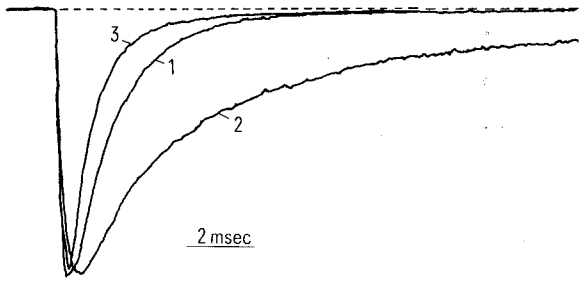

Figure 1. The influence of the transmembrane osmotic pressure gradient on sodium TTX-sensitive current. The current was measured first in the reference external solution containing glucose (1). Then the osmolality of external solution was twice increased (2) and twice decreased (3) by varying the concentration 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.

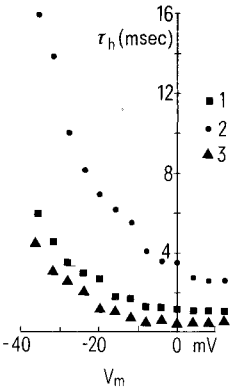

Figure 2. The influence of the osmotic pressure gradient on the inactivation time constant (τ_h) of sodium TTX-sensitive current. 1 - $\tau_h(V)$ for the reference solution; 2 - osmolality of external solution twice increased; 3 - osmolality of external solution twice decreased. The τ_h value were calculated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

G.M. Wright, F.W. Keeley and J.H. Youson

Department of Zoology, University of Toronto, Toronto (Ontario, Canada M5S1A1), and Department of Biochemistry, University of Toronto, and Research Institute, The Hospital for Sick Children, Toronto (Ontario, Canada M5G1X8), August 20, 1982

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.

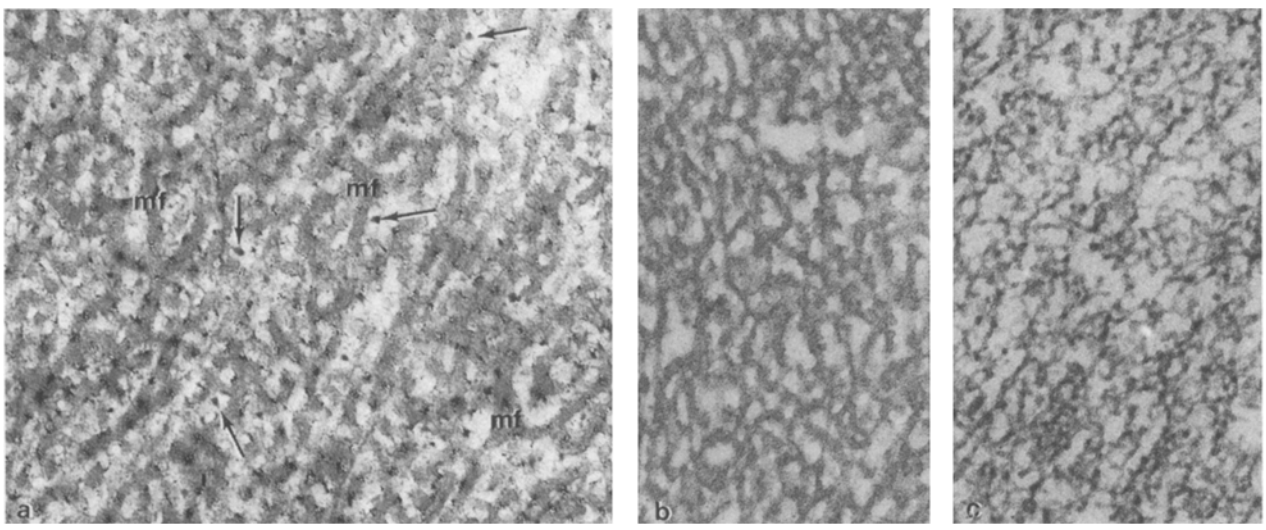
Most of the cartilaginous elements of the lamprey skeleton are contained within the head. In order to determine

