Neuroscience Letters, 97 (1989) 18–22
Elsevier Scientific Publishers Ireland Ltd.

NSL 05863

Immunocytochemical localization of $(Na^+ + K^+)$-ATPase in the rat hippocampus

Harold J. Sheedlo[1], George J. Siegel[2] and Stephen A. Ernst[3]

[1]Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC (U.S.A.) and Departments of [2]Neurology and [3]Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI (U.S.A.)

(Received 19 September 1988; Revised version received 10 October 1988; Accepted 11 October 1988)

Key words: Rat; Hippocampus; $(Na^+ + K^+)$-ATPase; Immunocytochemistry; Pyramidal cell

The adult rat hippocampus was investigated by light microscopic immunocytochemistry for $(Na^+ + K^+)$-ATPase. In the CA1, CA2 and CA3 hippocampal regions, dense immunostaining for $(Na^+ + K^+)$-ATPase, exhibiting a punctate appearance, was demonstrated along the soma plasmalemma of hippocampal pyramidal cells in the stratum pyramidale, thus outlining these cells distinctly, and along dendrites extending into the stratum radiatum. $(Na^+ + K^+)$-ATPase immunostaining was dense in the neuropil of the strata oriens and radiatum of the rat hippocampus, but much lighter in the corpus callosum. Immunostaining at the periphery of pyramidal cell soma may be associated with the plexus formed by axon terminals of hippocampal basket cells.

The membrane-bound enzyme $(Na^+ + K^+)$-ATPase, which maintains Na^+ and K^+ ion concentrations across membranes, is heavily concentrated in neuropil of cerebrum, cerebellum and spinal cord [13, 15, 16]. This enzyme has been localized immunocytochemically at the node of Ranvier, the plasmalemma of neuronal somata and in astrocytic processes [1, 10, 16, 17]. Also, immunocytochemical studies have shown $(Na^+ + K^+)$-ATPase to be in particularly high concentrations in regions of high synaptic density such as in retinal plexiform layers [7], Purkinje cell basket terminals and glomeruli in cerebellum [15] and at the soma plasmalemma of trigeminal ganglion cells [13].

The highly ordered structure of the hippocampus makes it an ideal region to study the histological distribution of $(Na^+ + K^+)$-ATPase. Axons of hippocampal basket cells, which are found near the border of strata pyramidale and oriens, form a dense series of axosomatic synapses with pyramidal cells [11]. Similarly, basket cell axon terminals in the cerebellum form a dense plexus in the axon hillock region of Purkinje cells where dense immunostaining for $(Na^+ + K^+)$-ATPase has been detected [15]. Because of this similarity in structural organization, a corresponding distribution of

Correspondence: H.J. Sheedlo, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103, U.S.A.

$(Na^+ + K^+)$-ATPase might be expected among pyramidal cell bodies. The immunocytochemical approach to localization of $(Na^+ + K^+)$-ATPase has not yet been applied to the hippocampus. The purpose of this study was to determine the immunocytochemical distribution of $(Na^+ + K^+)$-ATPase in the adult rat hippocampus.

In this study 4 male Wistar rats (200 g) and 4 male Sprague–Dawley rats (200–250 g) were anaesthetized with sodium pentobarbital and perfused through the left ventricle with either 4% paraformaldehyde or PLP (0.01 M sodium periodate, 0.1 M lysine, 4% paraformaldehyde) [8], fixed for an additional 4–6 h and embedded in paraffin the next day. Five μm sections were immunostained, in sequence, as described previously [12, 14]. Briefly, sections were incubated in 20% normal goat serum (NGS) for 60 min, 1:200–1:750 $(Na^+ + K^+)$-ATPase antisera or rabbit preimmune serum, 1:250 goat anti-rabbit IgG–horseradish peroxidase conjugate, 0.025% diaminobenzidine–0.01% hydrogen peroxide for 5 min. The rabbit antiserum was raised against bovine brain $(Na^+ + K^+)$-ATPase catalytic subunit purified on denaturing gels and was characterized as specific for the catalytic subunit in bovine and rodent brain preparation on Western blots and by immunocytochemistry in rodent brain and kidney [4]. Antiserum was diluted in 1% NGS and incubations were performed at 4°C for 16–18 h. The immune serum was replaced by rabbit preimmune serum for the controls. The sections were rehydrated, mounted in Permount and photographed under bright-field optics.

