Histol Histopathol (2007) 22: 497-504

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Localization of connexins in neurons and glia cells of the *Helix aspersa* suboesophageal brain ganglia by immunocytochemistry

M.J. Azanza[1], N. Pes[1], R.N. Pérez-Bruzón[1], J. Aisa[1], M. Raso[1], C. Junquera[1], M. Lahoz[1], C. Maestú[2], C. Martínez-Ciriano[1], C. Pérez Castejón[1], A. Vera-Gil[1] and A. del Moral[3]

[1]Laboratorio de Magnetobiología, Departamento de Anatomía e Histología Humanas, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain, [2]Fundación Humanismo y Ciencia, Madrid, Spain and [3]Laboratorio de Magnetismo, Departamento de Física de Materia Condensada e ICMA,Universidad de Zaragoza-CSIC, Zaragoza, Spain

Summary. The aim of the present study was to examine the distribution of cells expressing connexin 26 (Cx26) in the suboesophageal visceral, left and right parietal and left and right pleural ganglia of the snail *Helix aspersa* by immunocytochemistry. Altogether we have found approximately 452 immunoreactive neurons which represent the 4.7% of the total neurons counted. The stained large neurons (measured diameter 55-140 μm) occurred mostly on the peripheral surface of the ganglia while the small immunostained cells (5-25 μm diameter) were observed in groups near the neuropil. The number of large neurons giving positive Cx26-like immunostaining was small in comparison with that for medium (30-50 μm diameter) and small sized cells. The expression of Cx26 was also observed in the processes of glia cells localized among neurons somata and in the neuropil showing that the antiserum recognized epitopes in both protoplasmic and fibrous glia cells of *Helix aspersa*. The neuropils of all ganglia showed fibers densely immunostained. While we have observed a good specificity for Cx26-antiserum in neurons, a lack of reaction for Cx43 antiserum was observed in neurons and glia cells. The reaction for enolase antiserum in neurons was light and non-specific and a lack of reaction in glia cells and processes for GFAP antiserum was observed. Although the percentage of positive neurons for Cx26 antiserum was low is suggested that in normal physiological conditions or under stimulation the expression of connexin could be increased. The observed results can be considered of interest in the interpretation of *Helix aspersa* elemental two neuron networks synchronizing activity, observed under applied extremely low frequency magnetic fields.

Key words: Connexins, Immunocytochemistry, *Helix aspersa*, Synchronization

Offprint requests to: Dr. M.J. Azanza, Departamento de Anatomía e Histología Humanas, Facultad de Medicina, Universidad de Zaragoza, C/ Domingo Miral s/n, 50009 Zaragoza, Spain. e-mail: mjazanza@unizar.es

Introduction

The neurons of gastropods have been object of several cytological, cytochemical, biochemical and physiological studies. The neuronal network regulating cardiac activity is scattered throughout the visceral and right parietal ganglia of *Helix pomatia* (Rózsa, 1976). *Helix* ganglia can contribute to the knowledge of the cellular bases of the behaviour (Balaban, 2002). They constitute a good experimental model for the study of the effects of applied static and extremely low frequency (ELF) weak magnetic fields (MF) (Azanza and del Moral, 1994, 1998) and for the study of the generation and transmission of synchronizing activity in elemental neuron networks under applied ELF-MF (Azanza et al., 2002). Giant neurons of snails are excellent for investigating rhythmical activity. Among giant neurons studied there are many which show rhythmic activity and some of these generate the spikes clearly without any synaptic influence, i.e., they are spontaneously firing cells, the so-called pacemaker neurons (Vadász and Salánki, 1976). In the determination of the dynamical structure of neural circuits, rhythms and oscillations are a prominent feature. Coherent oscillations have been reported to be displayed by a network of olfactory interneurons in the cerebral ganglion of the terrestrial mollusc *Limax maximus* (Gelperin and Tank, 1990). Interneurons have been identified in the procerebrum of *Helix aspersa* as small neurons (5-8 μm) whose

processes were restricted into the ganglion and were synaptically interconnected (Ratté and Chase, 1997). Either in response to internal or to external stimuli, single oscillatory neurons can trigger some other neurons in a network through the synchronous activity of a group of neurons in which interneurons could play an important role. Gap junctions are intercellular channels which directly link the cytoplasm interior of cells, allowing the passage of ions and other small molecules between neighbouring cells. They are in this way involved in intercellular communication. One of their physiological meanings is that they form the so called electrical synapses, since the bioelectric impulse can be transmitted between adjacent cells without the delay being important in inducing the synchronous rhythmic activity in the central nervous system (CNS). Gap junctions are common between glia cells, but they are also present between at least some populations of neurons and between neurons and glia cells (Dermietzel and Spray, 1993; Fróes et al., 1999). The observation of synchronizing activity in mollusc's elemental two neuron networks under applied ELF-MF (Azanza et al., 2002) opened the interest about the study of the development of gap junctions between neurons and neuron-glial cells in the suboesophageal ganglia complex of the snail *Helix aspersa*. The aim of this work has been to evaluate whether neurons and glia cells from these ganglia are able to express connexin 26, which is one of the protein monomers of the neuron-neuron and neuron-glia gap junction connexons. The results about the expression and distribution of positive immunostained nerve cells may contribute to the knowledge of the role played by gap junctions in the synchronizing process in *Helix aspersa* neuron networks, either in normal physiological conditions or induced under an externally applied stimulus like an extremely low frequency magnetic field.

