IMMUNOLOCALIZATION OF THE SODIUM CHANNEL PROTEIN IN THE NEURAL PLATE OF RAT EMBRYOS.

Sánchez F.[1*], Villegas, G.M.[2], Villegas, R.[2]

[1] Departamento de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC),
[2] Centro de Biociencias y Medicina Molecular, Instituto de Estudios Avanzados (IDEA), Caracas-Venezuela.

*Corresponding author. Apartado Postal 21827, Caracas 1020A - Venezuela
Tel. 58-212-5041724; fax: 58-212-5041444
E-mail address: fsanchez@ivic.ve

RESUMEN

Las proteínas de los canales de sodio (Na^+Ch) están relacionadas con la generación del potencial de acción en los tejidos excitables y con varios procesos celulares dinámicos tales como: proliferación y migración celular. La formación del Sistema Nervioso Central (SNC) es uno de estos casos. La placa neural, primera estructura formada durante el desarrollo del SNC, se forma en los embriones de rata a los 10 días de desarrollo (E10). En este trabajo se emplearon las técnicas de Microscopía Electrónica de Transmisión (TEM) y Barrido (SEM) para describir los detalles morfológicos-ultraestructurales de la placa neural de embriones de ratas al momento de su formación (E10) y describimos su posible relación con la expresión de las proteínas de los Na^+Ch en estos tejidos. Para ello se emplea la microscopía de fluorescencia y el anticuerpo *pan*-policlonal SP19, anticuerpo dirigido contra una región conservada de las proteínas de los Na^+Ch de vertebrados. Como conclusión se postula que existe una posible relación entre las características ultraestructurales de la placa neural, el desarrollo del SNC y la expresión de las proteínas de los Na^+Ch.

SUMMARY

Sodium channels (Na^+Ch) are implicated in the generation of the action potential in excitable tissues and also in some dynamic cell processes like proliferation and cell migration. The formation of the Central Nervous System (CNS) is a well studied dynamic event involving rapid cell proliferation and migration. The neural plate is the first structure formed during the development of the CNS and appearing at day 10 in rat embryo. Here we document the earliest appearance of the neural plate of rat embryos and describe in it the immunolocalization of the voltage-gated Na^+Ch protein by fluorescent microscopy, using the *pan*-polyclonal antibody SP19. We also characterize the typical polarized epithelial morphology and ultrastructure of this tissue, at the same stage of development, with conventional transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and postulate organellar distribution changes with the presence of Na^+Ch proteins .

Palabras claves: SNC, placa neural, desarrollo, TEM, SEM, Na^+Ch.

Introduction

Voltage-gated sodium channels (Na^+Ch) mediate regenerative inward currents that are responsible for initiating depolarization of the action potential in the neuron and the conduction of electrical impulses throughout the nerve [1] More recently Na^+Ch have been implicated in other cellular processes during the cell cycle, such as proliferation, migration and adhesion [2, 3] and in the metastatic characteristics in some cancer events [4, 5, 6, 7, 8].

The Na^+Ch protein is composed of particles of 10 nm in diameter (9, 10). In adult rat brain they are heterotrimeric complexes consisting of an ion selective and voltage-sensitive α subunit (260 kD),

noncovanlently associated with a β1 (36kD) and β2 (33 kD) subunits that modulate channel function. The β1 subunit carries out this modulation by accelerating the inactivation kinetic of the channel and by shifting its voltage dependence towards the hyperpolarizing direction [11], whereas the β2 subunit works by only slightly accelerating inactivation [12, 13, 14, 15].

The α subunit consists of 4 homologous domains, each containing 6 transmembrane segments [9, 16, 17, 18, 19, 20]. From this subunit, at least 9 full length Na $^{+}$Ch cDNAs have been cloned: rat brain I and II [16] IIA [21], III [17] rat $Na^{+}6$ [22].

In the early stages of CNS development, a differential temporal and spatial expression of Na^{+}Ch protein has been reported [23, 24]. The Na^{+}Ch type I is expressed predominantly at late postnatal stages, Na^{+}Ch type II expression increases throughout development and the Na^{+}Ch type III is expressed predominantly at embryonic and early postnatal stages. Similar results were obtained by using saxitoxin specific binding [25]. The physiological implication of these findings is not clear, though some studies have shown that each type of Na^{+}Ch protein has a distinct role in determining the electrical excitability [26, 27, 28]. Studies done in other tissues have shown a direct relationship between the expression of Na^{+}Ch proteins and malignant phenotypes [2, 5, 6, 29], and in migration process [29, 30].

Neurogenesis is initiated at the beginning of the gastrulation with the commitment of dorsal ectoderm to neural plate formation under the inductive influence of the mesoderm [30, 31, 32]. In effect, inhibitory proteins, the neural inducers, such as chordine, noggin, follistatin and others, secreted by the organizer of Spemann and Mangol [33] play a key role for inducing the formation of neural cells and then the neural plate [34]. The dorsalization of the mesoderm and formation of dorsal ectoderm cells made these latter as to be genetically commited to become neural cells, fate being achieved with the concourse of the so called neural inducers. These are proteins in charge of inhibiting another set of proteins belonging to the family of the Transforming Growth Factor-β (TNF-β) and known as Bone Morphogenetic Proteins (BMP). The neural inducers project the fate of the dorsal ectodermic cells to become neural cells. The neural plate is transformed by invagination into the neural tube, later, and the cerebral hemispheres, as well as the other CNS structures, will develop from this structure. These events occur in the rat embryo between the 10 - 11 days of gestation [35, 36]. During morphogenesis, the neuroepithelial cells further differentiate into various kinds of neuronal and glial cells [37, 38] as a result of migration or segregation of cells which in turn, establish their particular fields or cell-cell interactions.