In the CA1 hippocampal region, pyramidal cells appeared to be immunostained for $(Na^+ + K^+)$-ATPase along their plasmalemma (Fig. 1A), which is more clearly revealed at higher magnification (Fig. 1B). Immunostaining for $(Na^+ + K^+)$-ATPase in the CA2 region was also detected along the plasmalemma of cell bodies in the stratum pyramidale. This immunostaining appeared punctate or in clumps, possibly associated with axon terminals of hippocampal basket cells contacting the pyramidal cell body plasmalemma and/or enwrapping glial elements. $(Na^+ + K^+)$-ATPase immunostain in the stratum oriens and stratum radiatum was of uniform density, interrupted by unstained cell bodies and blood vessels. The intensity of reaction was greatest in stratum oriens, moderate in stratum radiatum and light in the corpus callosum (Fig. 1C). At higher magnification pyramidal cell apical dendrites in the stratum radiatum appeared to be immunostained for $(Na^+ + K^+)$-ATPase along their plasmalemma (Fig. 1D). Mossy fibers arising from dentate granule cells and converging on the inner dendritic field of pyramidal cells in the CA2/CA3 region did not appear to be immunostained for $(Na^+ + K^+)$-ATPase, thus giving this region a lightly stained appearance (Fig. 2A). However, pyramidal cell bodies and their dendrites were immunostained as in the CA1 and CA2 regions (Fig. 2B). Cells of the dentate gyrus were also immunostained for $(Na^+ + K^+)$-ATPase along their plasmalemma (data not shown). Sections of rat hippocampus incubated with rabbit preimmune serum exhibited no specific immunostaining (not shown).

The focal or punctate appearance of $(Na^+ + K^+)$-ATPase immunostain along the plasmalemma of pyramidal cell somata is consistent with the possibility that this enzyme may be associated with axon terminals at axosomatic synapses. It is of interest that glutamic acid decarboxylase (GAD), which synthesizes the neurotransmitter

