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DEVELOPMENT AND REGIONAL DISTRIBUTION OF TWO MOLECULAR FORMS OF THE CATALYTIC SUBUNIT OF THE NA,K-ATPase IN RAT BRAIN

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SUMMARY: Two molecular forms of the catalytic subunit of the Na,K-ATPase can be isolated from brain (1). Only the α form was detected in rat embryo brain at 13 days of gestation (E13). The $\alpha(+)$ form, which is characteristic of myelinated axons, appeared at E15 before myelination begins. Hence its expression is not dependent on prior myelination. Axonal transport of the $\alpha(+)$ form was demonstrated in 4 day-old rats. The ratio of $\alpha(+):\alpha$ was 1:1 in adult retina, cortex and cerebellum and 10:1 in brain stem. Although $\alpha(+)$ is characteristic of myelinated axons, this regional difference was present not only in enzyme extracted from crude microsomes, that contain myelinated axon fragments, but also in enzyme from isolated synaptosomes. Hence, the $\alpha(+):\alpha$ ratio is an inherent characteristic of the neuron and does not depend on regional differences in myelination.

The α and $\alpha(+)$ forms of the catalytic subunit of the Na,K-ATPase can be distinguished by apparent molecular weight in SDS-polyacrylamide gels, by Ki for ouabain and by cellular localization. Both forms are phosphorylated by ^{32}P-ATP in a sodium-dependent, ouabain-inhibited manner, and dephosphorylated by potassium. A large proportion of brain Na,K-ATPase activity is present in astroglia and these cells express only the α form of the catalytic subunit; axons contains only the higher molecular weight form, $\alpha(+)$, but non-myelinated sympathetic neurons contain only α. (1) The cellular distribution of these forms suggests that the expression of $\alpha(+)$ may be dependent on, or induced by, myelination. The ratio of $\alpha(+):\alpha$ also varies with the region of the brain from which it was prepared. The present study was addressed to the three issues: (i) When does $\alpha(+)$ first appear in brain? (ii) Is expression of $\alpha(+)$ dependent on myelination? (iii) Is regional variation in $\alpha(+)$ due to different proportions of myelinated axons, since these have a high $\alpha(+)$ content, or is it an inherent characteristic of the neuronal membrane?

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METHODS

Animals. Adult and timed-pregnant-rats for embryo studies were obtained from Charles River Breeding Laboratories. Neonatal rats were bred in the laboratory. A total of 24 pregnant females were used for embryo studies and 54 neonates for postnatal studies.

Tissue samples. Postnatal rats were sacrificed and the brains placed on an ice-filled Petri dish for dissection under a stereo microscope. The retinae, lateral geniculate nucleus and superior colliculus, frontal and visual cortex and orbit-chiasm segment of the optic nerve were dissected. For embryonic studies, the paired uteri were removed from pregnant rats and placed in cold homogenization solution. Brain samples dissected from the embryos varied according to embryonic age. 13 days-old:eye (minus lens), telencephalon, diencephalon-mesencephalon, metencephalon. 15 days-old: eye (minus lens), cortical hemispheres, diencephalon, metencephalon. 18 days-old: retina, cortical hemispheres, diencephalon, metencephalon. Samples were frozen rapidly and stored at -70°C.

Enzyme purification and SDS-polyacrylamide gel electrophoresis. The Na,K-ATPase was purified from a microsomal pellet and visualized exactly as previously described (2). In some cases, the protein bands in gels were visualized by silver staining using Bio-Rad reagents.

Estimation of $\alpha(+):\alpha$ ratio. Gels were scanned at 650 nm in a Gilford scanner. The relative protein content of bands was estimated by weighing the area traced by the absorbance curve. The peak absorbance was linear from 0.2 -2 μg/band. The $\alpha(+):\alpha$ ratio was also estimated visually by comparison with Coomassie Blue stained gels containing known amounts of standard proteins (Bio-Rad).

Axonal transport. In some experiments, the Na,K-ATPase was labeled by intraocular injection of 250 μCi ^{35}S-methionine (New England Nuclear, 1107 Ci/mmol.) Animals were sacrificed at 24 h and the enzyme was prepared as usual. The radioactive enzyme was visualized in gels by fluorography with Kodak XAR-5 film; exposures were 2-4 weeks at -70°C.

Synaptosomal enzyme: The microsomal pellet was used for preparation of synaptosomes by a modification of the method of Cohen et al. (3) and Na,K-ATPase was extracted from the resuspended synaptosomes as usual.