Material and methods

We examined 8 samples of *Helix aspersa* suboesophageal visceral, left and right parietal and left and right pleural brain ganglia complex. The specimens were bought in the local market. Many of the snails maintained in laboratory conditions are inactive; it has been shown that with very little neuron bioelectric spontaneous activity (Kerkut and Walker, 1961a). It could be expected in such conditions a low expression of certain proteins so that the animals were maintained active in the following manner. During approximately two weeks before dissection, the snails were maintained in a jar containing 1/4 inch fresh tap water for about 30 min/day. The results of the humid atmosphere led the animals to emerge from their shells and crawl round the jar. They were then transferred to a cage and fed with lettuce *ad libitum* (Kerkut and Walker, 1961b). The circumoesophageal ring of ganglia were removed and the suboesophageal ganglia complex cut and fixed for 16 hours in 4% formaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4), at 4°C, dehydrated in a graded series of ethanol and embedded in paraffin wax. Serial sections of 5μm were routinely stained with haematoxylin-eosin.

Immunocytochemical staining was performed on serial paraffin sections 5 μm thick fixed in formaldehyde using the immunocytochemistry EnVision® (Dako) method. We used a panel of polyclonal antibodies raised against mammal antigens. In order to determine neuron and glia cells connexin expression: connexin 26 (Cx26) (dilution 1/1000, rabbit-antimouse, AB 1717 Chemicon). For characterizing neuron and glia cells: neuron-specific enolase (dilution 1/3000, rabbit-antimouse, AB 951 Chemicon); astrocytes-specific connexin 43 (Cx43) (dilution 1/500, rabbit-antimouse, AB 1728 Chemicon) and glial fibrillary acidic protein (GFAP, dilution 1/400, Z 0334 Dako). The antibodies were diluted with Dako diluent (S2022).

The tissue sections were deparaffined in xylene (10 min. twice) and rehydrated in a graded ethanol series up to distilled water. Before all assays, for heat-induced antigen retrieval, the samples were treated during 6 min. in an 800-W microwave oven with 10% citrate buffer (Dako S2031) in distilled water and at 360 W for 5 additional minutes. After washing with PBS, 3 min twice, the sections were treated with endogenous peroxidase blocking (Dako S2001) for 20 min washed in distilled water and PBS 3 min twice. The blocking was repeated for a second time. The sections were incubated with the primary antibodies solution for 30 min followed by a rinse in blocking buffer (100 ml PBS, 2 ml triton x100, 0.25 ml BSA (A4503 SIGMA)), for 3 min. twice. The visualization was made by incubating with Envision® peroxidase-based visualization kit (Dako K5007) during 30 min, washed in PBS, for 3 min. twice according to manufacturer's direction. To confirm the presence of immunocomplexes, 3,3'-diaminobenzidine was used as chromogene and hydrogen peroxide as substrate. The samples were washed twice in distilled water, contrasted with Mayer's haematoxylin for 7 min., washed in tap water for 15 min., dehydrated in a graded series of ethanol, cleared in xylene and cover slipped with DPX. Digital microscope images were captured by means of an Olympus BX 51 microscope. The number of immunoreactive cells was estimated from serial sections by counting immunostained cell bodies. The diameter of a neuron was taken from its cross section containing the nucleus. Both measurements were made with the program Olympus DP-Soft.

The research of possible co-localization of the immunoreactive stainings for Cx26, Cx43, enolase and GFAP antisera was performed on 5 μm-thick serial tissue sections.

Positive controls were made by applying the same protocol on Wistar rat brain tissue and rectum sections. Negative controls were made by omission of primary antibody on *Helix* and rat brain tissue sections.

Results

For the interpretation of the results obtained by immunocytochemistry it must be taken into account that we have used in an invertebrate a panel of antisera which were raised against mammal antigens. We have observed a positive reaction for Cx26, but not for Cx43 or for GFAP antisera and a non-specific, light reaction for enolase antiserum. This means that the nervous cells of *Helix aspersa* suboesophageal brain ganglia complex only recognize epitopes for Cx26 antiserum.

The distribution of neurons giving Cx26-like immunostaining was not homogeneous throughout the suboesophageal ganglia complex. Immunocytochemistry results are given as the percentage of cells showing immunoreactive-like staining for the studied antisera. We have counted a total of 9626 neurons in the serial tissue sections stained with Cx26 antiserum. Altogether we have found approximately 452 immunoreactive neurons which mean that about 4.7% have given a positive reaction. In this percentage the positive glía cells were not enclosed since they are too small and difficult to count. The immunostaining revealed immunoreactive structures in the cytoplasm of the soma and in fibers in the neuropil. Up to now there have been no data on morphological dissimilarities between nerve cells differing in the character of their physiological rhythmic activity. The cellular morphology did not reveal any gross structural organization and in the neuropil, axons were not predisposed to run at any particular position along any of the axes, they seemed to do so in a random fashion. According to these characteristics we have considered the size of neurons as identification criteria. With a few exceptions, the entire population of Cx26 neurons consisted of small (5-25 μm), medium (30-50 μm) and large-size (55-140 μm) neurons.