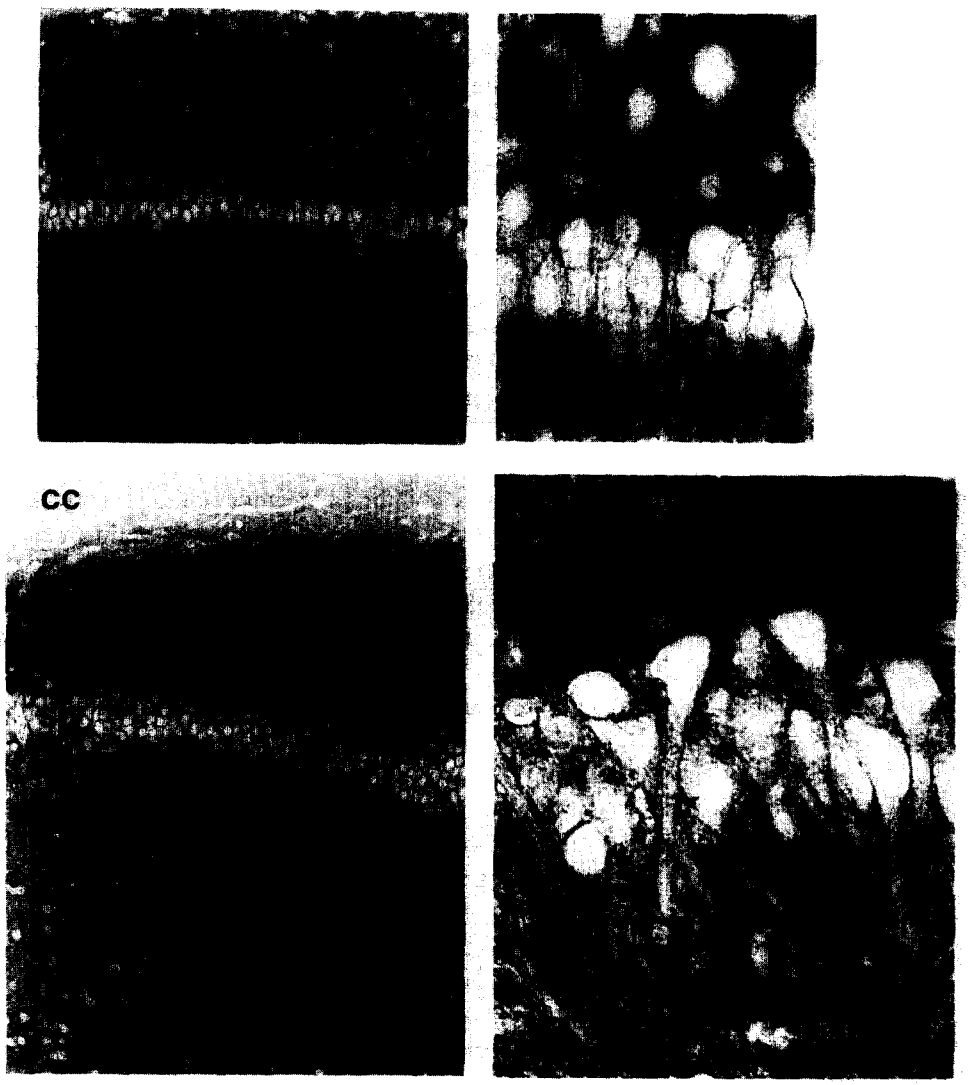

Fig. 1. Immunocytochemical localization of $(Na^+ + K^+)$-ATPase in the CA1 and CA2 region of the adult rat hippocampus. A: pyramidal cell bodies in the CA1 region appear to be immunostained at their periphery (arrowheads). B: in the same region as C, but at higher magnification, immunostaining surrounding pyramidal cell bodies were more clearly revealed (arrowheads). C: $(Na^+ + K^+)$-ATPase immunostaining in the CA2 hippocampal region was dense in the stratum oriens (so) and at the periphery of cell bodies in the stratum pyramidale (sp), less dense in the stratum radiatum (sr) and light in the corpus callosum (cc). D: at high magnification immunostaining for $(Na^+ + K^+)$-ATPase is clearly discerned at the periphery of pyramidal cell bodies (arrows) and along dendrites (arrowheads) in the stratum radiatum (sr). Final magnification: A, C: ×95; B, D: ×375.

γ-aminobutyric acid (GABA), also has been detected in axon terminals and boutons contacting rabbit hippocampal pyramidal cell bodies [6]. The pattern of immunostaining seen at the light microscopic level in that study is identical to that reported here for $(Na^+ + K^+)$-ATPase. As shown by electron microscopic immunocyto-