RESULTS

Development of the $\alpha(+)$ subunit. The α and $\alpha(+)$ forms were identified by comigration with enzyme from adult rat brain in a 5% Laemmli system gel (4,5). The apparent molecular weights were 92 Kd and 94 Kd. At embryonic age E13 only the α form was detected in all brain regions analyzed (Fig. 1).

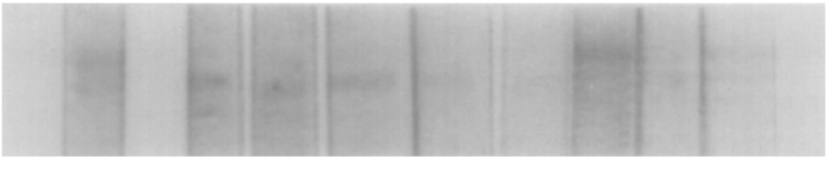

Figure 1. SDS-polyacrylamide gel electrophoresis showing $\alpha(+)$ and α catalytic subunits of Na,K-ATPase isolated from fetal and adult rat brain. From left to right: adult cortex, 13 embryonic days-old (E13) eye, telencephalon, diencephalon, metencephalon; E18 retina, diencephalon, cortex, metencephalon. The lines at the position of $\alpha(+)$ in E13 eye and telencephalon are shadows and not protein bands.

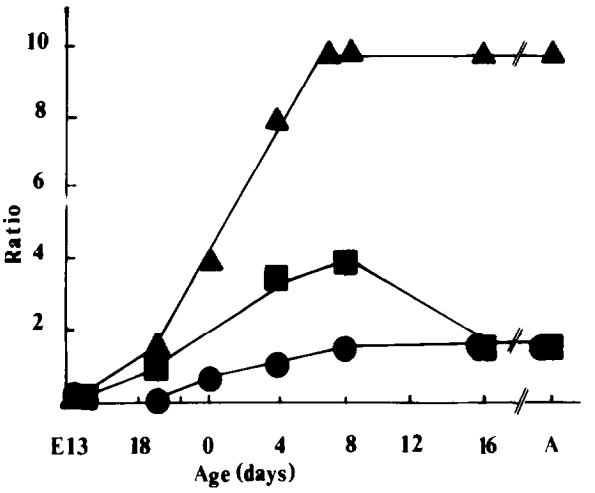

Figure 2. Developmental change in α(+):α ratio in Na,K-ATPase isolated from rat brain from age E13 to adult (A). The vertical arrow marks the day of birth. Key: ▲ diencephalon-lateral geniculate nucleus/superior colliculus; ■ telencephalon-cortex; ● eye-retina.

The α(+) form was present in all regions but eye at E15; this was confirmed by both Coomassie Blue and silver stain. The α(+) form was first detected in all regions at E18. The α(+):α ratio continued to increase in all regions until 10 days postnatal. The cortical ratio decreased slightly after 10 days to its adult value (Fig. 2).

Premyelinated optic nerve. The rat optic nerve does not begin to myelinate until 6 days postnatal (6). Enzyme was isolated from 4 day-old nerve to determine if α(+) is present before myelination. Retinae were labeled by intraocular injection of ^{35}S-methionine to improve the probability of detecting the retinally-synthesized form of the enzyme in optic nerve (2). The enzyme was not visible by Coomassie Blue staining, but both α(+) and α were detected by fluorography of the gel containing radioactive, axonally-transported enzyme (Fig. 3A). Hence, prior myelination is not necessary for expression of the α(+) form.

Regional and subcellular distribution. The ratio of α(+) to α was roughly equal in adult retina, cerebral cortex and cerebellum, but 10:1 in brain stem, i.e., lateral geniculate nucleus, superior colliculus, medulla and mesencephalic tegmentum (Fig. 3C). To determine if these regional