Antibodies to Na^{+}Ch protein, raised against specific peptide sequences, have been used to localize voltage-activated Na^{+}Ch in rat brain. Consequently, antibody peptide sequences SP11 (aa 577– 496) and SP20 (aa 1116–1136) have been used for immunocytochemical localization of two different subtypes of sodium channels in central neurons of rat [39], the SP11 sequence being different in the two channel forms, whereas the antibody to SP20 recognized both forms. On the other hand, antibodies against sequences SP11, SP12 (aa 1997 – 2015), SP16 (aa 41 – 57), SP19 (aa 1501 –1518), and SP20 have been used to detect the effects of these segments on the electrical properties of rat brain. Only the antibody against SP19, a highly conserved intracellular segment of the channel between two transmembrane domains, was effective in slowing Na^{+}Ch inactivation [40]. All amino acid numbering used by these authors was based on the rat brain type-I sequence [16].

In the present study, we use both fluorescent microscopy and electron microscopy for mapping the distribution of the Na^{+}Ch protein in the 10 day rat embryo neural plate wall. Also, we make a correlation of such distribution with the ultrastructural characteristics observed at same stage of the developing embryonic neuroepithelium. The possible significance of the onset of the ionic channels on

modulating the ultrastructural features of the developing embryonic neuroephitelium is discussed**.**

Materials and methods

The anti-pan α-Na^+Ch protein antibody used in the present experiments (*Alomone labs. Jerusalem, Israel*) is a polyclonal antibody raised in rabbit against highly purified peptide SP19(*TEEQKKYNAMKKLG SKK*), corresponding to residues 1501-1518 of rat brain type I Na^+Ch [15]. This sequence is situated at the intracellular loop between domains III and IV of voltage – gated α Na^+Ch subunit. That antibody was found to immunoreact in several diverse animal species, including insect CNS tissues from locust [41] grasshopper, cockroach, fly, and moth [42].

Timed pregnant Sprague-Dawley rats were sacrificed by CO_2(g), and the neural plates from ten day rat embryos (E10) were removed. Whole pieces were fixed in 4% Paraformaldehyde in saline phosphate buffer (PBS), pH 7.4, for 1 h; then rinsed in PBS, preincubated in 0.5 % Triton – X100 in PBS during 30 min, rinsed in PBS and then blocked with 2% BSA, 5% goat serum during 1 h at 4°C in a moist chamber. The whole samples were then incubated with the primary antibody (antipan α Na^+Ch 1/100) during 1h at room temperature, after which, they were rinsed in PBS and then incubated with the secondary antibody (1/200 anti–rabbit Ig* FITC), for 1 more hr. Finally, they were rinsed in PBS and mounted in phenylene diamine in PBS/Glycerol. The existence of lacunar spaces separating the cells facilitates antibody penetration when using the whole tissue method. Observations were made with a Fluorescent Microscope Zeiss Axiophot 100. Negative controls were similarly prepared, except for the primary antibody step, where PBS was used instead. The whole procedure was carried out at 21–22 °C.

For the Conventional Transmission Electron Microscopy (CTEM) E10 embryos neural plates were sectioned in 1-2 mm^3 pieces, fixed for 2 h in 2.5% glutaraldehide in 0.1M cacodylate buffer, pH 7.4 at 4 °C, and postfixed for 1h in 1% OsO_4 in the same cacodylate buffer at 4 °C. They were dehydrated in a graded ethanol series, embedded in Polybed 812 resin (Polysciences, – USA), and polymerized at 60 °C for 48h. Thin sections (60 nm) were cut in an Ultracut Reichert-Jung ultramicrotome with a diamond knife (IVIC, Venezuela), double stained with uranyl acetate and lead citrate and examined in a Philips 400T transmission electron microscope, at 80KV. From the same block semi-thin sections (1μm) were cut, stained with toluidine blue and analyzed in a light microscope Polyvar (Reichert-Jung).

For Scanning Electron Microscopy (SEM), whole E10 embryo neural plates were fixed for 2h in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4 at 4 °C, postfixed for 1h in 1% OsO_4 in 0.1M cacodylate buffer, then dehydrated with a graded series of acetone and dried in a Balzers Critical Point Drying machine. Samples were then shadowed with Au/palladium and observed in a Scanning Electron Microscope (Philips S-500)

Results

The neural plate is a simple structure that can be considered to be the earliest neural tissue and hence, viewed as the developmental beginning of the nervous system. A typical SEM image of a neural plate at E10 is shown in Fig. 1. The diameter at the base is 0.6 mm. The lower half corresponds to the future telencephalic region that will appear at E12, the upper half will give rise to the median cerebrum and the top posterior zone to the posterior cerebrum. The two valve–like parts in the lower zone measure about 0.1 mm in thickness. Semi-thin sections (1 μm), stained with toluidine blue and obtained from this lower zone (Fig. 2) revealed a regular columnar epithelium, with the cells bodies occurring at different distances from the surface. Some cells can be seen extending all along across the neuroepithelium wall. Numerous apparently empty intercellular spaces occur throughout the tissue confering it a delicate consistency. No blood vessels were observed (Fig. 2). TEM observations (Fig. 3) confirm the presence of microvilli at the outer surface