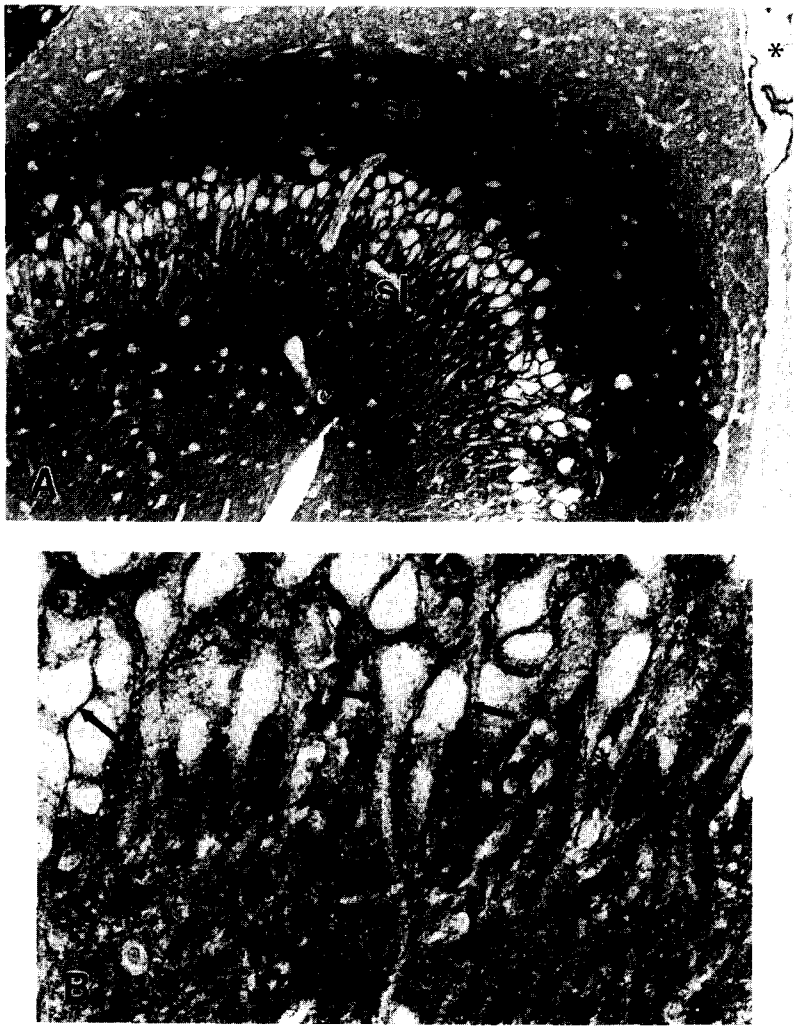

Fig. 2. Immunocytochemical localization of $(Na^+ + K^+)$-ATPase in the stratum lucidum of the adult rat hippocampus. A: in the stratum lucidum (sl), which is in the CA2/CA3 region of the hippocampus, mossy fibers did not appear to immunostain for $(Na^+ + K^+)$-ATPase. The choroid plexus in the lateral ventricle was immunostained at the apical border (asterisk). B: however, immunostaining was detected at the periphery of cell bodies (arrows) in the stratum pyramidale and along dendrites (arrowheads) in the stratum radiatum of the CA2/CA3 region. Final magnification: A: ×95; B: ×375.

chemistry, astroglial elements, such as cell processes and endfeet, and neuronal cell body plasmalemma have been shown to exhibit immunostaining for $(Na^+ + K^+)$-ATPase [1].

Two histochemical studies of the hippocampus using the K^+-stimulated *p*-nitrophenylphosphatase (pNPPase) method have been reported [3, 9]. Neither study showed activity corresponding to $(Na^+ + K^+)$-ATPase associated with pyramidal cell bodies or basket endings in the pyramidal layer, in direct contrast to the immunocy-

tochemical results in this study. This major difference in results obtained by pNPPase and immunocytochemical methods with regard to CNS perikarya has been noted and discussed previously [15]. The hippocampal pyramidal cell layer is another example of this difference and the reason for this difference remains unknown.

It is possible that immunocytochemical studies of $(Na^+ + K^+)$-ATPase as a membrane marker may contribute to detecting changes in the pyramidal cell layer in diseases of the hippocampus such as epilepsy [2] and dementia [5].

This research was supported by Grant NS15935 from the National Institutes of Health. We thank Dr. J.E. Turner (Department of Anatomy, Bowman Gray School of Medicine) for the use of his laboratory facilities and T.J. Desmond for his excellent technical assistance.

1 Ariyasu, R.G., Nichol, J.A. and Ellisman, M.H., Localization of sodium/potassium adenosine triphosphatase in multiple cell types of the murine nervous system with antibodies raised against the enzyme from kidney, J. Neurosci., 5 (1985) 2581–2596.

