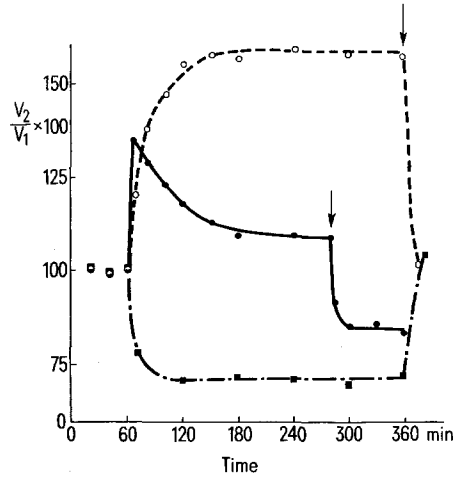

Fig. 1. Effects of hyper-osmotic stress ($\pi_1/\pi_2 = 0.5$) on the volume of *Eriocheir sinensis* axons (■--■) and effect of hypo-osmotic stress ($\pi_1/\pi_2 = 2$) on the volume of *Eriocheir sinensis* (●—●) and *Homarus vulgaris* ○—○) axons. The axons are returned to control conditions at the time marked by the arrow.

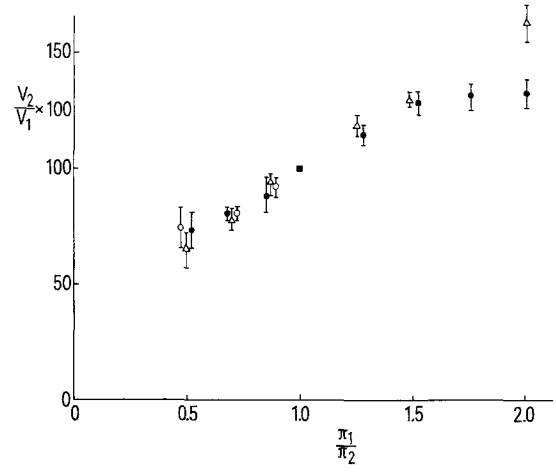

Fig. 2. Volume changes of isolated axons of *Homarus vulgaris* (Δ) and *Eriocheir sinensis* acclimatised to sea water (●) or to fresh water (○) when placed in media of different osmotic pressures. Ordinate: ratio (×100) of the final volume (V_2) to the initial volume (V_1). Abscissa: ratio of the osmotic pressure of the control saline (π_1) to the osmotic pressure of the experimental saline (π_2). In the case of *E. sinensis*, only the peak volume reached during hypo-osmotic stress has been considered.

amount of intracellular osmotic effectors as indicated by the fact that volume shrinks to values lower than control ones upon return of the axons to control saline. Modification of the intracellular fluid osmolarity may be the result of a leak of various solutes from the swollen cells. Amino acids are important osmotic effectors in tissues of marine invertebrates[1-3] and an increase in alanine efflux has been shown to occur in *Callinectes sapidus* axons submitted to hypo-osmotic stress[5]. However we consider that a modification of the amino-acid oxidative metabolism is also involved in the volume readjustment process[11]. Modifications in metabolic activity occur during both hypo- and hyper-osmotic stresses[11], while efflux changes can only be recorded during hypo-osmotic shock[5]. This may account for the fact that volume regulation cannot be observed during hyper-osmotic stress. Indeed, an increase in amino- acid amount large enough to produce volume regulation in hyper-osmotic conditions and effected solely by decrease in catabolism may take longer than the time of our observations.

Lack of volume regulation during hyper-osmotic shock does not appear to be due to non-availability of osmotic effectors in the incubating saline, since volume readjustment cannot be observed in salines supplemented with a 10 mmolar mixture of amino acids. It remains, however, possible that some factor present in the blood of the animal would be required to initiate an increase in uptake of osmotic effectors. This possibility is at present under investigation.

Résumé. Les axones isolés du crustacé euryhalin *Eriocheir sinensis* sont capables d'effectuer une régularisation de volume lorsqu'ils sont soumis à des chocs hypo-osmotiques. Les mécanismes pouvant être impliqués dans ce processus sont discutés.