In a panoramic section of the anterior surfaces of the visceral and left and right parietal ganglia 107 positive immunoreactive cells for Cx26 antiserum have been counted with the following distribution: 48 in the left parietal, 45 in the visceral and 14 in the right parietal ganglia (Fig. 1). In this tissue section it has been possible to recognize several mapped neurons in the right parietal ganglion, F1, F2, F34, F54, F53 and F77 which are regularly the object of electrophysiology recordings (Kerkut et al., 1975). The neuropil was richly supplied with immunoreactive fibers. In a more posterior section, at the level of the left pallial nerve sorting (Kerkut and Walker, 1962), 63 immunostaining neurons have been

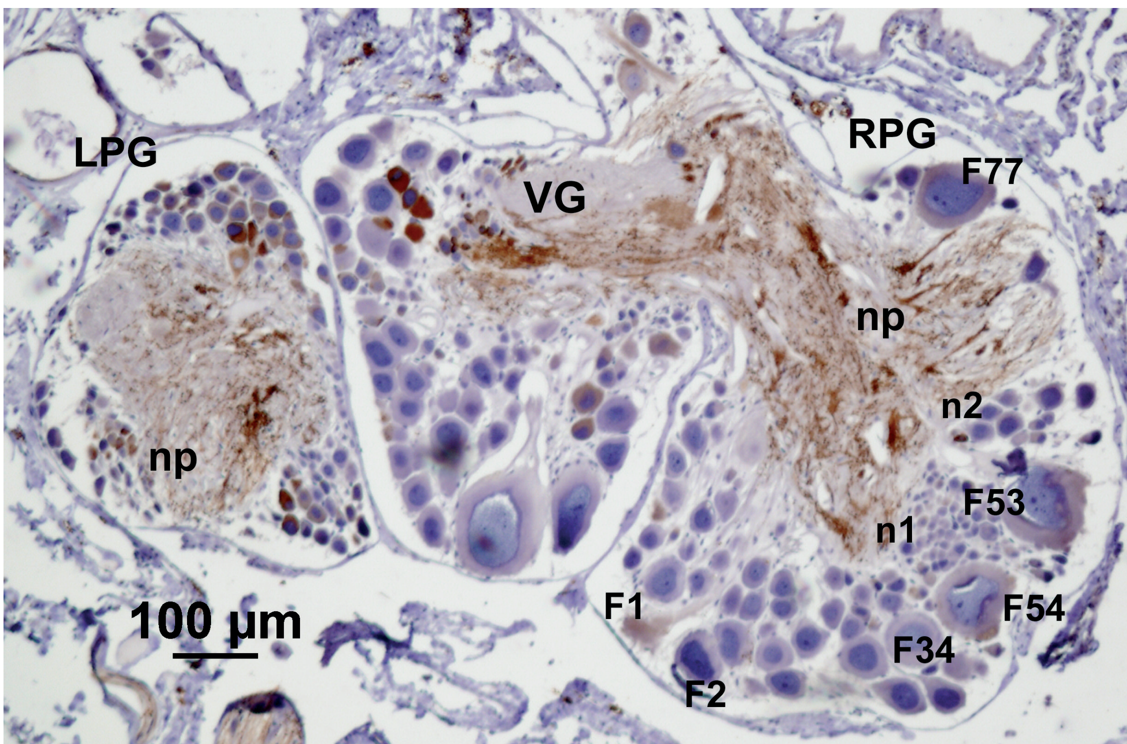

Fig. 1. Panoramic view of an anterior section of the visceral (VG), left (LPG) and right parietal (RPG) suboesophageal ganglia. 107 immunoreactive cells for Cx26 antiserum are observed with the following distribution. LPG, 48 small sized neurons: 27 in the dorsal portion (11 with somatic diameters of about 25 μm and 16 with diameters in the range of 10-15 μm); 9 neurons in the medial-lateral portion with diameters of about 10 μm; 12 neurons in the ventral-medial portion (4 of about 25 μm and 8 with diameters in the range of 8-10 μm). VG, 45 small sized neurons: 31 in the dorsal portion (5 of about 25 μm and 26 with very small diameters of 5-10 μm); 10 neurons in the medial portion (1 of about 25 μm and 9 of 5-10 μm); 4 small neurons in the ventral portion. RPG, 14 positive neurons: 1 large neuron in the dorsal portion slightly stained (F77, of about 100 Ìm diameter); 10 neurons in the medial portion (9 with diameters of about 10 μm and 1 large neuron slightly stained, F53, of about 100 μm); 3 neurons in the ventral portion (1 small of about 10 μm and 2 large slightly stained, F2 and F54, of about 100 μm). The neuropil (np) was richly supplied with immunoreactive fibers.

observed, 43 in the left parietal, 15 in the visceral and 5 in the right parietal ganglia. With the exception of one large neuron of 110 μm, localized in the dorsal portion of the visceral ganglion, all the neurons in this section were of small size. The left parietal ganglion is smaller than the corresponding right one but it shows a higher number of stained neurons.