whether these cartilage structures contained a protein resembling insoluble elastin, adult lamprey heads were extracted with 0.1 M NaOH for 45 min at 100 °C (25 ml/g, wet wt), the classical method for the isolation of insoluble elastin[10]. The insoluble residue after such a treatment included the intact annular cartilage, dorsal plate and piston cartilage together with smaller cartilaginous structures and teeth, all other tissues having been solubilized. The amino acid composition of the alkali-insoluble annular cartilage, determined after hydrolysis in 5.7 M HCl for 24 h at 110 °C under vacuum, resembled that of insoluble elastin in some respects, but was significantly different in others (table). Like elastin, the insoluble residue contained only small amounts of acidic amino acids. Furthermore, proline, glycine, alanine, valine and leucine together made up over 80% of the amino acid residues. However, unlike elastin the residue contained substantial amounts of tyrosine and histidine and only traces of hydroxyproline. No hydroxylysine could be detected. Tryptophan, measured after hydrolysis in 2 M KOH, could not be detected. Although the lysine content was low, desmosine (DES) and isodesmosine (IDES) could not be detected. This alkali-insoluble residue made up 44% of the dry weight of the annular cartilage dissected free of surrounding tissue.

Because of the absence of methionine in the alkali-insoluble residue, attempts were made to isolate the protein by digestion of the annular cartilage with cyanogen bromide, a technique which should degrade and solubilize any contaminating proteins containing methionine. Such a technique has also been used for the isolation of insoluble elastin[11]. Finely divided annular cartilage tissue was suspended in 70% formic acid (1 mg/ml) and the solution was bubbled with nitrogen. Cyanogen bromide was added to a final concentration of 12 mg/ml and the sample was incubated at 20 °C for 16 h. Undigested residue was removed by centrifugation. The insoluble residue after cyanogen bromide treatment of the annular cartilage made up 51% of the dry weight of the tissue and had an amino acid composition very similar, but not identical to that of the insoluble residue after treatment of the tissue with hot alkali (table). The amino acid composition of the unextracted annular cartilage dissected free of surrounding tissue is shown in the table and is very similar to those of the insoluble residues prepared by the 2 different procedures. This would suggest that the residual material seen after either cyanogen bromide or hot alkali treatment is the major protein component of the annular cartilage. The decrease in the proportion of hydroxyproline and hydroxylysine seen after cyanogen bromide or hot alkali treatment probably reflects removal of the small amounts of collagen which can be seen histologically to be present only in the periphery of the cartilage matrix[5].

Amino acid compositions of pig aorta elastin and protein from lamprey annular cartilage (residues/1000 total amino acid residues). Values are not corrected for losses during hydrolysis

	Total catilage	Residue after cyanogen bromide treatment	Residue after NaOH treatment	Pig aorta elastin[7]
HYP	2.6	1.4	0.9	8.7
ASX	20.1	20.8	14.5	6.4
THR	37.7	31.1	16.2	15
SER	28.1	27.4	15.2	12
GLX	37.8	38.1	22.1	19
PRO	91.1	95.6	95.3	113
GLY	279	282	303	313
ALA	144	156	180	244
VAL	74.7	78.7	77.7	128
MET	2.9	< 0.2	< 0.2	< 1
ILU	22.6	19.8	19.6	18
LEU	121	119	125	54
TYR	58.6	53.3	63.4	19
PHE	12.6	13.8	10.2	33
IDES	< 0.2	< 0.2	< 0.2	1.9
DES	< 0.2	< 0.2	< 0.2	1.3
HIS	42.0	38.0	42.2	1.0
HYL	0.5	< 0.2	< 0.2	-
LYS	9.9	7.3	4.8	5.2
TRP	-	-	< 0.1	-
ARG	15.4	17.4	9.5	7.9



Electron micrographs (× 69,500) of the extracellular matrix of lamprey cartilage. *a* Portion of untreated cartilage showing matrix fibrils (mf) and matrix granules (arrows), fibril diameter 150–400 Å; *b* portion of cartilage matrix after treatment with cyanogen bromide, fibril diameter 100–270 Å; *c* portion of cartilage after extraction with hot alkali, fibril diameter 60–100 Å. All samples for electron microscopy were fixed for 2–2.5 h at 4 °C in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate at pH 7.4. Tissues were then washed in buffer and post-fixed for 1 h at room temperature in buffered 2% osmium tetroxide, washed again in buffer, dehydrated in ethanol and propylene oxide and embedded in epon/araldite. Ultrathin sections were placed on copper grids and stained with uranyl acetate and lead citrate.

