ELSEVIER

Brain Research 869 (2000) 244–250

BRAIN RESEARCH INTERACTIVE

www.elsevier.com/locate/bres

Interactive report

Morphological bases for a role of nitric oxide in adult neurogenesis[1]

Bernardo Moreno-López, Jose Angel Noval, Luis Germán González-Bonet, Carmen Estrada*

Área de Fisiología, Facultad de Medicina, Universidad de Cádiz, Plaza Fragela 9, 11003 Cádiz, Spain

Accepted 9 May 2000

Abstract

The subventricular zone (SVZ) of the adult mouse brain retains the capacity to generate new neurons from stem cells. The neuronal precursors migrate tangentially along the rostral migratory stream (RMS) towards the olfactory bulb, where they differentiate as periglomerular and granular interneurons. In this study, we have investigated whether nitric oxide (NO), a signaling molecule in the nervous system with a role in embryonic neurogenesis, may be produced in the proximity of the progenitor cells in the adult brain, as a prerequisite to proposing a functional role for NO in adult neurogenesis. Proliferating and immature precursor cells were identified by immunohistochemistry for bromo-deoxyuridine (BrdU) and PSA-NCAM, respectively, and nitrergic neurons by either NADPH-diaphorase staining or immunohistochemical detection of neuronal NO synthase (NOS I). Nitrergic neurons with long varicose processes were found in the SVZ, intermingled with chains of cells expressing PSA-NCAM or containing BrdU. Neurons with similar characteristics surrounded the RMS all along its caudo-rostral extension as far as the core of the olfactory bulb. No expression of NOS I by precursor cells was detected either in the proliferation or in the migration zones. Within the olfactory bulb, many small cells in the granular layer and around the glomeruli expressed either PSA-NCAM or NOS I and, in some cases, both markers. Colocalization was also found in a few isolated cells at a certain distance from the neurogenesis areas. The anatomical disposition shown indicates that NO may be released close enough to the neuronal progenitors to allow a functional influence of this messenger in adult neurogenesis.

Theme: Development and regeneration

Topic: Cell differentiation and migration

Keywords: Neuronal precursor; Nitrergic neuron; Nitric oxide synthase I; Olfactory bulb; PSA-NCAM; Subventricular zone

1. Introduction

Progenitor stem cells that can give rise to neurons and glia have been found recently in certain areas of the adult brain in several species, including humans [5,16], leading to the new concept of neurogenesis occurring throughout life and not only during the developmental period. Neuronal and glial progenitors as well as multipotent stem cells have been identified in the adult rodent subventricular zone (SVZ), lining the external wall of the lateral ventricles [4,16,20]. Neuronal progenitors migrate tangentially along the ''rostral migratory stream'' (RMS) to reach the olfactory bulb, where the daughter cells differentiate into granular and periglomerular interneurons [17,18]. Identification of molecules controlling proliferation, migration or differentiation of neuronal precursors in the adult brain is presently under investigation.

Nitric oxide (NO) is a free radical molecule with signaling functions in the central nervous system [8]. In the brain parenchyma, NO is synthesized by widely distributed specific neurons that express the neuronal isoform of NO synthase (NOS I) [2,26]. The transient expression of NOS I in different neural structures during development [1,9,12,27,30] suggests that NO plays a role in embryonic neurogenesis. The participation of NO in the formation of the nervous system may be related to its involvement in neuronal programmed cell death [6,31,32], in the organization of axonal projection patterns [23,34], or in the control of cell proliferation [13]. In neuronal cell lines, NOS I and II isoforms are induced by differentiating agents [22,24,25] and, in some cases, the NO antiproliferative action was

[1]Published on the World Wide Web on 25 May 2000.

*Corresponding author. Tel.: +34-956-015-252; fax: +34-956-015-214.

E-mail address: carmen.estrada@uca.es (C. Estrada)

0006-8993/00/$ – see front matter
PII: S0006-8993(00)02474-4

shown to be a prerequisite for differentiation [25]. Concerning neurogenesis in the SVZ, we have recently demonstrated that NO is an antimitotic agent in primary cultures of proliferating cells isolated from this region [7].