The immunostaining reaction for Cx26 antiserum was like a fine brown precipitate widespread in the cytoplasm of the largest neurons, the medium and small sized cells appeared more densely stained (Figs. 1, 2a). As shown in figures, the number of large neurons giving positive Cx26-like immunostaining is smaller that the one for medium and small size cells (Figs. 1, 2a,b, 3a). The small sized immunostained cells occur mostly in groups near the neuropil, sometimes forming clusters (Figs. 2c, 3a). Some of the axons could be traced into the neuropil (Figs. 2c, 3a). Among superficially located non-stained neuron somatas of medium and large size, were localized protoplasmic glia cells processes positive for Cx26 antiserum (Fig. 2d). A common observation is the high density of immunostained fibers in the neuropil for the low number of positive neurons localized in the same tissue section. A percentage of these fibers must come from neurons located at different tissue levels in the ganglia and from fibrous glia cell expansions (Figs. 1,

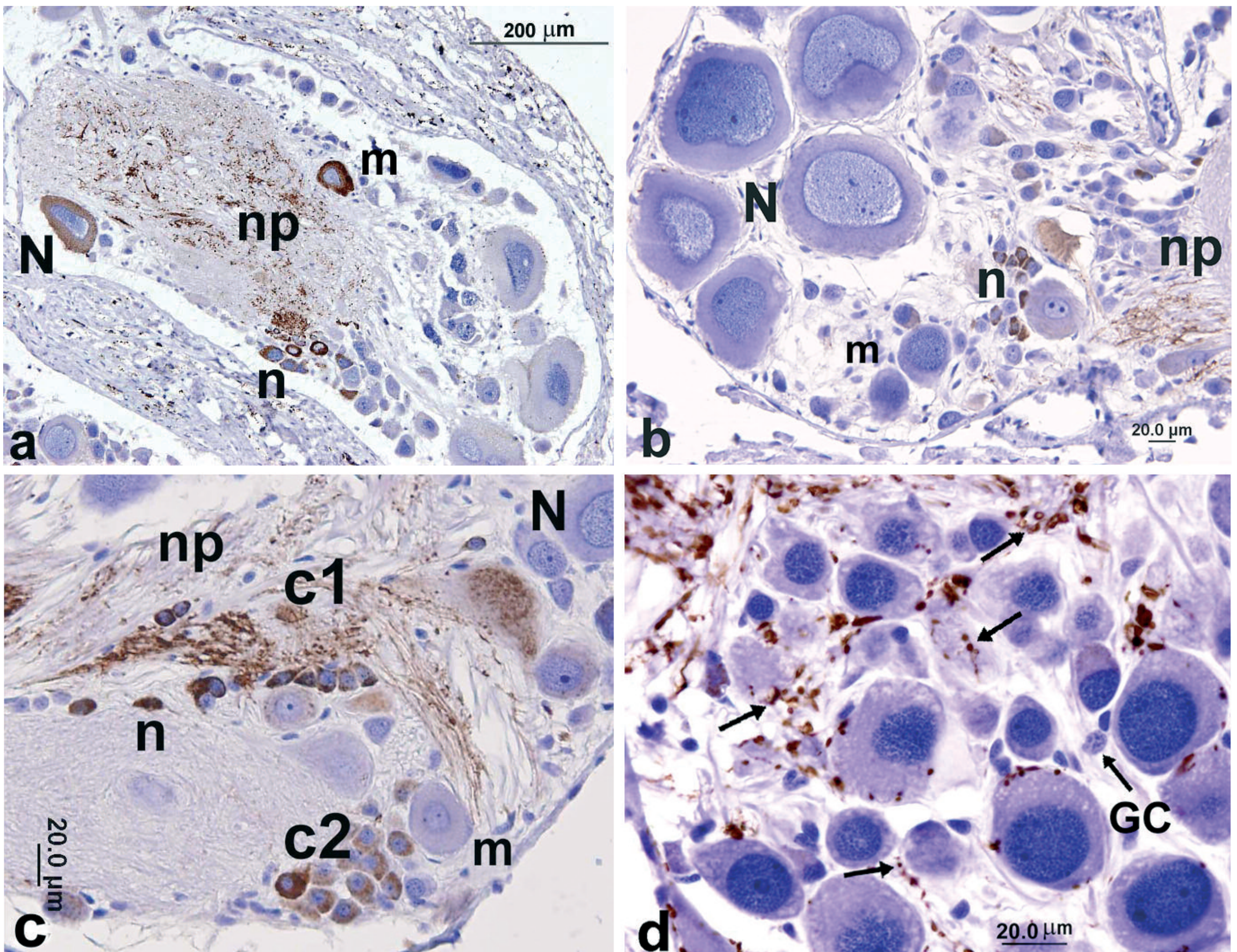

Fig. 2. Immunocitochemistry reaction with anti-Cx26. **a)** panoramic section of the right parietal ganglion, posterior portion, shows: 1 large neuron (N) localized in the left-superficial dorsal portion; 1 medium neuron (m) in the right-medial portion and 7 small sized neurons (n) densely stained in the left-medial portion. **b)** section of the left parietal ganglion, medial-anterior portion, shows: 5 