Elemental Distribution of Na, P, Cl and K in Different Structures of Myelinated Nerve of *Rana esculenta*

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Summary. Measurements of the distribution of Na, P, Cl and K were performed in different structures of the myelinated nerve. Whereas the axon shows a typical intracellular distribution pattern for Na, Cl and K, the interstitial space and the myelin sheath show a typical extracellular pattern. These measurements have demonstrated that Na is present in the myelin sheath close to the node of Ranvier.

In order to explain the mechanism of Na^+-current 'activation' and 'inactivation' during an action potential in myelinated nerves, a new hypothesis (synapse hypothesis) has recently been discussed[2,3]. In this hypothesis, the initial Na^+ inward current is assumed to be controlled by structures of the paranodal region. An essential part of this hypothesis is that Na is stored close to the axon membrane in the paranodal region of a node of Ranvier. Evidence that Na is available in sufficient concentrations in this region is, as yet, not available.

In order to obtain some information about the electrolyte distribution in different regions of myelinated nerves, electron microprobe analysis was performed on freeze dried sections of frog sciatic nerve (*Rana esculenta*). An isolated bundle of motor fibres was bathed for about 20 sec in 20 g/100 ml albumin Ringer's solution before shock freezing in liquid propane (–180 °C). The frozen material was cut into sections of 1–2 μm thickness using a cryomicrotome (Reichert/Shandon). The sections were then sandwiched between 2 collodion films and freeze dried. During sectioning and freeze-drying, the temperature was kept below –70 °C. The analysis of the sections was performed using a scanning electron microscope (Cambridge) to which an energy dispersive X-ray detector (EDAX) was attached. Sample areas of 1–10 μm^2 were scanned for 400 sec at an acceleration voltage of 15 kV and a beam current of 0.5 nA. The X-rays emmitted from these areas were analyzed in the energy range between 0.6 and 4.0 keV.

The concentrations of Na and Cl were evaluated by comparing the X-ray peaks from the tissue with those of the adherent albumin layer, of known extracellular electrolyte composition. For the other elements (P, K)

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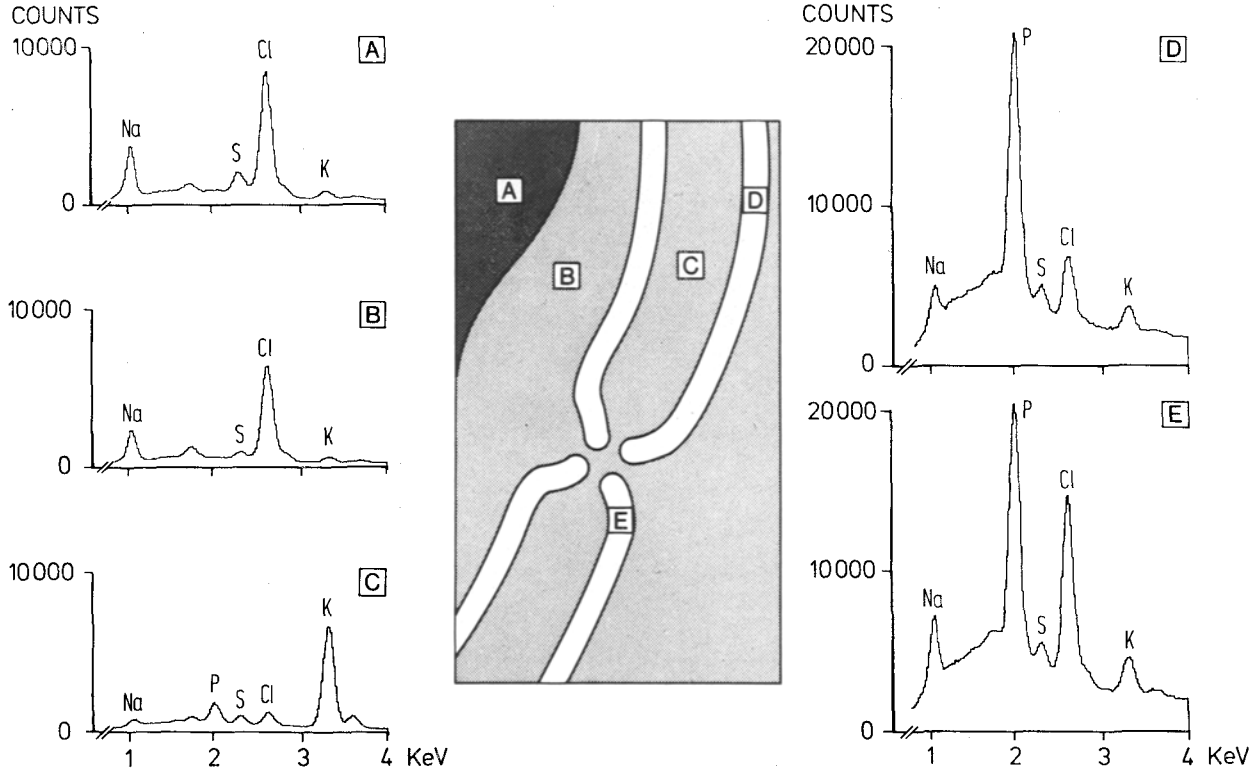