A criterion needed for proposing a role for NO in adult neurogenesis is the identification of possible sources of NO in the vicinity of the precursor cells. Therefore, the purpose of the present study was to analyze the anatomical relationship between nitrergic neurons and neuronal progenitors of the SVZ–olfactory bulb neurogenic system. For this, we have used double-labeling immunofluorescence identification of NOS I and the polysialylated form of the adhesion molecule NCAM(PSA-NCAM), which is expressed by neuronal progenitors [29] and is required for tangential migration [11]. We provide morphological bases for a paracrine action of NO in the proliferation and migration zones, and for a paracrine/autocrine action at the site of final differentiation.

2. Materials and methods

2.1. Animals and tissue preparation

Adult male CD1 mice were anesthetized with 7% chloralose, intraventricularly injected with heparin, and perfused transcardially first with isotonic saline solution, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, at 4°C. The brains were removed, post-fixed for 2 h in the same fixative solution, and cryoprotected by overnight immersion in PB containing 30% sucrose. Coronal or sagittal brain sections (40 μm) were obtained using a freezing microtome and these were processed for the different histochemical treatments. Stained sections were analyzed by light or fluorescent microscopy, using a BX60 microscope and the image analysis software Microimage, from Olympus.

2.2. NADPH-diaphorase staining

Brain sections were washed in 0.1 M PB and incubated for 20 min in a shaking bath at 37°C in a solution containing 1 mM β-NADPH, 0.8 mM nitroblue tetrazolium and 0.1% Triton X-100 in 0.1 M Tris buffer, pH 8. After extensive washing, the tissue was mounted with DePeX and analyzed under light microscopy.

2.3. NOS I immunohistochemistry

Brain sections were washed in phosphate-buffered saline (PBS) and incubated for 30 min at room temperature in 2.5% (w/v) bovine serum albumin, 0.25% (w/v) sodium azide and 0.1% (v/v) Triton X-100 in PBS, followed by overnight incubation at 4°C with the primary antibody (1:500; specific anti-NOS I rabbit polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in the same solution. The tissue was rinsed with PBS and incubated for 1 h at room temperature with a FITC-labeled anti-rabbit IgG (1:200; Jackson ImmunoResearch, West Grove, PA, USA). After washing, sections were mounted with Vectashield and observed under fluorescence microscopy.

2.4. PSA-NCAM immunohistochemistry

The polysialylated form of the neuronal adhesion molecule, PSA-NCAM, which is expressed by the SVZ neuronal progenitors in vivo [29], was made visible by means of an IgM monoclonal antibody raised against the capsular polysaccharides of meningococcus B [28] (a kind gift from Dr. G. Rougon, Marseille, France). For single labeling, the avidin–biotin–peroxidase technique was used. Brain sections were first incubated in a solution containing 2% (v/v) hydrogen peroxide and 60% (v/v) methanol in PBS, to block endogenous peroxidase activity. Then, the tissue was incubated as described above with a 1:4000 dilution of the primary antibody, using as secondary antibody a biotinylated anti-mouse IgM (1:200; Sigma). Biotin was detected by means of the avidin–biotin–peroxidase system (ABC kit, Vector Laboratories) using 3,3′-diaminobenzidine tetrahydrochloride as the chromogen. Tissues were mounted on slides, dehydrated, covered with DePeX and visualized under light microscopy.

The anatomical relationship between NO-producing neurons and neuronal precursors was studied by NADPH-diaphorase staining followed by PSA-NCAM immunohistochemistry, or by the simultaneous immunohistochemical detection of NOS I and PSA-NCAM. For both types of double-labeling technique, a Cy3-labeled anti-mouse IgM (1:100; Jackson ImmunoResearch) was used as the secondary antibody for PSA-NCAM. Omission of either primary antibody resulted in no detectable specific staining in all cases.