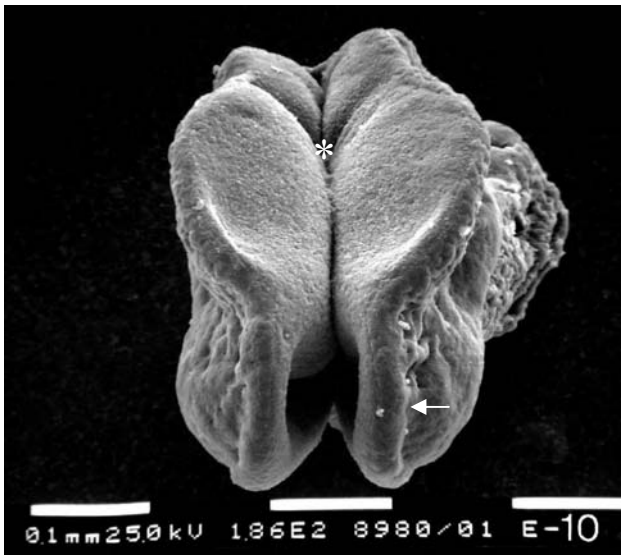

Fig.1: scanning electron microscopy of the neural plate at E10 embryos. At the inner zone is observed the future telencephalic region (arrow) and at the superior region the future medium brain (*) Magnification: 130 X.

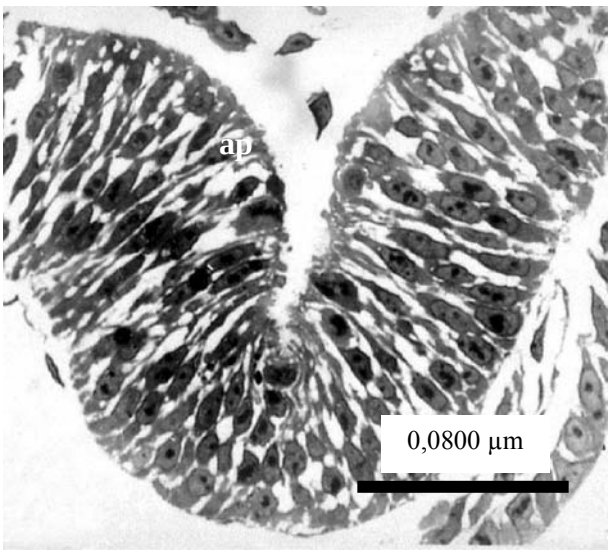

Fig. 2: Transversal semithing section (1 µm) of the neural plate E10 as seen with the light microscope (312X). Cell nuclei (N) are sited at different distances form the epithelium borders and many of then are in mitosis.