Quantitation of glucosamine and galactosamine in limited hydrolysates of cartilage[12] indicate that the glycosaminoglycans present in annular cartilage contain mainly N-acetyl galactosamine and comprise less than 5% of the dry weight of the cartilage.

Samples of untreated cartilage as well as cartilage that had been treated with cyanogen bromide or hot alkali were prepared for electron microscopy (fig.). The matrix of untreated cartilage consisted of a dense network of branched fibrils and matrix granules (fig., a). The matrix fibrils showed no cross-banded periodicity and had a diameter of 150–400 Å. Matrix granules were spherical to polygonal shaped having a diameter of 100–400 Å. After treatment with either cyanogen bromide (fig., b) or hot alkali (fig., c), the fibrillar nature of the matrix was retained, though the diameter of the fibrils was reduced and matrix granules removed. Extraction of lamprey cartilage with 4 M guanidine hydrochloride which removes most proteoglycans from mammalian cartilage[13] removed matrix granules; however the matrix fibrils were unchanged.

The fibrous nature of this cartilage protein and its insolubility both suggest that it may be regarded as a structural protein. However, its amino acid composition clearly distinguishes it from other previously identified structural proteins including elastin[7], abductin[14], collagen, resilin, elastoidin or silk fibroin[15]. Although the physical properties of the protein imply that it exists as a crosslinked polymer, such crosslinks do not seem to be of the types present in insoluble elastin.

Although collagen represents 40–80% of the dry weight of most vertebrate cartilages[16], it clearly can be no more than a minor component of lamprey cartilage. Thus we have shown that lamprey cartilage is not a hyaline cartilage, nor does it contain any elastin. The present amino acid analysis and corresponding electron microscopic examination demonstrates that the cartilage of the adult lamprey is an unusual form of vertebrate connective tissue composed primarily of a previously unrecognized but major structural protein of unique composition which we have termed lamprin.

Despite the position of lampreys among the vertebrates, they are specialized in many ways[17] and this new type of connective tissue may not necessarily represent a primitive kind of cartilage but one which has developed through the 280 million years of the evolution of lampreys.

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Plasma catecholamines in children

G. Planz, J. Bieber, H.J. Mencke and G. v. Bernuth

Abteilungen Innere Medizin II, Orthopädie und Kinderkardiologie, Klinikum der Rheinisch-Westfälischen Technischen Hochschule Aachen, Goethestrasse 27–29, D-5100 Aachen (Federal Republic of Germany), May 13, 1982

Summary. Plasma catecholamine concentrations in 46 children of various ages were determined by a sensitive radioenzymatic assay. Noradrenaline levels were found to be in the same range as in adults, whereas adrenaline levels in a few of the children were abnormally high.

Little information is available concerning the physiology and pathophysiology of the sympatho-adrenal system in children. Recently, radioenzymatic methods have been introduced which allow to determine even in little children in small volumes of blood the plasma adrenaline (A) and noradrenaline (NA) concentrations[1]. The concentrations of A and NA in plasma are commonly accepted as the best and most reliable parameters of sympatho-adrenal activity.

Subjects and methods. 32 'healthy' children from the orthopedic clinic and 14 children from the cardiologic clinic were investigated (see fig.). The 'healthy' children had been hospitalized for conservative treatment or surgical correction of club-foot, hip luxation or os tibiale externum. Before conservative or surgical treatment was started, a blood sample of 3 ml was drawn from the child's cubital vein immediately after venipuncture. During this procedure all the children were lying in bed and their arm was fixed by an assistant. These 'healthy' children had received no medication, and the collection of blood for the catecholamine (CA) assay was carried out in all the children at the same time of day (8.30–10.30 h). The children from the cardiologic clinic were hospitalized for cardiac catheterization. None of the children was in frank heart failure at the time of the study, although half of them were receiving digoxin. The blood sample of 3 ml was taken from the right heart during catheterization. These children were premedicated with atropine and sedated with diazepam and/or fentanyl. Concentrations of A and of NA in the blood sample were determined by a radioenzymatic assay[1].

Results and discussion. As may be seen from the figure, the