2.5. BrdU immunohistochemistry

Mice were injected with BrdU (three doses of 200 mg/kg i.p., separated by 3-h intervals) and perfused 3 h after the last injection. The brain sections were processed as indicated above after DNA denaturalization. A monoclonal anti-BrdU antibody (1:100; Dako, Glostrup, Denmark) and a biotinylated anti-mouse IgG (1:250; Sigma) were used as primary and secondary antibodies, respectively. For double-labeling staining, a Cy3-labeled anti-mouse IgG (1:4000; Amersham) was used.

3. Results

3.1. Distribution of nitrergic neurons in the SVZ

Neuronal cell bodies and abundant neuropil positive for

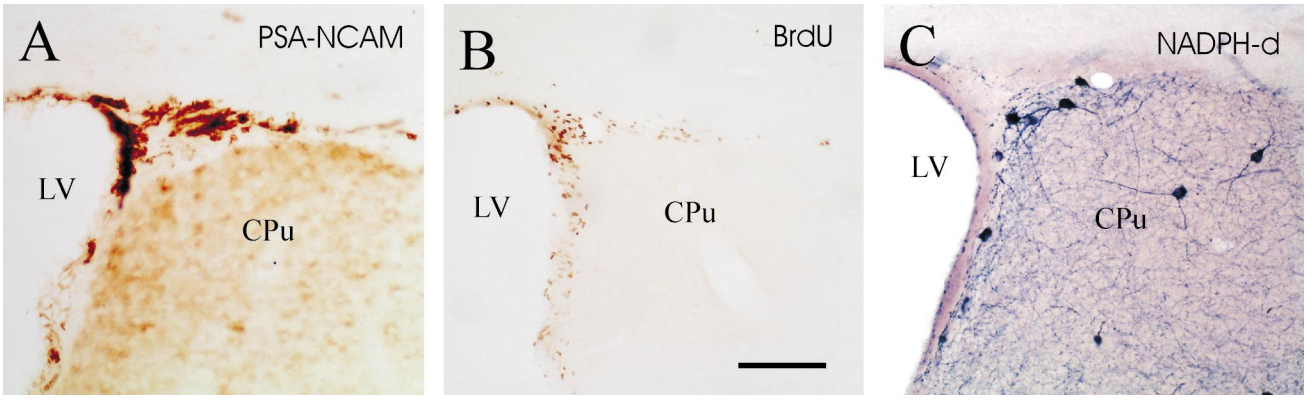

Fig. 1. Distribution of proliferating precursor cells and differentiated nitrergic neurons in the subventricular zone (SVZ). Photomicrographs showing adult mouse SVZ coronal sections processed for PSA-NCAM immunohistochemistry (A), bromo-deoxyuridine (BrdU) immunohistochemistry (B), and NADPH-diaphorase (NADPH-d) histochemistry (C), labeling SVZ precursor cells, recently divided cells, and nitrergic neurons, respectively. CPu, caudate/putamen; LV, lateral ventricle. Calibration bar: 150 μm.

NADPH-diaphorase staining were observed along the lateral walls of the lateral ventricle, in the area where BrdU-containing and PSA-NCAM-expressing cells were found (Fig. 1). Double-labeling immunofluorescence studies using antibodies against NOS I and PSA-NCAM showed that both markers never colocalized within the same cell in this region (Fig. 2A, B and C). Nitrergic axons containing varicosities were intermingled with chains of precursor cells that were positive for PSA-NCAM immunohistochemistry (Fig. 2D, E and F). Double staining for NOS I and BrdU also showed that both markers were present in neighboring cells, but never in the same neuron (Fig. 2G, H and I).

3.2. NOS I expression in the RMS and olfactory bulb core

The RMS was made visible by either PSA-NCAM- or BrdU staining in both sagittal and coronal brain sections, extending rostrally between the striatum and the corpus callosum. Neurons expressing NOS I surrounded the stream (Fig. 3A, B and D), with neurites running parallel to the migration pathway (Fig. 3A and C). A similar disposition was observed at the entrance of the olfactory bulb, where the RMS was enlarged and a greater number of nitrergic cell bodies was found (not shown). In the core of the olfactory bulb, chains of PSA-NCAM-positive cells were visualized, together with a few large nitrergic cells that were heavily stained for NOS I following immunohistochemistry (not shown). Colocalization of NOS I with BrdU or PSA-NCAM was never observed in the RMS, nor at the entrance or in the core of the olfactory bulb.