non-stained large neurons (N) at the surface of the ganglion and 17 small-sized positive neurons (n) near the neuropil (np). **c)** section of the right parietal ganglion, posterior-dorsal portion, shows: 1 superficially located large non-stained neuron (N); 3 medium-sized scattered stained neurons (m) and 27 small neurons disposed in clusters (c1, c2) near the neuropil (np). **d)** positive glia cell processes distribute among superficially localized non-stained neuron somatas (CG, glia cell nuclei).

Fig. 3. Comparison of immunostaining reaction for Cx26, Cx43 and enolase antisera in serial sections of the left pleural ganglion, dorsal portion. The results show: **a)** a good specificity for Cx26 in 2 medium-sized neurons, an small group of 5 neurons (n1) and two clusters of 8 (c1) and 6 (c2) small neurons; **b)** lack of reaction for Cx43; **c)** non-specific reaction for enolase. Serial tissue sections allow recognizing neurons 1, 2, 3, 4 and 5, the group of small neurons n1 and clusters c1 and c2 of small neurons (np, neuropil).

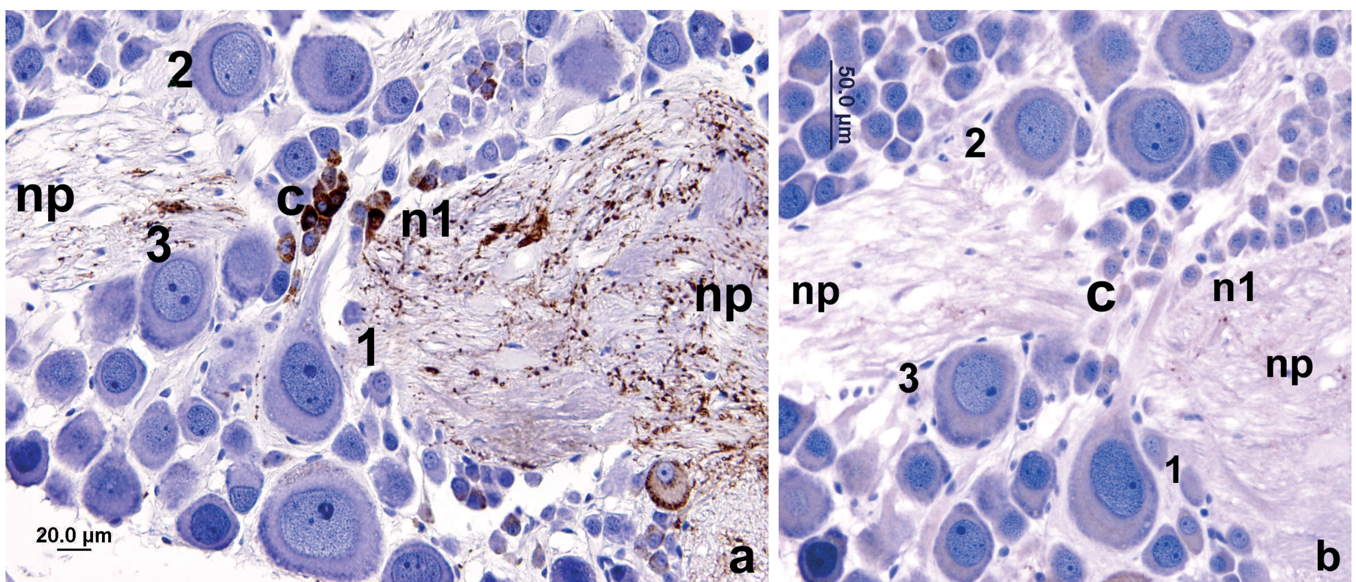

Fig. 4. Serial tissue sections of the right pleural ganglion, posterior-dorsal portion, shows: **a)** a cluster (c) of 8 small neurons plus 18 small and 1 medium sized neurons positive for anti-Cx26; **b)** the negative reaction in glia cells and processes for anti-GFAP. Serial tissue sections allow recognizing neurons 1, 2, 3, the cluster of small neurons (c) and the group of small neurons n1. np: neuropil.