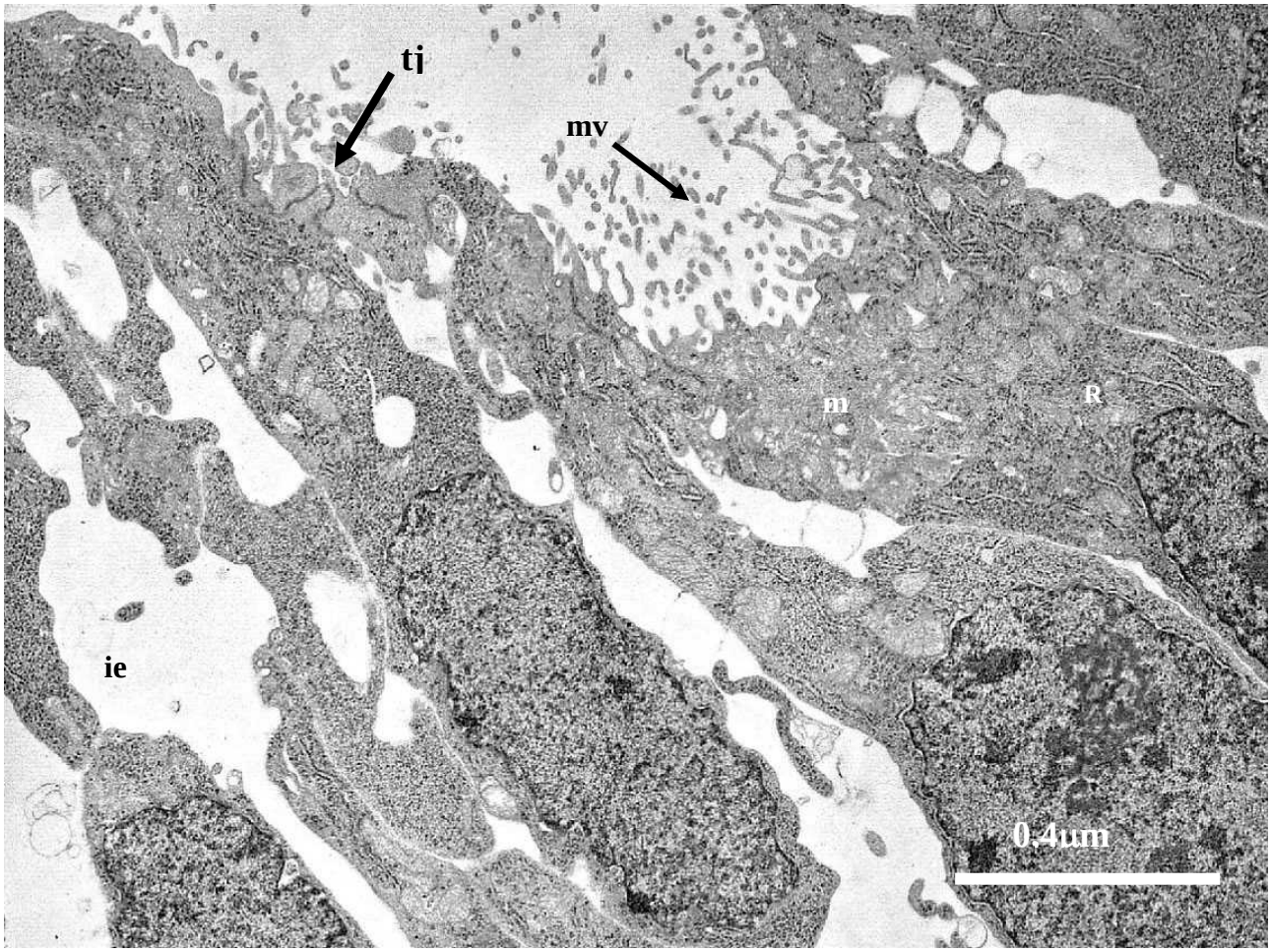

Fig. 3. Electron micrograph of the apical zone of the neurophitelium wall of an E10 rat embryo (10.800X). At the apical tips, the cells are separated by large intercellular space closed at the end by tight junctions. The apical cytoplasm appears full of mitochondria's (m) and endoplasmic reticulum cisterns (R). Abundant microvill (mv) project into the intracellular space.

of the neural plate, which will become the luminal or ventricular surface. Cells exhibit voluminous nuclei and appear connected by tight junctions at their apical or luminal ending. Mitochondria preferentially occur at the apical end of the cell. Mitosis are seen close to the surface of the neural plate wall (fig. 4). At the distal end of the cells (fig.5), a basal lamina lines the surface and delimits the large lacunars spaces separating the cell bodies. At this CNS early stage of development the use of the highly specific anti-pan α-Na^+Ch antibody permitted to see a distribution of the Na^+Ch, as revealed by the immunofluorescence label (fig.6, 7, 8).

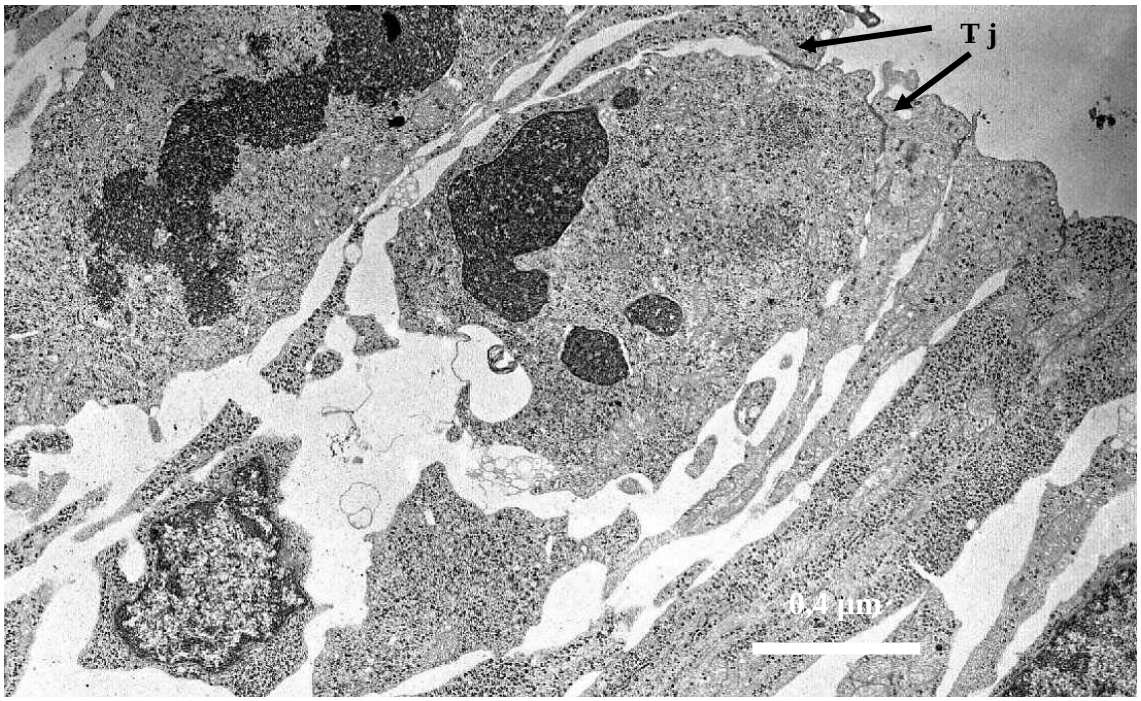

Fig. 4. The apical zone showing two neuroephitelial cells with mitotic nuclei (N) and the tight junction (TJ)c losing those at the apical zone (7.200X).