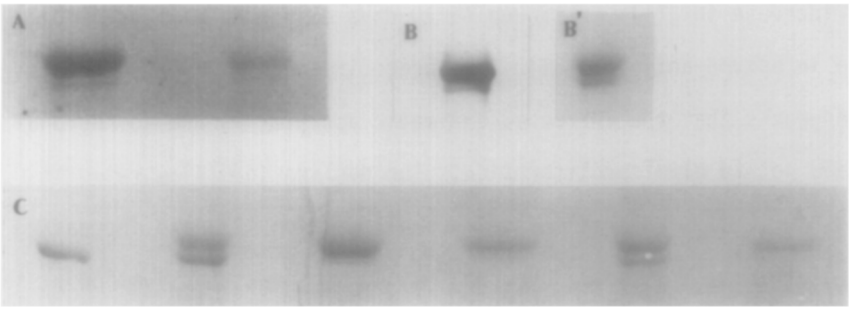

Figure 3. Regional variation in α(+):α ratio shown by SDS-polyacrylamide gel electrophoresis. A: Autoradiography of axonally-transported α(+) and α in lateral geniculate nucleus/superior colliculus (left) and optic nerve (right) of 4 day-old rat. B: Silver-stained gel showing α(+) and α isolated from synaptosomes of lateral geniculate/superior colliculus (left) and cerebral cortex (right). C: Coomassie Blue stained gel. The first and last slots contain a 92 Kd molecular weight marker. From left to right, α(+) and α isolated from adult rat cerebral cortex, medulla, midbrain tegmentum and cerebellum.

differences were due to different proportions of myelinated axons, the Na, K-ATPase was purified from synaptosomal membranes. The microsomal pellet from which enzyme was usually prepared was further fractionated on a 4-step sucrose gradient, thus separating myelinated axons from synaptosomes. The synaptosomes were then precipitated and used for enzyme preparation. The regional difference in α(+):α ratio was also seen in enzyme isolated from purified synaptosomes (Fig. 3B) and is thus an inherent characteristic of the neuronal membrane.

DISCUSSION

The only form of the catalytic subunit of the Na,K-ATPase detected in early embryonic brain is α. This is probably neuronal, since few glial cells are present. The appearance of α(+) precedes myelination and probably precedes synaptogenesis. The neurones of the rat retina and lateral geniculate nucleus are born between E13 and E14 (7). The optic nerve arrives in the lateral geniculate nucleus during E15 and immediately begins to form synapses (8). Retinal synaptic connections are formed postnatally (9), as are those of the visual cortex (10). Hence, it is conceivable that innervation of a region, e.g., the lateral geniculate nucleus, may accelerate the development of α(+), but innervation is not a necessary condition for its expression.

The increase in $\alpha(+):\alpha$ ratio up to 10 days coincides with a 15 fold increase in enzyme activity (11). The predominance of $\alpha(+)$ during this period suggests that the increased enzymatic activity is due to neuronal enzyme and not to glial proliferation. The decline in $\alpha(+):\alpha$ ratio in cortex after 10 days may reflect glial proliferation (12) and increasing content of the glial α form. Eye opening and subsequent activation of the visual system pathway at 12-14 days after birth does not produce any obvious change in α $(+):\alpha$ ratio, although enzyme activity increases 6-7 fold (3).

The developmental relationship of α and $\alpha(+)$ is similar to that of the $\alpha 1$ and $\alpha 2$ forms of the catalytic subunit described in brine shrimp (13). In both cases, the more slowly migrating form increases relative to the other during early development, however both forms are always co-existent in the brine shrimp whereas only α is found in early rat embryos.

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REFERENCES

1. Sweadner, K.J. (1979) J. Biol. Chem. 254, 6060-6067.
2. Specht, S.C. and Sweadner, K.J. (1984) Proc. Natl. Acad. Sci. USA. 81, 1234-1238.
3. Specht, S.C. (1982) J. Neurochem. 39, 310-314.
4. Laemmli, U.K. (1970) Nature 227, 680-685.
5. Ames, G.F.-L. (1974) J. Biol. Chem. 249, 634-644.
6. Skoff, R.P., Price, D.L., and Stocks, A. (1976) J. Comp. Neurol. 169, 291-312.
7. Bruckner, G., Mares, V. and Biesold, D. (1976) J. Comp. Neur. 166, 245-256.
8. Lund, R.D. and Bunt, A.H. (1976) J. Comp. Neurol. 165, 247-264.
9. Weidman, T.A. and Kuwabara, R.T. (1968) Arch. Ophthalmol. 79, 470-484.
10. Lund, R.D. and Mustari, M.J. (1977) J. Comp. Neurol. 173, 289-306.
11. Bertoni, J.M. and Siegel, G.J. (1978) J. Neurochem. 31, 1501-1511.
12. Caley, D.W. and Maxwell, D.S. (1968) J. Comp. Neurol. 133, 45-70.
13. Peterson, G.L., Churchill, L., Fisher, J.A. and Hokin, L.E. (1982) Ann. N.Y. Acad. Sci. 402, 185-205.