3.3. Coexpression of NOS I and PSA-NCAM in the olfactory bulb

Small nitrergic cells with large nuclei and cytoplasms that were lightly stained for either NADPH-diaphorase or NOS I following immunohistochemistry were found in the granular layer and around the glomeruli in the olfactory bulb. Approximately 30% of the periglomerular nitrergic neurons also expressed PSA-NCAM (208 double-labeled neurons out of a total of 663 nitrergic neurons counted in 14 coronal sections of the olfactory bulb), although they constituted a small percentage of the periglomerular PSA-NCAM-positive neurons (Fig. 4A, B and C).

3.4. Coexpression of NOS I and PSA-NCAM in other locations

Scattered cells expressing PSA-NCAM were found in the corpus callosum and the striatum, in proximity to the RMS, but outside the well-defined tubular pathway. In the dorsal hippocampus, isolated PSA-NCAM-labeled cells were also observed, far from the neurogenesis area in the dentate gyrus. Some of these cells also expressed NOS I (Fig. 4D–I). With this staining, a differentiated neuronal morphology with several long processes was identified in all cases.

4. Discussion

In this study, we provide anatomical evidence supporting a possible role for NO in adult neurogenesis based on the close proximity between nitrergic neurons and neuronal precursors in the SVZ and RMS, and on the expression of NOS I by some of the progenitor cells, once arrived at the site of their final differentiation.

All neuronal precursors were within the sphere of influence of a NO source

Due to its diffusibility through cell membranes, messages transmitted by NO are multidirectional, and communication between cells is established in the absence of