Energy dispersive X-ray spectra obtained on different structures of a freeze dried frog nerve section together with a schematic view of the sites of analysis.

where the concentration in the albumin Ringer is low, quantification was performed by comparing the X-ray peaks of the tissue with that of the Cl peak in the albumin layer, taking into account the experimentally determined differences in the X-ray yield for equal concentrations of the elements. The Figure presents 5 typical X-ray spectra together with a schematic view of a nerve fibre section with a node of Ranvier. The capitals indicate the different locations of the scanned areas on which the analysis was performed. The Table gives the corresponding count rates for the characteristic X-rays and the continuous radiation. The spectrum obtained from the albumin layer (A) shows high Na and Cl but low K and P peaks, corresponding to a typical extracellular pattern. A similar spectrum is obtained in the interstitial space of the nerve (B). In contrast, the spectrum obtained in the axon of a nerve fiber (C) shows small peaks for Na and Cl, but a large K peak. Compared to cellular spectra from other tissues[4], the P peak is relatively small. The wet weight concentrations (mmol/kg wet wt.) computed using the albumin layer as a standard are Na = 90, P = 2, Cl = 84, K = 4 for the interstitial space, and Na = 17, P = 17, Cl = 10, K = 81 for the axon. The values obtained for the axon are in agreement with those determined by other methods[5]. The spectra obtained from the myelin sheath of the nerve fibre (D, E) are characterized by high peaks of P, Cl and Na and a low K peak. As indicated by the high background radiation, the mass content of this structure exceeds several fold that of the albumin. Since the relation between the respective intensities of the characteristic X-rays obtained from samples with large differences in mass content is not known, a comparison of the myelin X-ray spectra with the albumin spectrum cannot provide reliable quantitative data for the element-distribution within the myelin sheath. Nevertheless it is obvious from the Figure that the spectra from these structures show an extracellular pattern with high Na, Cl and low K peaks. The very high P peaks are consistent with the high content of phospholipids in the myelin sheath[6]. It It should be noted that the myelin sheath close to the node of Ranvier exhibits higher peaks for Na and Cl than the internodal one as seen in the Figure (D, E). This observation seems to be a real effect and cannot be accounted for by differences in the mass content in the excited volumes, since the background radiation in both spectra (D, E) is of similar size.

The presence of a high Na concentration in the myelin sheat, particularly in the paranodal part of this structure is consistent with the 'synapse hypothesis' which assumes a Na storage in the paranodal region of the node of Ranvier. However, these measurements do not provide any information about the state of Na present in the myelin sheath, whether it is bound or exchangeable. Therefore, the functional significance of this Na remains uncertain.

Count rates of the K-α radiation of Na, P, Cl and K and the 'Bremsstrahlung' obtained on different structures of a freeze dried frog nerve section.

	Na	P	Cl	K	'Bremsstrahlung'
	(counts/sec)				
A) albumin layer	38.6	3.5	140.2	9.0	198
B) interstitial space	25.7	2.2	112.0	6.3	150
C) axonal space	5.2	20.6	14.2	125.9	127
D) myelin, internodal	33.5	334.8	82.5	35.2	1142
E) myelin, close to node	61.6	314.4	234.5	50.0	1275

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Lower Limit of Cerebral Autoregulation in Normotensive and Spontaneously Hypertensive Rats

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Summary. Cerebral autoregulation was examined in NTR and SHR. The lower blood pressure limit of autoregulation being 95 mm Hg in SHR was shifted upwards from 62 mm Hg in NTR.

Cerebral blood flow is kept constant despite a wide range of cerebral perfusion pressure in normal humans as well as animals under normocapnia, whereas the lower limit of autoregulation (the pressure below which cerebral blood flow decreases) varies depending on the habitual blood pressure level[1]. In the present study, we tested the cerebral autoregulation in normotensive and spontaneously hypertensive rats to find whether or not the lower limit of autoregulation is shifted upwards in hypertensive rats.

Methods. 6 normotensive rats (NTR) and 11 spontaneously hypertensive rats (SHR), weighing from 350 to 400 g, were anesthetized with i.p. amobarbital of 10 mg per 100 g of body weight. Tracheotomy was performed, and their respiration was controlled mechanically. One femoral artery was cannulated for blood pressure recording with an electromanometer and for blood sampling. After a midline incision of scalp, a small hollow screw was introduced into 2 mm anterior of the confluence sinus through a 2 mm burr hole allowing cerebral venous blood sampling. After completing the operation, a resting period of 30 min was allowed before the experiment. $^1/_2$ ml of each arterial and cerebral venous blood was withdrawn anaerobically for gas analysis by IL meter of Model 113.

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