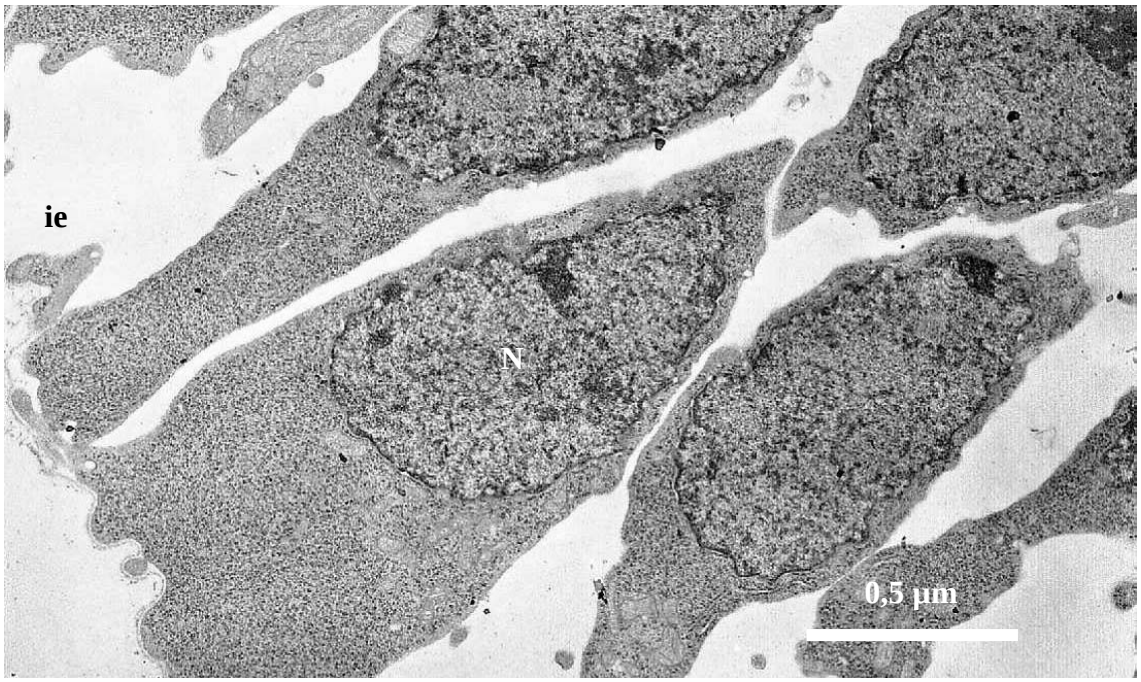

Fig.5. EM of the basal zone of the neuroephiteium wall showing the morphologic characteristic of large cells exhibiting a dense cytoplasm full of ribosome's and mitochondria's below the nuclei at the large intercellular spaces separating then (7.200X)

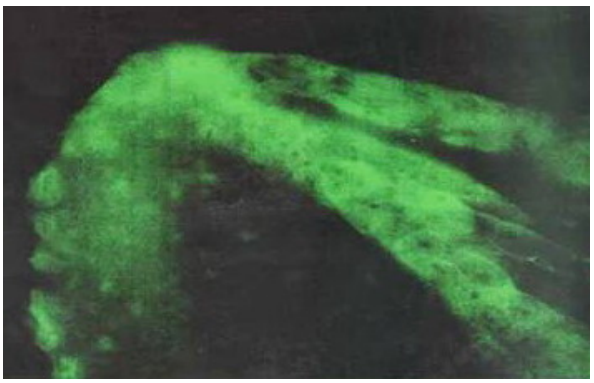

Fig.6. Fluorescent micrograph of the open neural plate of E10 embryo treated with neural SP19 antibody. As seen, the label appears at the border of the wall (170X).

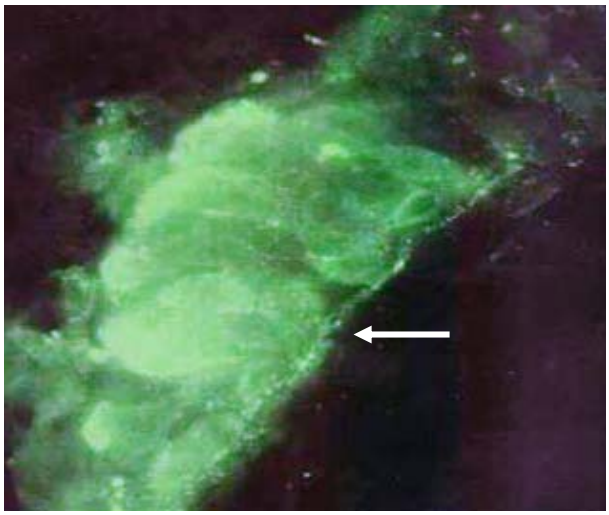

Fig.7. Fluorescence micrograph of the wall of the neuroephiteium of an E10 neural plate taken with a 40X lens. Punctuated labeling of the SP19 antibody at the level of basal membrane is observed. Also note the intense labeling of the whole cells (725X).

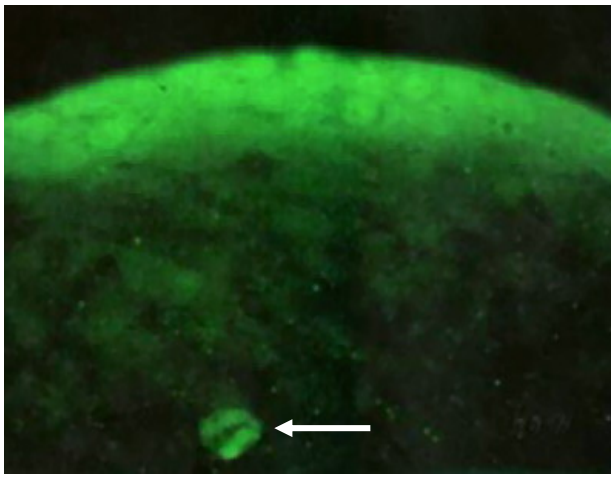

Fig.8. Fluorescence micrograph of the external surface of the neural plate of a 10 day embryo showing a selective label at the polar region (PR). Cell with mitotic appearance and with an intense labeling is marked (⟵) (170X)

In transversal sections of the neural plate wall, the Na^+Ch label was found at both the apical and the basal zone and at the inner wall of the neural plate. At the basal membrane, strong punctuated label (fig. 7) is observed, whereas at the apical zone, the label expression appeared more homogeneous and generally distributed. In addition, Na^+Ch proteins are also localized in neuroepithelial cells undergoing mitosis.

