Na^+,K^+-ATPase Subunit Isoforms of the Developing Central Nervous System of the Lizard *Gallotia galloti*

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In the central nervous system, the gradients generated by the Na^+,K^+-ATPase are used for electrical membrane potential changes, uptake of neurotransmitters, and, in astrocytes, uptake of K^+ from the extracellular space after depolarization of neurons. Radial glia forms the scaffold that guides migrating neurons and the outgrowth of axons during development.[1] In the adult brain, $\alpha 2$ predominates in astrocytes and $\alpha 3$ predominates in neurons, $\alpha 1$ and $\beta 1$ are ubiquitous, $\beta 2$ is an adhesion molecule on glia involved in neuron-astrocyte adhesion, and $\beta 3$ in the central nervous system is expressed only in photoreceptors and oligodendrocytes.[2] The patterns of developmental specification are extremely complex, and both the control of biosynthesis and the intrinsic enzyme properties are affected by the choice of α and β.[3]

We propose to establish the differential expression patterns of the Na^+,K^+-ATPase isozymes of radial glia along developmental stages of the lizard *Gallotia galloti.* A panel of isoform-specific antibodies (6F, McB2, XVIF9-G10, SpET$\beta 1$, SpET$\beta 2$, RTN-$\beta 3$)[2,4] and the glia marker GFAP (glial fibrillary acidic protein)[4] were used for the localization in frozen sections of the developing mesencephalon of the lizard by double-label immunofluorescence.

During ontogeny, immunoreactivity for Na^+,K^+-ATPase subunits isoforms varied along stages. At embryonic stage 35 (E35), only $\alpha 2$ and $\beta 2$ isoforms appeared in radial glial processes and glial cell somas (coexpressing with GFAP) in pretectum, optic tectum (in strata), cerebellar ventriculi, and raphe. From E37 to hatching, there is a relative decay of specific immunoreactivity for $\beta 2$ as the $\beta 1$ expression signal increases from faint to high. No $\beta 3$ was found at any stage of development. A faint expression of $\alpha 1$ and $\alpha 2$ appears in isolated cellular groups not yet characterized.

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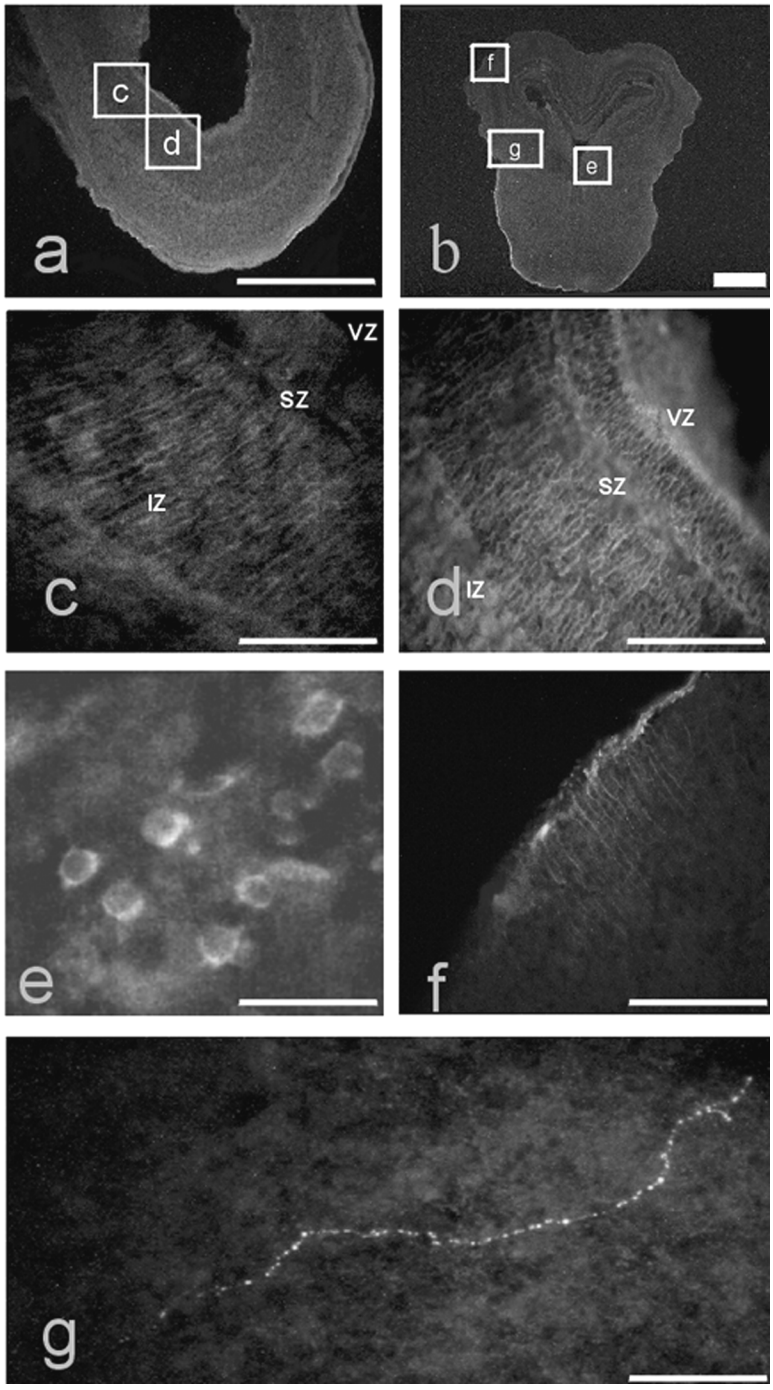

FIGURE 1. *See following page for legend.*

(FIG. 1). The diversity of Na^+,K^+-ATPase isozymes with different affinities for its physiological ligands and their complex spatial and temporal patterns of cellular expression in radial glia suggest that these isozymes are cast along development in such a way that affinity for K^+ increases and affinity for Na^+ decreases and the apparent number of total copies per cell increases.

Noteworthy is the finding of a bright signal for β2, like sparks following a line, from immediately before hatching to further stages in synaptic contacts; this points out a role for β2 in the synaptic setup.

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FIGURE 1. Immunolocalization of Na^+,K^+-ATPase α and β subunit isoforms in developmental stages of *Gallotia galloti*. Sections of mesencephalon at stages E35 (**a**) and hatching (**b**) marking the squares containing the areas of the corresponding pictures in **c** to **g**. Scale bar = 200 μm. (**c**) α2 immunofluorescent labeling of radial glia processes in E35. Scale bar = 40 μm. (**d**) β2 labeling of radial glia processes and plasma membrane of ventricular cell somas in E35. Scale bar = 50 μm. (**e**) α2 staining of plasma membrane in cells of optic tectum at hatching. Scale bar = 5 μm. (**f**) β1 labeling of radial glia processes in hatching. Scale bar = 25 μm. (**g**) β2 immunofluorescent labeling of synaptic contacts (buttons) in hatching. Scale bar = 10 μm. VZ, ventricular zone; SZ, subventricular zone; IZ, intermedial zone; CP, cortical plate; ML, molecular layer.