2 Corsellis, J.A.N. and Meldrum, B.S., Epilepsy. In W. Blackwood and J.A.N. Corsellis (Eds.), Greenfields Neuropathology, Edward Arnold, London, 1976, pp. 771–795.

3 Haglund, M.M., Stahl, W.L., Kunkel, D.D. and Schwartzkroin, P.A., Developmental and regional differences in the localization of Na,K-ATPase activity in the rabbit hippocampus, Brain Res., 343 (1985) 198–203.

4 Hieber, V., Siegel, G.J., Desmond, T.J., Liu, J.L.-H. and Ernst, S.A., Mouse brain Na,K-ATPase: preparation of α-subunit RNA probe and cellular localization of mRNA and polypeptide in cerebellum, retina and kidney, submitted.

5 Hyman, B.T., Van Hoesen, G.W., Kromer, L.J. and Damasio, A.R., Perforant pathway changes and the memory impairment of Alzheimer's disease, Ann. Neurol., 20 (1986) 472–481.

6 Kunkel, D.D., Hendrickson, A.E., Wu, J.Y. and Schwartzkroin, P.A., Glutamic acid decarboxylase (GAD) immunocytochemistry of developing rabbit hippocampus, J. Neurosci., 6 (1986) 541–552.

7 McGrail, K.M. and Sweadner, K.J., Immunofluorescent localization of two different Na,K-ATPases in the rat retina and in identified dissociated retinal cells, J. Neurosci., 6 (1986) 1272–1283.

8 McLean, J.W. and Nakane, P.K., Periodate-lysine-paraformaldehyde fixative: a new fixative for immunoelectron microscopy, J. Histochem. Cytochem., 22 (1974) 1077–1083.

9 Nasu, F., Ultracytochemical demonstration of ouabain-sensitive, K^+-dependent *p*-nitrophenylphosphatase $(Na^+ + K^+)$-ATPase activity in the rat hippocampal formation, Acta Histochem. Cytochem., 16 (1983) 368–373.

10 Schwartz, M., Ernst, S.A., Siegel, G.J. and Agranoff, B.W., Immunocytochemical localization of $(Na^+ + K^+)$-ATPase in the goldfish optic nerve, J. Neurochem., 36 (1981) 107–115.

11 Schwartzkroin, P.A., Kunkel, D.D. and Mathers, L.H., Development of rabbit hippocampus; anatomy, Dev. Brain Res., 2 (1982) 453–468.

12 Sheedlo, H.J., Starosta-Rubenstein, S., Desmond, T. and Siegel, G.J., $(Na^+ + K^+)$-ATPase in C6 glioma and rat cerebrum, J. Neuroimmunol., 15 (1987) 173–184.

13 Sheedlo, H.J. and Siegel, G.J., Comparison of the distribution of $(Na^+ + K^+)$-ATPase and myelin-associated glycoprotein (MAG) in the optic nerve, spinal cord and trigeminal ganglion of shiverer (Shi/Shi) and control (+/+) mice, Brain Res., 415 (1987) 105–114.

14 Sheedlo, H.J. and Siegel, G.J., Myelin-associated glycoprotein (MAG) in the CNS of adult shiverer mice, J. Neurosci. Res., 16 (1986) 629–641.

15 Siegel, G.J., Holm, C., Schreiber, J.H., Desmond, T.J. and Ernst, S.A., Purification of mouse brain $(Na^+ + K^+)$-ATPase catalytic unit, characterization of antiserum, and immunocytochemical localization in cerebellum, choroid plexus, and kidney, J. Histochem. Cytochem., 32 (1984) 1309–1318.

16 Stahl, W.L. and Broderson, S.H., Localization of $(Na^+ + K^+)$-ATPase in brain, Fed. Proc., 35 (1976) 1260–1265.

17 Wood, J.G., Jean, D.H., Whitaker, J.N., McLaughlin, B.J. and Albers, R.W., Immunocytochemical localization of the sodium, potassium activated ATPase in knifefish brain, J. Neurocytol., 6 (1977) 571–581.