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Multi-Lamellar Astroglial Wrapping of Neuronal Elements in the Hypothalamus of Rat

Cajal proposed specific glio-neuronal contact relationships in the central nervous system, a conception which was generally accepted. Though such relationships could not be confirmed by electron microscope studies of neocortical tissue[1,2], specific spatial affinity between astroglia and receptive surfaces of neurones or synapses has been recognized in many subcortical regions of the central nervous system[2-5]. The affinity of the astrocyte contacts seems to be restricted not only to special regions but also to distinct neuron types, e.g. in the cerebellar cortex the Purkinje cells are regularly covered by astroglia, the granular and basket cells are only poorly covered if at all[4].

In the nucleus suprachiasmaticus, n. ventromedialis and the area praeoptica, many neuronal elements are surrounded by more than one astroglial lamella. The 2–10 layers result from interdigitations of different lamellar extensions of astrocytic processes. Sporadically one very extended lamella forms a spiral cover around a neuronal element or a synapse. This kind of wrapping occurs on different parts of the neuron, i.e. the dendrites (Figure 2), somata and presynaptic elements. Synapses are enveloped irrespective of their type, i.e. their position on the postsynaptic neuron (soma, dendrite, intrabouton-al spinule) and the structure of their presynaptic elements (different vesicle types, etc., see Figure 1). Thus all discernible types of synapses may be covered in

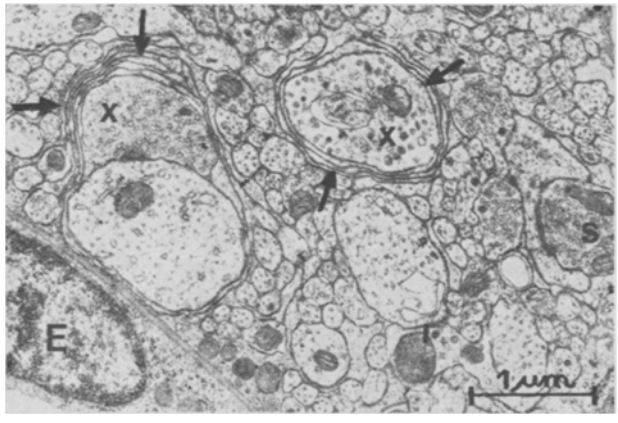

Fig. 1. Multi-lamellar wrapping of 2 synapses (×) by astroglial lamellae (arrows) near a capillary in the n. ventromedialis. Note another synapse (s) with only uni-lamellar, partial covering. E, endothelial cell; cal. bar, 1 µm.

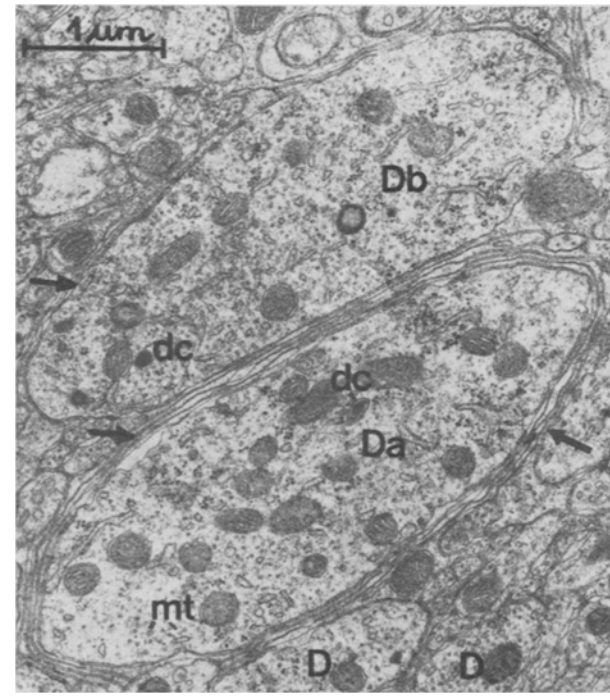

Fig. 2. Dendrites (Da, Db) covered by several astrocytic lamellae (arrows) in the n. suprachiasmaticus. Note other dendrites (D) only partially covered, if at all. dc, dense core vesicles; mt, microtubules; cal. bar, 1 µm.