Discussion

One of the main characteristics of the neural differentiation is the development of electrical excitability related to the expression of voltage-dependent ion channels. However, ion channels also have an important role in many other cellular functions and disease states such as cell cycle (43, 44) and apoptosis (45, 46). The mammalian embryonic nervous system is a proliferative neuroepithelium actively engaged in cell replication and cell migration from the apical side (proliferative zone) to the basal side of the neural plate. These activities are regulated by different protein factors. The expression of Na^+Ch is not only responsible for electrical and cellular activities, but it has also an important role in the development of some morphological and physiological activities. Na^+Ch have been localized by previous workers by using immunocytochemistry with antibodies raised against the entire Na^+Ch protein and also against specific peptide sequences such, at sites as the nodal region in rat optic nerve, astrocyte cell bodies and processes (47), Schwann cells (47, 48), rat brain (39) and insect central nervous system (49). Molecular biology and biochemistry studies have revealed the presence of different types of Na^+Ch at early stages of development in rat CNS and a temporal and spatial distribution of them (23). At E10 the expression of Na^+Ch I mRNA is very low, but it increases progressively with CNS maturation, even into postnatal stages of development (25). Unique electrophysiological properties of rat Na^+Ch I suggest that this channel is capable of more rapid firing of the action potential when compared to rat Na^+Ch II. Additionally, the electrophysiological

properties of rat Na^+Ch I are more compatible than those of rat Na^+Ch II with the role of mediating the rapid inactivation transient current in cerebellar Purkinje cells (50). The results presented here show a correlation between the morphological and physiological polarizations in the neuroepithelium of embryonic rat brain. With fluorescent microscopy, we were able to demonstrate the presence of Na^+Ch protein in the neuroepithelial cells at this early stage of development (E10 rat embryos). The fluorescent mark appears around cell bodies and also differentially distributed at the apical and basal zone. The same distribution pattern of the diverse organelles, existing in same tissue at E10, was seen by transmission electron microscopy. The anti-pan α antibody against SP19 sequence has been also effective at the nodal region of mammalian myelinated axons (47) as well as in insect CNS (49), cell adhesion (51, 52, 53), cell movement (51, 54, 20, 50, 55), proliferation (56), fertilization (57), cystic fibrosis and prostate cancer (4, 5, 7, 6). The anti-pan α antibody against SP19 sequence has been also effective at the nodal region of mammalian myelinated axons (47) and insect CNS (49). Several reports using blastomeres have shown that, during development a morphological polarization occurs, being characterized by a redistribution of mitochondria and nuclei towards the basal end of the cells (58. 59). The localization of the cytoplasmic organelles may be influenced by transcellular currents via the resulting intracellular ionic concentration changes, the electric fields or the vectorial water movement (60, 58). Most studies on the development of ion channels in mammals have been carried out in primary culture of embryonic CNS neurons, or in neuronal cell lines, that is, in cells already destined to become excitable. In vertebrates, the neural plate is initially established during gastrulation, when the ectoderm becomes subdivided into the neural and nonneural domains. Our present findings, that show the presence and distribution of the Na^+Ch at such an early stage of vertebrate CNS development, suggests that Na^+Ch potentially play a role in influencing the migration and distribution of cell organelles within cells of the developing neuroepithelium, as well as they are known to do in the excitability events and in the excitability events.

Acknowledgements

We are grateful to Dr. Bechara Kashar from the Section on Structural Biology, National Institute of Health (NIH), Maryland-USA for the collaboration in the use of the fluorescent microscope and of his laboratory facilities. To Mrs Inna Belyanteva of same laboratory for training to make the imumunolabel and for her excellent technical assistance,

Bibliography

[1] Hille B. (1984). *"Ionic Channels of Excitable Membrane"* (Saunderland, MA; Sinauer Associates, INC).

[2] Pinto FM., Intado CG., Merid A., Hidalgo A., Cardenas ML (2000). *"Differential expression of amloid-sensitive Na^+ channel subunit menssenger RNA in the rat uterus."* LifeSci. 22: 313-317

[3] Malhortra JD., Kazen-Gillesper K., Hortsch M., Isom LL (2000). *"Sodium channel beta subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact"*. J.Biol Chem. 275: 11383-11388

[4] Leniado M.E., Lalani E.N., Fraser S.P., Grimes J.A., Bhangal G., Djamgoz M., Abel P.D (1997) *"Expression and functional analysis of voltage-activated Na^+ channels in human prostate cancer cell lines and their contribution to invasion in vitro"*. Am. J. Pathol. 150: 1213 – 1221

[5] Smith P., Rhodes N.P., Adam P., Shorthand S.P., Mustafa B.A., Youqian K., Foster C (1998). *"Sodium channel protein expression enhances the invasiveness of rat and human prostate cancer cells"*. FEBS Letters. 423: 19-24

[6] Diss JK., Archer S.N., Hirano J., Fraser S.P., Djamgoz M.B (2001). *"Expression profile of voltage-gate Na^+ Channel α-subunit genes in rat*

and human prostate cancer cell lines". Prostate 48: 165-78

[7] Djamgoz M.B.A., Mycielska M., Madeja Z., Frazer SP., Korohoda W (2001). *"Directional movement of rat prostate cancer cells in direct–current electric field: involvement of voltage gated Na^+ channel activity"*. J,Cell.Sci. 114: 2697-2705

[8] Abdul R., Hoosein N (2002). *"Voltage-gated sodium channel in prostate cancer: expression and activity"*. Anticancer Res. 22: 1727-1730

[9] Sato Ch., Sato M., Iwasaki A., Takeshi D., Engel A (1998). *"The sodium channel has four domains surrounding a central pore"* J Struct Biol. 121: 314-325

[10] Ellisman M.H., Miller J.A., Agnew W.S (1983). *"Molecular morphology of the tetrodotoxin–binding sodium channel protein from Electrophorus electricus in solubilized and reconstituted preparations"*. J. Cell Biol. 97: 1834 -1840