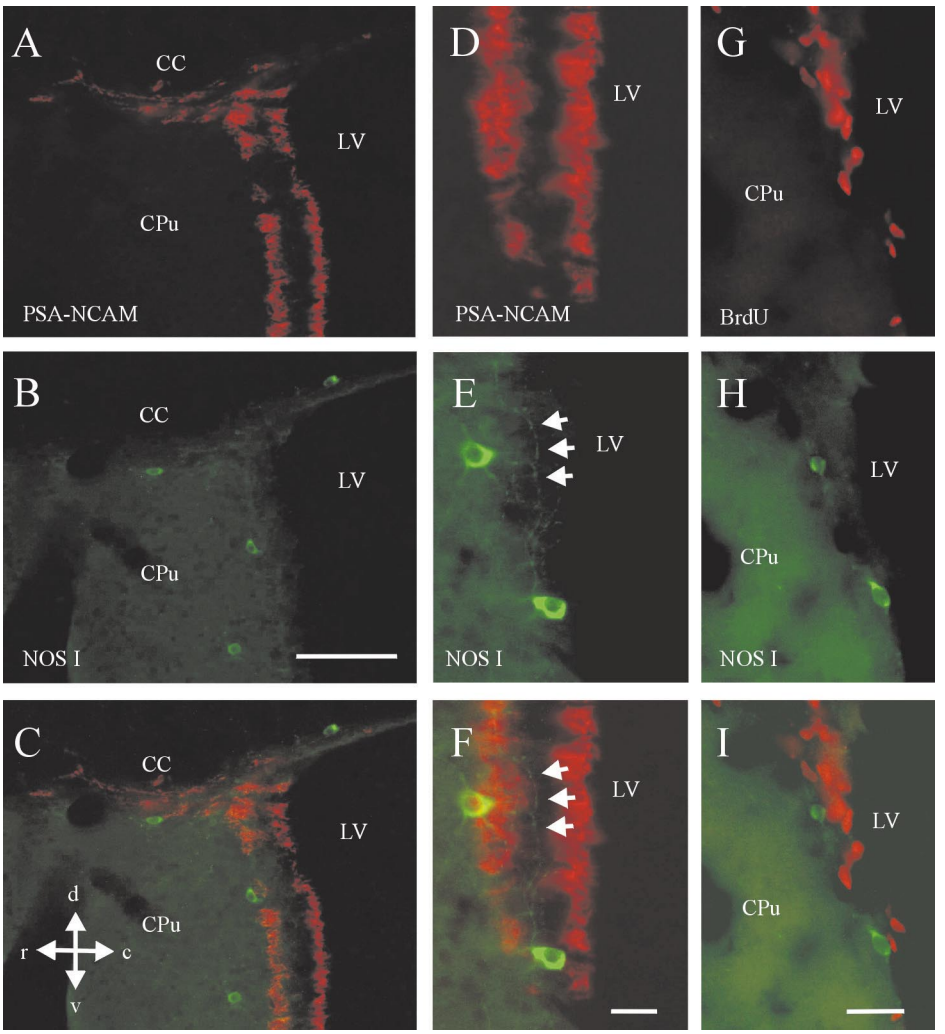

Fig. 2. Relationship between nitrergic neurons and neuronal precursors or proliferating neural cells in the subventricular zone (SVZ). A–F, Photomicrographs of adult mouse SVZ sagittal sections double-labeled for PSA-NCAM and NOS I. Staining for PSA-NCAM (A and D), NOS I (B and E), and superimposition of both (C and F), are shown. G–I, Photomicrographs of coronal sections through the adult mouse SVZ double-labeled for BrdU and NOS I. Staining for BrdU (G), NOS I (H), and superimposition of both (I), are shown. Note the proximity of nitrergic neurons and neuronal precursors, and the absence of colocalization of NOS I with PSA-NCAM or BrdU in the same cell. Nitrergic varicose nerve fibers, indicated by arrows in E and F, formed a network that was intermingled with the chains of PSA-NCAM containing cells throughout the SVZ. c, caudal; CC, corpus callosum; CPu, caudate/putamen; d, dorsal; LV, lateral ventricle; r, rostral; v, ventral. Calibration bars: B, 100 μm; F, 25 μm and I, 50 μm.

cell–cell contact, provided that the target cell is within the sphere of influence of NO. Any activated NO source produces increases of NO concentration in a volume of parenchymal tissue that has been estimated as a sphere with a radius of ~100 μm around the NO point source [33]. Therefore, the condition for proposing a functional role of NO in the SVZ–olfactory bulb neurogenesis system, i.e. that neural precursors are within the volume of influence of a possible NO source, was fulfilled in all regions studied.

NOS I is expressed by differentiated neurons in proliferation and migration areas

In spite of the general finding mentioned above, the relative disposition of nitrergic neurons and neuronal precursors varied in the different regions studied. In the SVZ, NOS I expression was detected in well-differentiated neurons presenting abundant varicose processes, which were intermingled with the chains of precursor cells. Although the number of nitrergic somas was not high, it is very likely that, due to the dense neuropil, the whole proliferation area is under the influence of NO when the nitrergic neurons are activated. The NO function in the SVZ is still to be determined, but it may be related to neuronal cell death, since the interaction of NO with several apoptotic mechanisms has been reported in cultured neurons [6,31,32], and approximately 60% of the constitutively proliferating cells in this region undergo apoptosis [21].

