It is clear from figure 1 that IAA stimulated very weakly the ethylene production of maize root segments incubated in the light or in the dark. This differed significantly from the large enhancement of ethylene production observed when pea root segments were treated with IAA[2,3]. The effects of IAA on elongation of maize root segments are shown in figure 2. In the dark, IAA progressively and strongly inhibited root elongation from 10^{-6} to 10^{-4} M. When root elongation was already inhibited by light, IAA reduced the elongation further at 10^{-5} and 10^{-4} M. It has been reported that ethylene, applied from 100 to 1000 nl/l inhibited maize root elongation only weakly[8] and had no effect at the lowest concentration tested (100 nl/l). But it is of course difficult to compare the consequences of ethylene application (expressed per l of air) and the ethylene production (calculated per a given number of root segments). Nevertheless it can be concluded that, at least for maize root segments but probably for all the roots of monocotyledonous plants, the stimulation of ethylene production induced by IAA is far too weak to affect elongation. Thus the inhibition of root growth induced by IAA can only be attributed to a direct effect of the auxin itself.

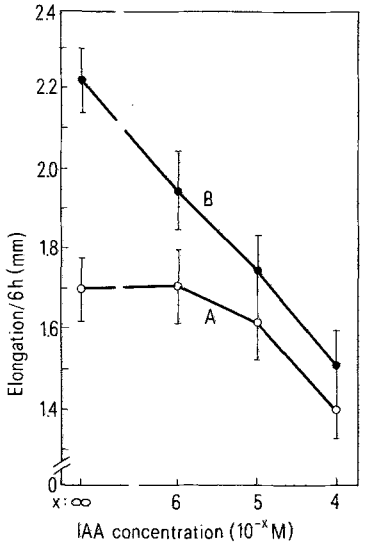

Figure 2. Elongation (in mm ± SE) after 6 h of apical maize root segments. Segments were incubated in a vertical position, in light (A) or in darkness (B).

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The transmembrane gradient of osmotic pressure modifies the kinetics of sodium currents in perfused neurons

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Summary. Sodium TTX-sensitive current was investigated in isolated, internally perfused neurons of rat spinal ganglia. The transmembrane osmotic pressure gradient was found to slow the kinetics of sodium current inactivation when the external osmolality was increased and to speed up the kinetics when the external osmolality was decreased.

Animal cells are usually good osmometers: the surface membrane is permeable to water and normally a steady-state osmotic pressure gradient cannot be created and maintained under normal conditions. However, with the comparatively small mammalian spinal ganglia neurons (diameter 20–30 μm) perfused through a pipette of 5–8 μm in diameter[1,2] an effective exchange of artificial solutions from both sides of the membrane is possible. It is large enough to ensure the removal of increased water flow through the membrane. As judged from the changes in ionic currents, the internal saline was completely substituted within 1 min. This rate is obviously sufficient to prevent accumulation or depletion of water in the cell. This was confirmed by measuring the size of the perfused cells (unchanged) while large osmotic pressure gradients were created. The volume control was done with screen displays of cell images received from a video microscope camera.

We have investigated the effect of the osmotic pressure gradient across the membrane on the sodium TTX-sensitive inward current using the method of intracellular perfusion. Neurons were isolated (in vitro) from rat spinal ganglia pretreated with pronase[2]. In some experiments the cells of mouse neuroblastoma C-1300 (clone N-18)[3] were investigated. The composition of the reference extracellular solution was: NaCl 30 mM, KCl 3.7 mM, $CaCl_2$ 2.6 mM, $MgCl_2$ 1.1 mM, Tris HCl 'Serva' 10 mM (pH 7.4), and TMA chloride 110 mM or nonelectrolyte (glucose, sucrose) 220 mM. The osmolality of the external solution at 20–22 °C was varied in both directions by changing the concentration of TMA from 0 to 500 mM or nonelectrolyte from 0 to 1 M. The intracellular reference solution contained 160 mM of Tris fluoride or phosphate (at pH 7.3). Its osmolality was increased by adding TMA, glucose or sucrose and decreased by the dilution. The direction of the osmotic pressure gradient was defined as 'positive' when the osmolality of external solutions exceeded that intracellularly and 'negative', vice versa. The data were digitized and the averaging procedure P/4[4] employed in order to subtract leakage and capacitance currents.