[11] Isom L.L., DeJongh K.S., Patton D.E., Reber B.X., Offord J., Charbonneau H., Walsh K., Golding A.L., Catterall W.A (1992). *"Primary structure and functional expression of the β1 subunit of rat brain sodium channel"*. Science 256: 839-842

[12] Isom L.L., Ragsdale D.S., De Jongh K.S., Westenbroeck R.E., Reber BX., Scheuer, T., Catterall W.A (1995). *"Structure and function of β2 subunit of brain sodium channels, a Tranmembrane glycoprotein with a CAM motif"*. Cell 83: 433-442

[13] Hartshorner R.P., Catterall W.A (1994). *"The sodium channel from rat brain: purification and subunit composition"*. J.Biol.Chem. 257, 13888 – 13891

[14] Messner D.J., Catterall W.A (1985). *"The sodium channel from rat brain. Separation and characterization of subunits"*. J.Biol.Chem. 261, 211-215

[15] Robert R., Barchi R (1987). "*The voltage-sensitive sodium channel from rabbit skeletal muscle. Chemical characterization of subunits"*. J. Biol. Chem. 262: 2298 – 2303

[16] Noda M., Ikeda T., Kayano H., Suzuki H., Takeshima M., Kurasaki M., Takahashi H., Numa S (1986). *"Existence of distinct sodium channel messenger RNAs in rat brain"*. Nature 320:188 – 192

[17] Kayano T., Noda M., Flockerzi V., Takahashi H., Numa S (1988). *"Primary structure of rat brain sodium channel III deduced from the cDNA sequence"*. FEBS Lett. 228:187-194

[18]Trimmer J.S., Cooperman S.S., Tomiko S.A., Zhou J.Y., Crean S.M., Boyle M.B., Kallen R.G., Sheng Z.H., Barchi R.L., Sigworth F.J., Goodman R.H., Agnew W.S., Mandel G (1989). *"Primary structure and functional expression of a mammalian skeletal muscle sodium channel"*. Neuron 3:33-49

[19] Tanabe T., Takashima H., Mikami A., Flockerzzi V, Takahashi H, Kangawa K, Kojima M, Matsuo H., Hirose T., Numa, S (1987*).."Primary structure of the receptor for calcium channel blockers from skeletal muscle"*. Nature 328: 313-318

[20] Miyazaki S., HiraI S (1991) *"Fast polyspermia block and activation potential. Correlated changes during maturation of starfish"*. Dev. Biol. 70: 327-340

[21] Auld V.J., Goldin A.L., Krafte D.S, Marshall J., Dunn L.M., Catterall W.A., Lester H.A., Davidson N, Dunn R.J (1988). *"A rat brain Na^+ channel α subunit with novel gating properties"*. Neuron 1: 449– 461.

[22] Schaller K.L., Krzemien D.M., Yarowsky P.J., Krueger B.K., Caldwell J.H (1995*)."A novel, abundant sodium channel expressed in neurons and glia"*. J. Neurosci.15: 3231– 3242

[23] Beck S (1990*)."Differential expression of sodium channel mRNA in rat peripheral nervous system and innervated tissue" .FEBS*.Lett. 262: 317-322

[24] Brysch W., Creutzfeldt O.W., Luno K., Schlingensiepen R., Schlingensiepen K.H (1991). *"Regional and temporal expression of sodium*

channel messenger RNAs in the rat brain during development". Exp. Brain. Res 86: 562-567

[25]Villegas R., Castillo C., Póo M.E., Schnell S., Piernavieja C., Balbi D., Villegas G.M (1994). *"Expression of sodium cannels with different saxitoxin affinity during rat forebrain development"*. Dev. Brain. Res. 81: 26-40

[26] Scheiman R., Auld V.J., Goldin A.L., Davidson N., Dunn R., Caterall W.A. (1989). *"Development regulation of sodium channel expression in the rat forebrain"*. J. Biol.Chem. 18: 10660-10666

[27] Felts P.A., Yokoyama S., Dib-hajj S., Black J.A., Waxman S.G (1996). *"Sodium channel α-subunit mRNAs I, II; III, Na6 and hNE(PN1): different expression patterns in developing rat nervous system"*. Mol. Brain Res.45: 71-82

[28] Mandel G (1993*). "Sodium channel regulation in the nervous system: how the action potential keeps in shape"*. Curr. Opin y Neurobiol. 3: 278-282

[29] Grimes J.A., Fraser S.P., Stephens J.E.G, Downing M.E., Laniado C.S., Foster P.D., Abel P.D., Djamgoz M.B.A (1995). *"Differential expression of voltage-activated Na^+ currents in two prostatic tumor cell lines: contribution to invasiveness in vitro"* FEBs Letter 369:290-294

[30] Gordon D., Merrick D., Auld,V., Dunn R., Goldin A.L., Davidson N., Caterall W.A (1987). *"Tissue specific expression of the TI and RII sodium channel subtypes"*. Proc. Natl. Sci USA 84:8682–8686

[31] Kirschner M.W (1992) *"The biochemical nature of cell cycle"*.Important Adv. Oncol. 3-16

[32] Okamoto H., Takahashi K., Yamashita N (1977). *"Ionic currents through the membrane of the mammalian oocyte and their comparison with those in the tunicate and sea urchin"*. J.Phisiol. (Lond). 267: 465 - 495

[33] Spelmann H., Mangold H (1924). *"Uber Induktion von Embryonalanlagen durch Implantation artfremder Organsatoren"*. Arch.f. mikr. Anat. U .Entw. mech. 100: 599-638

