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Short Communications

Differential axonal transport of individual Na,K-ATPase catalytic (α) subunit isoforms in rat sciatic nerve

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Three isoforms of the Na,K-ATPase catalytic (α) subunit are present in neurons, demonstrated by in situ hybridization of neurons and Western blot of nerve. We used Western blot with antibodies specific for α1, α2 and α3 peptides to measure the accumulation of individual peptides at a ligature on the sciatic nerve. α1 peptide accumulated with kinetics suggesting rapid axonal transport of that isoform within nerve. α2 and α3 peptides did not accumulate at the ligature. These studies provide insight into the dynamics of axonal Na,K-ATPase isoforms.

Na,K-ATPase is the membrane enzyme found in all eukaryotic cells which exchanges 2 Na⁺ for 3 K⁺, and in the nervous system is responsible for maintaining the transmembrane gradient of Na⁺ and K⁺, the membrane electrical potential, and cell volume. The functional enzyme is a heterodimer of catalytic (α) and glycosylated (β) subunits, although all enzyme and pumping activities are found in the α subunit. Three isoforms of the α subunit have been identified in the nervous system. These isoforms are the products of 3 different members of a multigene family. Although a variety of pharmacologic differences between isoforms have been identified in vitro, the specific function of the different isoforms in the nervous system has not been defined.

In situ hybridization studies with riboprobes specific for α1, α2 and α3 isoforms have demonstrated the presence of mRNAs for each of those isoforms in neurons. It appears that all neurons contain α2 and α3 isof orm mRNA, while some neurons also contain α1 mRNA. Glia contain only α1 and α2 isof orm mRNAs. Electron microscopic immunocytochemical studies have demonstrated the presence of Na,K-ATPase both at the node of Ranvier, and in the internodal axolemma of large myelinated fibers, though the ultrastructural distribution of individual isoforms along the axolemma of large myelinated fibers has not been unambiguously defined.

Because protein synthesis in neurons is restricted to the cell body, all axonal proteins are delivered from the cell body into the axon by axonal transport. Membrane proteins and those proteins located within membrane bound organelles are carried at approximately 400 mm/day from the cell body towards the axon terminal. The transport of specific peptides can be identified by the accumulation of those peptides at a ligature tied around the nerve. Na,K-ATPase is carried by rapid axonal transport in sciatic nerve, as demonstrated by the accumulation of ouabain binding sites at a ligature placed on the nerve, but that study predated the identification of catalytic subunit isoforms.

In order to better define the dynamics of isoform distribution within the axon, we exploited the phenomenon of axonal transport to study the movement of individual isoforms within the axon.

Male Sprague–Dawley rats (250–300 g) were anesthetized with chloral hydrate and the sciatic nerve exposed in the gluteal region. Two ligatures of 4-0 prolene were tied approximately 1 cm apart along the nerve in the exposed region. 24 h later the animals

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were sacrificed by decapitation, the nerve removed, and cut into 3 mm segments. Four segments proximal to the first ligature, 3 segments distal to the second ligature, and one 3 mm segment between the ligatures were collected. The tissue was homogenized in 75–100 μl of 5 mM NaCl, 50 mM Tris (pH 7.0), and debris removed by centrifugation at 10,000 rpm for 15 min. A membrane fraction was prepared from the supernatant by centrifugation at 100,000 x g for 1 h. We have previously shown that all of the immunoreactive Na,K-ATPase in sciatic nerve is found in this fraction. The amount of α isoform-specific peptide in each segment was determined using Western blot. 15 μg of membrane fraction protein was separated by 6% SDS-PAGE and transferred to a nitocellulose membrane (Hybond-ECL, Amersham). The membranes were blocked with 5% dried milk in Tris buffered saline Tween (TBS-T) and incubated overnight with polyclonal antibodies (1 : 1000) directed specifically against α1, α2 or α3 peptides (Upstate Biotechnologies). The specificities of the antibodies, which were raised against synthetic peptides, has been previously reported. Each experiment was performed with 3 different animals for each isoform (because the majority of the membrane protein from an individual segment was applied to the gel for the Western blot), and was repeated twice. The results of all experiments were identical, and representative blots from control and ligated nerves are shown. 24 h after the ligature had been applied, an accumulation of immunoreactive α1 peptide in the segment immediately proximal to the ligature was found (Fig. 1), consistent with the rapid axonal transport of that catalytic subunit isoform. There is no accumulation of the α1 peptide distal to the second ligature, suggesting that there is no significant retrograde axonal transport of that isoform. α1 subunit mRNA is found in motor neurons of spinal cord and in DRG neurons, as well as in Schwann cells of sciatic nerve. The results imply that the major portion of α1 peptide in the nerve is axonal, since glial Na,K-ATPase would not be transported, and would not accumulate at the ligature.

α2 isoform is found in both glia and in neurons. 24 h after ligature the amount of α2 peptide was essentially similar in all nerve segments (Fig. 1). Because these antibodies do not function in electron microscopic immunocytochemistry, it was not possible for us to determine whether this distribution represents the stability of subunit isoforms present in Schwann cells, or the relative stability and immobility of axolemmal α2 subunits.

A similar picture was seen with the α3 isoform, which showed no evidence of accumulation at the ligature (Fig. 1). Unlike the α2 isoform, α3 mRNA is found exclusively in neurons, and is not found in the Schwann cells of the sciatic nerve. α3 must therefore be transported from the neuronal cell body to the axonal membrane, but the small incremental amount added over the 24 h that the ligature is present is not detected, so that an apparent rate of transport cannot be determined. α3 peptide immunoreactivity in individual segments must represent axonal α3 which is relatively stable in the membrane.

Although we do not have immunocytochemistry with these antibodies, the accumulation proximal to the

![Fig. 1. Western blot of individual Na,K-ATPase α isoforms in sequential 3 mm segments of sciatic nerve, 24 h after application of a ligature about the nerve, as indicated in the diagram. The cell body is indicated schematically, and segments proximal to the ligature are to the left, those distal to the ligature to the right. 15 μg of membrane fraction protein was run in each lane.](image-url)
ligature of α1 does not represent an artifact of inflammatory cells. If that were the case, we would expect to see an accumulation about the second ligature as well, and there is no such accumulation of α1 peptide.

In the control nerve, the amount of peptide in individual segments is relatively constant across the length of the nerve (Fig. 2). Although the amount of α1 peptide in the segments of the ligated nerve, excluding the segment just proximal to the ligature, appears to be less than the level in the contralateral nerve, this was not the case. When segments from the ligature and control nerve were run together on a single gel, no differences between the sides was apparent (data not shown). In addition, dot blot analysis of α1 isoform mRNA levels in the dorsal root ganglia compared to control nerve showed no difference between the two sides (data not shown).

The results of this study have important implications for our understanding of the dynamics of Na,K-ATPase isoform distribution within neurons. α1 and α3 are both found predominantly in neurons, but while virtually all α1 appears to be carried by rapid axonal transport, α3 is predominantly non-mobile over a 24 h period. The induction of α1 mRNA by electrical activity in hypothalamic neurons has led us to propose that α1 isoform functions to pump Na⁺ which enters with electrical depolarization. We would propose that α1 is found predominantly at the nerve terminals and at particular nodes of Ranvier, and for that reason is rapidly transported. α3, which is relatively stable in the membrane, may represent an axolemmal form, present along both the internodal axolemma and the axolemma of unmyelinated fibers principally in a non-mobile form.