The changes is osmolality did not influence substantially either the I-V relationship or the h_∞ (V) dependence of the sodium current but were found to produce a prominent effect on its inactivation kinetics. Figure 1 demonstrates the changes in sodium inward current due to the 2-fold changes in the osmolality of external solution. When the direction of the osmotic pressure gradient was made positive, the rate

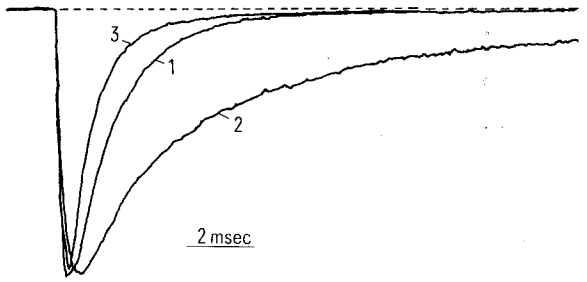

Figure 1. The influence of the transmembrane osmotic pressure gradient on sodium TTX-sensitive current. The current was measured first in the reference external solution containing glucose (1). Then the osmolality of external solution was twice increased (2) and twice decreased (3) by varying the concentration of glucose. The curves are normalized to facilitate the comparison of the changes in kinetics. The peak values of current were 0.7 nA (1), 0.6 nA (2), and 0.8 nA (3). P/4 procedure; holding potential −108 mV; testing potential −20 mV. The peak sodium currents were close to maximum values at this testing potential.

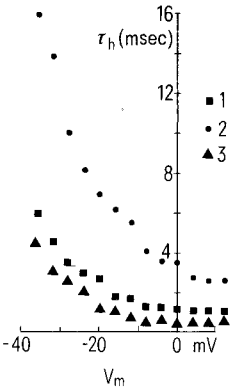

Figure 2. The influence of the osmotic pressure gradient on the inactivation time constant (τ_h) of sodium TTX-sensitive current. 1 - $\tau_h(V)$ for the reference solution; 2 - osmolality of external solution twice increased; 3 - osmolality of external solution twice decreased. The τ_h value were calculated using the least square fit procedure.

of sodium current inactivation was markedly decreased. This effect was independent of the way in which the osmotic gradient was created. Negative gradient always induced an opposite effect. Similarly directed but relatively smaller changes in the activation kinetics were also detected. The same effects were observed when the corresponding changes in the osmotic pressure gradient were created by varying the osmolality of internal solutions.

The effect of osmotic pressure on the kinetics of sodium current inactivation was observed in the entire range of the membrane voltages in which activity of sodium channels is observable. Figure 2 summarizes the values of inactivation time constant τ_h obtained in the experiment similar to the presented in figure 1. The effect did not depend on the direction of ionic current which was tested by the introduction of sodium ions into the cell. Its prominence did not depend on the way in which the osmolality was changed (either by introducing ions or uncharged molecules). It was impossible to saturate the effect by causing a 4-fold increase in the osmolality of external saline. Extremely slow sodium TTX-sensitive currents were observed in the latter experiments. The effect was completely reversible. The experiments on the neuroblastoma cells gave the same results. Slow TTX-insensitive sodium channels present in certain spinal ganglia neurons[5] were affected by the osmotic pressure gradient in a qualitatively similar manner.

We suppose that the molecules of water flowing through the membrane interact with the gates of sodium channels speeding up or slowing down their movement. A model of the sodium channel employing the movable inactivation gating particle[4] could serve as a starting point for this hypothesis.

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Lamprin: a new vertebrate protein comprising the major structural protein of adult lamprey cartilage

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Summary. Chemical analysis of lamprey cartilage showed that its major constituent was a newly defined structural protein termed lamprin. Amino acid analysis of lamprin revealed that it has a unique composition which is distinct from previously identified structural proteins.

Lampreys are extant representatives of an ancient group of vertebrates, the agnathans. One of the structural features which places the lamprey low on the taxonomic scale is the simplicity of its cartilaginous skeleton[2] but there are no definitive reports of the chemical composition of this cartilage. Adult lamprey cartilage has been described as a cellular[3] form of hyaline cartilage[2] with chondroitin-6-sulphate as the major glycan moiety[4]. During a recent study of the lamprey skeleton, it was found that cartilaginous structures stained for elastin[5]. However, earlier comparative biochemical studies had concluded that elastin was absent in agnathans (lampreys and hagfishes)[6-9]. The present investigation was undertaken to determine the chemical composition of lamprey cartilage in order to explain this discrepancy. We report here the identification and partial characterization of lamprin, a new structural protein which constitutes 44–51% of the dry weight of lamprey annular cartilage.

Most of the cartilaginous elements of the lamprey skeleton are contained within the head. In order to determine