[34] Chang C., Hemmati-Brinvanlou A (1998*). "Cell fate determination in embryonic ectoderm"*. J. Neurobiol. 36: 128-151

[35] Witschi E (1962) *"Grow Including reproduction and morphological development. Development rat In"* Altman P.I and Dittmer D.S (eds). Washington: Fed Am. Soc. Exp. Biol: 304-314

[36] Angevine J.B., Sidman R.L (1969) *"Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse"*. Nature 192: 766-768

[37] Jacobson M (1979) Developmental Neurobiology. New York. Plenum,

[38] Jessel T.M., Melton D.A (1992) *"Diffusible factors in vertebrate embryonic induction"*. Cell 68:257-270

[39] Westenbroeck R.E., Merrick D.K, Catterall W.A (1989). *"Differential subcellular localization of the RI and RII Na^+ channel subtype in central neurons"*. Neuron 3: 695 – 704.

[40] Vassilev P, Scheuer T, Catterall W.A.(1989). *"Inhibition of inactivation of single sodium channels by a site directed antibody"*. Proc. Natl. Acad. Sci. USA 86: 8147 – 8151

[41] Gordon D, Merick D, Wollner D.A, Catterall, W.A (1988). *"Biochemical properties of sodium channels in a wide range of excitable tissue studies with site-directed antibodies". Biochemistry* 27: 7032 – 7038

[42] Gordon D, Moskowitz H, Zlotkin E (1990). *"Sodium channel in central nervous system of various insects identified with site–directed antibodies". Biophys. Biochem. Acta*. 1026: 80 – 86

[43] Wonderlin W.F, Woodforckr K.A, Strogl, J.S (1995) *"Changes in membrane potential during the progretion of MCF-1 human mammary tumor cell through the cell cycle"*. J.Cell Physiol. 165:177-185

[44] Van Dolah F.M, Ramsdell, J.S (1996) *"Maitotoxin, a calcium channel activator, inhibit cell cycle progretion throug the G1/S and G2/M transition and prevents CDC2 kinase activation in GH4Cl cell."* J.Cell Physiol 166:49-56

[45] Kondo S, Montag T, Tashima Y (1995) *"Benign prostate hypertrophy affects the endothelin receptor density in the human urinary bladder and prostate"*. Urol. Int. 54: 198-203

[46] Nagy P, Pany G, Jenei A, Bene L, Gaspar R, Matko JR, Damjanovich S (1995) *"Ion-Channel activities regulate transmembrane signaling in thymocite appoptosis and T cell activation"* .Immnol lett 44:91-95

[47] Black J.A, Westerbroeck R, Catterall, W.A, Waxman, S.G (1995*) "Type II brain sodium channl expression in non-neuronal cells: embryionic osteoblast"*. Mol Brain Res 34: 89-989

[48] Ritchie J.M, Black R.B, Waxman S.G, Angelides, K.J (1990) *"Sodium channel in the cytoplasm of schawn cells"*. Proc. Natl. Acad. Sci. USA 87: 9290-9204

[49] French A.S., Sander E.J., Duszky E., Prasad S., Torkkeli P.H., Haskins J., Murphy R.A (1993). *"Immunocytochemical localitation of sodium channels in an insect central nervous system using a site-directed antibody"*. J. Neurobiol 24: 939-948

[50] Beck S., Noda M., Lubbert H., Numa S (1989). *"Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development"*. EMBO J 8: 3611 – 3636

[51] Arcangeli A., Becchetti A., Mannini A., Muenai G, De Filippi P., Tarone G., Del Bene M.R., Barletta E., Wanke E., Olivoto M (1993). *"Integrin-mediated neurite outgrowth in neuroblastoma cell depends on the activation of potassium channels."* J cell. Biol. 122:1131-1143

[52] Stock C., Gassner B., Hauck, C.R., Arnold H., Mally., S, Eble., J.A., Dietrich P., Schwab A (2005) *"Migration of human melanoma cell depends on extracellular pH and Na^+/H^+ exchange"*. J.Physiol

[53] Isom LL (2002). *"The role of sodium channel in cell adhesion"*. Front. Biosci. 7: 12-23.

[54] Bolvist K., Eliasson L., Ammala C., Renstrom E., Rorsman P (1995) *"Co-localization of Ca^{+2} channels and insulin-containig secretory granules and its significance for the initiation of exocitosis"*. EMBO 3:50-57

[55] Sheng M., Tsaurt M.L., Jan Y.N., Jan L.Y (1994) *"Contrasting subcellular localization of Kv1.2K^+ channel subunit in different neuron of rat brain"*. 14:2408-2501

[56] Woodfork K.A., Wonderling W.F., Pterson V.A., Strobls J.R (1995) *"Inhibition of ATP sensitive potassium channel cause reversible cell-cycle arrest of human cancer cells in tissue culture"*. J.Cell physiol. 162:163-171

[57] Yamashita N (1982) *"Enhancement of ionic currents through voltage-gate channel in the mouse oocyte after fertilization"* .J. Physiol. (Lond) 329: 263-280

[58] Wiley C.M (1984) *"Cavitations in the mouse preimplantation embryo: Na+/K+ ATPasa and the origin of nascent blastocoel fluid"*. Dev.Biol 105: 330-342

[59] Reeve W.J., Kelly F.P (1983) *"Nuclear position in cells of the mouse early embryo"*. J. Embryol Exp Morphol 75:117-39

[60] Nuccitelli R., Wilye L (1985). *"Polarity of isolated blastomeres from mouse morulae: detection of transcellular ion currents"* Dev.Biol 109:452-463.