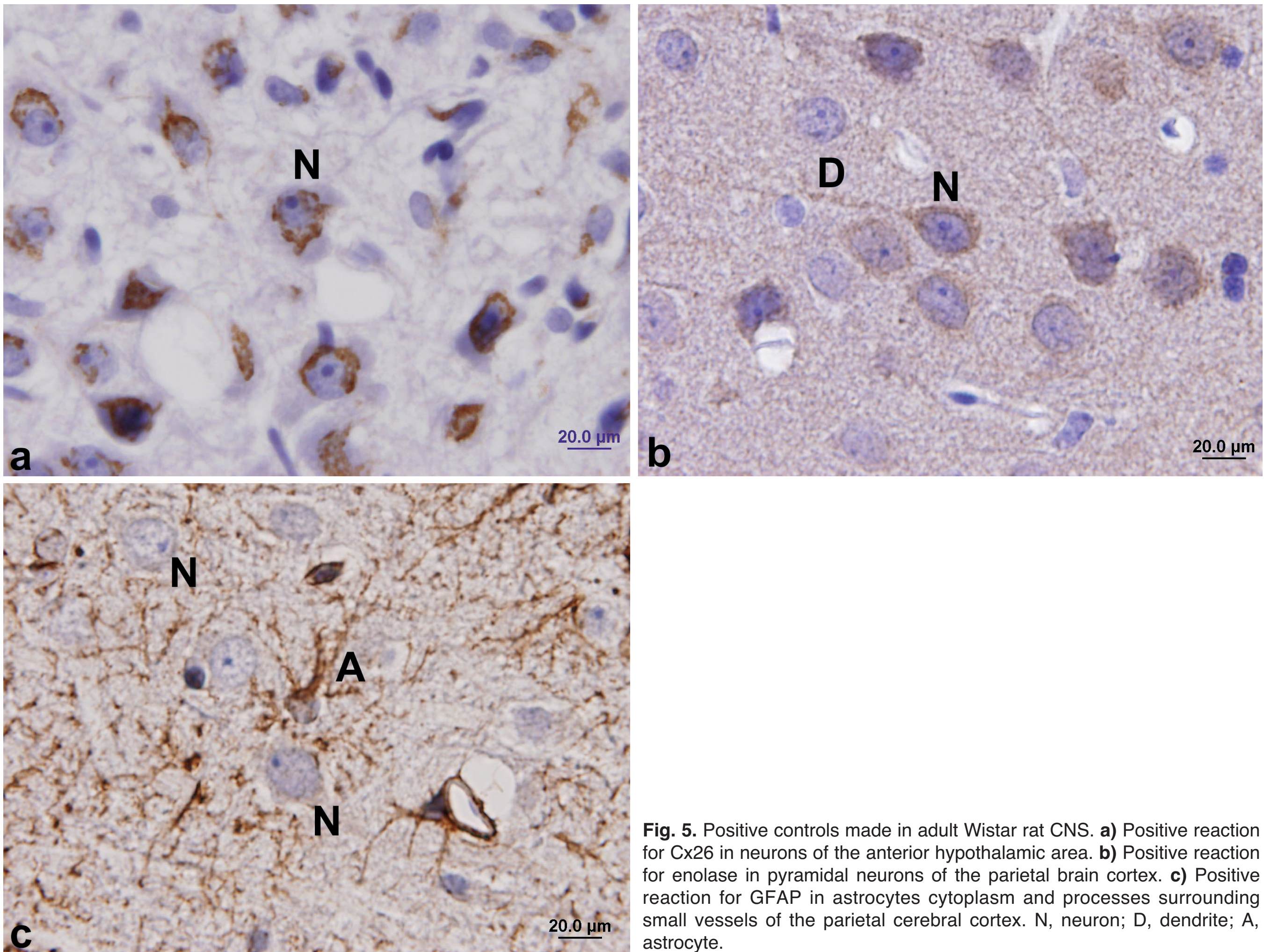

Fig. 5. Positive controls made in adult Wistar rat CNS. **a)** Positive reaction for Cx26 in neurons of the anterior hypothalamic area. **b)** Positive reaction for enolase in pyramidal neurons of the parietal brain cortex. **c)** Positive reaction for GFAP in astrocytes cytoplasm and processes surrounding small vessels of the parietal cerebral cortex. N, neuron; D, dendrite; A, astrocyte.

2a, 3a, 4a).

In order to characterize the kind of cells found in the CNS of *Helix aspersa*, we have studied the reaction for enolase antiserum, which is specific for mammal neurons (Dermietzel and Spray, 1993), Cx43 specific for mammal astrocytes (Micevych and Abelson, 1991; Dermietzel and Spray, 1993) and GFAP antiserum also specific for mammal astrocytes. In Figs.3 are shown the results obtained by comparing alternate serial sections of the left pleural ganglion, dorsal portion, treated with these markers. In comparison with the specificity for Cx26-antiserum (Fig. 3a), a lack of reaction for Cx43 (Fig. 3b) and a light, non-specific, reaction for enolase antiserum was observed (Fig. 3c). A lack of reaction was also observed for GFAP immunostaining in glia cells and processes in alternate serial sections of the medial portion of the right pleural ganglion (Fig. 4b).

The positive controls made in adult Wistar rat CNS showed positive reaction for Cx26 in the anterior hypothalamic area (Fig. 5a), for enolase in pyramidal neurons of the parietal cerebral cortex (Fig. 5b) and for GFAP in astrocytes of the parietal cerebral cortex (Fig. 5c). The results for Cx43 were negative in sections of the parietal cerebral cortex, a positive reaction was observed in the rectum.

Discussion

Considering the localization of the immunostaining reaction for Cx26 in the cytoplasm and fibers, it is of interest to consider the biosynthesis pathway. The first step of gap junction formation involves the assembly of newly synthesized connexins into connexons in either the endoplasmic reticulum or the trans-Golgi network. The connexons then travel in vesicular structures along microtubules to the plasma membrane where they insert. This insertion appears to occur randomly all over the plasma membrane instead of being specifically targeted to the gap junctions. The final step in gap junction formation involves the lateral diffusion of connexons in

plasma membrane to the outer margins of the plaques, where they can be anchored to form complete gap junction channels (Lauf et al., 2002). Our results are probably revealing several immunoreactive structures in the cytoplasm and fibers by the fact of the complex intracellular transport of connexins from the site of synthesis up to the final location in membranes. Immunocytochemistry seems to be a limited methodology which does not allow making a precise location of gap junction sites into membranes. Such an objective is even difficult to reach with conventional electron microscopy since the observation of gap-like specializations is that of a membrane thickness between neuron-neuron and neuron-glia cells with non specific structure. Nonetheless our images of electron microscopy corroborate our current results (Calvo et al., 2003).

Gap junction connexons are composed of six transmembrane protein subunits, connexins, which belong to a multigene family composed of at least 19 members in the human, being highly tissue specific. The reason to have selected Cx26 and Cx43 for characterizing *Helix aspersa* brain cells was that they are the two connexin proteins which have been more frequently linked to expression in progenitor cells in mammals. During early brain development the Cx26 is expressed in neuronal populations and has been detected in pinealocytes and neurons of prenatal rat brain (Dermietzel et al., 1989; Saez et al., 1991), although we have observed positive reaction in the anterior hypothalamic area of adult rat brain. It has been described that Cx43 is the most abundant connexin protein and is primarily found in astrocytes of the rat brain (Dermietzel et al., 1989; Micevych and Abelson 1991) being in adults mainly expressed in mature olfactory receptor neurons, substantia nigra, ventral posterolateral thalamic nuclei and globus pallidus (Theis et al., 2003).

According to our results we consider that enolase is not a good marker for the neurons of the CNS of *Helix aspersa*. This result could be due to certain non-specificity against the mammal antisera tested or they could rather be the expression of phylogenetic differences between the kinds of proteins expressed in molluscs with respect to the ones expressed in mammal neurons. The lack of results observed for Cx43 and GFAP tested antisera evidence the differences between mollusc and mammal glia cells. It has been described that immunocytochemical markers for astrocytes fail to cross-react with invertebrates in most cases, so that it has not been possible to define a class of invertebrate glia cells equivalent to vertebrate astrocytes. Instead, invertebrate glia has been described according to their position as plasmatic or protoplasmic, mainly at the periphery, and fibrous glia mainly associated with axonal tracts in the neuropil (Radojcic and Pentreath, 1979; Randsom, 1995). Direct electrical communication between glia cells has been confirmed in the leech central nervous system (Kuffler and Potter, 1964; Coggeshall, 1974). A characteristic of the *Helix* glia cells is that sometimes make membrane invaginations with large neuron somata called trophospongium, (Abbott, 1995; Calvo et al., 2003). This close ensheathment of neurons allows short-range interactions between neurons and glia, trophic support of neurons by glia and modulation of this support by signals liberated by neurons but is not related to connexin expression (Pentreath, 1982; Pentreath and Pennington, 1987).

Regarding the synchronizing processes in which gap junction could be involved; we have studied this process by recording the intracellular bioelectric activity from pairs of neurons randomly chosen from the visceral ganglion of *Helix aspersa*. The recordings were made in real time under exposure to sinusoidal low frequency (50 Hz) weak (1-15 mT intensity) MF (Azanza and del Moral, 1998). We have observed the synchronization effect in 27% of the neurons tested, out from a total number of approximately 120 studied neurons (Azanza et al., 2002). Our results about the expression of Cx26-like proteins in neurons and glia cells of the suboesophageal complex of *Helix aspersa* brain ganglia, shown in the present study, are of interest in order to evaluate the participation of gap junctions in the synchronization process. In a first approximation, the proportion of neurons positive for the Cx26 antiserum (of about 4.7%) could be considered not enough to explain in itself the synchronization behaviour observed in 27% of the neurons studied. It could be suggested that either in normal physiological activity conditions or under stimulation a higher number of neurons could be activated to express connexin proteins. The high density of immunostained fibers in the neuropil, corresponding in a certain percentage to positive fibrous glia cells, could be able to carry the activation signal to neuron pathways. It should also be considered that a percentage of the smallest observed positive neurons could be interneurons (Ratté and Chase, 1997). All these aspects will be considered in the evaluation of the studied synchronization activity in ongoing research.

Acknowledgements. This research has received financial support from “Fundación Humanismo y Ciencia” (Madrid) and from “Diputación General de Aragón” (Spain) under Project B43.

References

Abbott N.J. (1995). Morphology of nonmammalian glial cells: functional implications. In: Neuroglia. Kettenmann H. and Ransom B.R. (eds). Oxford University Press. New York. pp 97-116.

Azanza M.J. and del Moral A. (1994). Cell membrane biochemistry and neurobiological approach to biomagnetism. Prog. Neurobiol. 44, 517-601.

Azanza M.J. and del Moral A. (1998). ELF-magnetic field induced effects on the bioelectric activity of single neurone cells. J. Magn. Magn. Mat. 177-181, 1451-1452.

Azanza M.J., Calvo A.C. and del Moral A. (2002). Evidence of synchronization of neuronal activity of molluscan brain ganglia

induced by alternating 50 Hz applied magnetic field. Electromag. Biol. Med. 21, 209-220.

Balaban P.M. (2002). Cellular mechanisms of behavioral plasticity in terrestrial snail. Neurosci. Biobehav. Rev. 26, 597-630.

Calvo A.C., Junquera C., Martínez-Ciriano C. and Azanza M.J. (2003). Características ultraestructurales de los ganglios subesofágicos de *Helix aspers*a (mollusca). Actas del XII Congreso Nacional de Histología e Ingeniería Tisular. Valencia. pp 227.

Coggeshall R.E. (1974). Gap junctions between identified glial cells in the leech. J. Neurobiol. 5, 463-467.

Dermietzel R. and Spray D.C. (1993). Gap junctions in the brain: where, what type, how many and why?. Trends Neurosci. 16, 186-192.

Dermietzel R., Traub O., Hwang T.K., Beyer E., Bennett M.V.L., Spray D.C. and Willecke K. (1989). Differential expression of three gap junction proteins in developing and mature brain tissues. Proc. Natl. Acad. Sci. USA 86, 10148-10152.

Fróes M.M., Correia A.H.P., García-Abreu J., Spray D.C., Campos de Carvalho A.C. and Moura Neto V. (1999). Gap-junctional coupling between neurons and astrocytes in primary central nervous system cultures. Proc. Natl. Acad. Sci. USA 96, 7541-7546.

Gelperin A. and Tank D.W. (1990). Odour-modulated collective network oscillations of olfactory interneurons in a terrestrial mollusc. Nature 345, 437-440.

Kerkut G.A. and Walker R.J. (1961a). The resting potential and potassium levels of cells from active and inactive snails. Comp. Biochem. Physiol. 1, 76-79.

Kerkut G.A. and Walker R.J. (1961b). The effects of drugs on the neurons of the snail *Helix aspersa*. Comp. Biochem. Physiol. 3, 143-160.

Kerkut G.A. and Walker R.J. (1962). The specific chemical sensitivity of Helix nerve cells. Comp. Biochem. Physiol. 7, 277-288.

Kerkut G.A., Lambert J.D.C., Gayton R., Loker J.E. and Walker R.J. (1975). Mapping of nerve cells in the suboesophageal ganglia of *Helix aspersa*. Comp. Biochem. Physiol. 50A, 1-25.

Kuffler S.W. and Potter D.D. (1964). Glia in the leech central nervous system: physiological properties and neuron-glia relationship. J. Neurophysiol. 27, 290-294.

Lauf U., Giepmans B.N.G., Lopez P., Braconnot S., Chen S-C. and Falk M.M. (2002). Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. Proc. Natl. Acad. Sci. USA 99, 10446-10451.

Micevych P.E. and Abelson L. (1991). Distribution of mRNAs coding for liver and heart gap junction proteins in the rat central nervous system. J. Comp. Neurol. 305, 96-118.

Pentreath V.W. (1982). Potassium signalling of metabolic interactions between neurons and glia cells. Trends Neurosci. 5, 339-345.

Pentreath V.W. and Pennington A.J. (1987). Coupling of energy metabolism between neurons and glia cells in leech ganglia. J. Physiol. (Paris). 82, 218-228.

Radojcic T. and Pentreath V.W. (1979). Invertebrate glia. Prog. Neurobiol. 12, 115-179.

Randsom B.R. (1995). Gap junctions. In: Neuroglia. Kettenmann H. and Ransom B.R. (eds). Oxford University Press. New York. pp 299-318.

Ratté S. and Chase R. (1997). Morphology of interneurons in the procerebrum of the snail *Helix aspersa*. J. Comp. Neurol. 384, 359-372.

Rózsa K.S. (1976). Neuronal network underlying the regulation of heart beat in *Helix pomatia* L. In: Neurobiology of invertebrates. Gastropoda brain. Salánki J. (ed). Budapest. pp 597-613.

Sáez J.C., Berthoud V.M., Kadle R., Traub O., Nicholson B.J., Bennett M.V.L. and Dermietzel R. (1991). Pinealocytes in rats: connexin identification and increase in coupling caused by norepinephrine. Brain Res. 568, 265-75.

Theis M., Söhl G., Speidel D., Kühn R. and Willecke K. (2003). Connexin 43 is not expressed in principal cells of mouse cortex and hippocampus. Eur. J. Neurosci. 18, 267-274.

Vadász I. and Salánki J. (1976). Mechanisms of spike and burst generation in the bimodal pacemaker RPal neuron of *Helix pomatia* L. In: Neurobiology of invertebrates. Gastropoda brain. Salánki J. (ed). Budapest. pp 371-380.

Accepted November 17, 2006