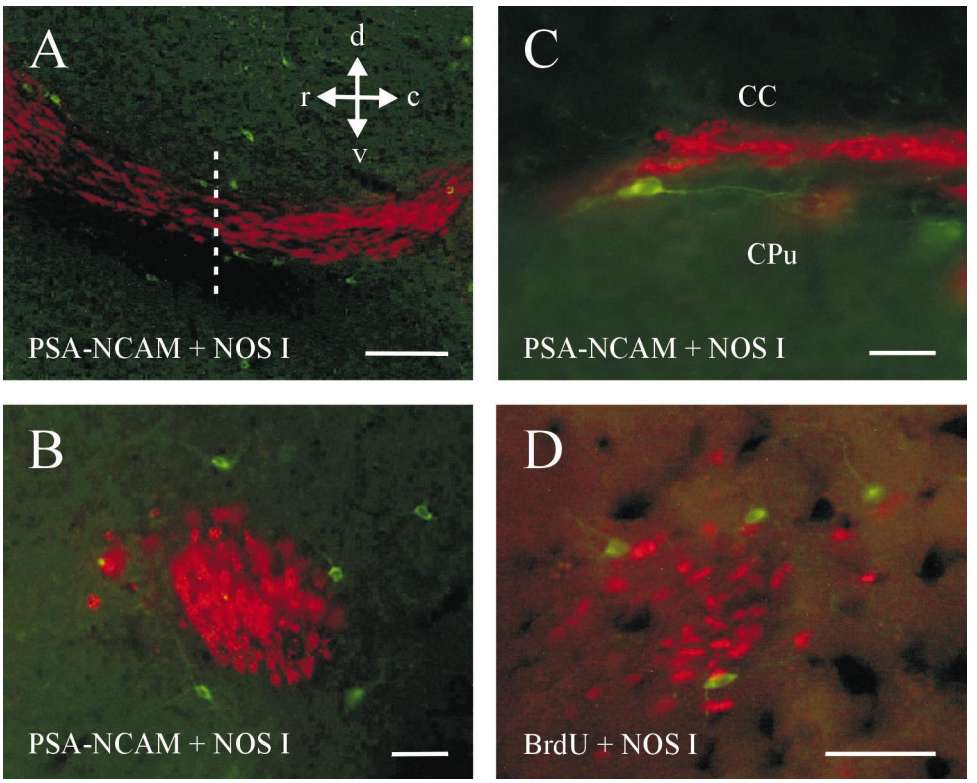

Fig. 3. Relationship between nitrergic neurons and neuronal precursors or proliferating cells in the rostral migratory stream (RMS).A–C, Double-labeling immunofluorescence for PSA-NCAM (red) and NOS I (green) showing parasagittal (A and C) and coronal (B) views of the RMS. The coronal section in B corresponds to the rostro-caudal level indicated in A. In C, one nitrergic neuron adjacent to the migratory cells extends its processes in a rostro-caudal direction parallel to the RMS. D, Double-labeling immunofluorescence for BrdU (red) and NOS I (green) showing a coronal view of the RMS. c: caudal; CC: corpus callosum; CPu: caudate/putamen; d: dorsal; r: rostral; v: ventral. Calibration bars: A, 150 μm; B and D, 50 μm; C, 30 μm.

In the RMS, where migrating neuronal precursors are ensheathed by a layer of glial cells [18], nitrergic somas were always found outside the stream, with processes extending parallel to the precursor chains. In a tridimensional reconstruction from the images obtained in coronal and sagittal sections, the NOS-I-containing structures form a loose network surrounding the RMS. Whether NO has any role in preventing cell migration outside the RMS is a possibility, but clarification of this is beyond the scope of the present work.

Neuronal precursors expressed NOS I at the sites of terminal differentiation

In contrast to the SVZ or RMS, where precursor cells never expressed NOS I, some neuronal precursors in their final destination in the granular layer or around the glomeruli of the olfactory bulb stained for both NOS I and PSA-NCAM. The expression of NOS I at the site of neuronal precursor final differentiation correlates well with our findings in SVZ-derived cells that were isolated and maintained in culture. We have observed that, under culture conditions inducing differentiation, as assessed by expression of specific lineage antigens, a large number of cells present NOS I immunoreactivity [7].

This particular feature may indicate that the newly formed neurons arriving from the SVZ differentiate towards a nitrergic phenotype in their final location, and that double-labeled cells represent intermediate stages of differentiation. However, the double-staining may also be due to a transitory NOS expression of differentiating neurons, whatever their final phenotype might be. In agreement with the latter possibility, induction of different NOS isoforms prior to, or concomitantly with, the differentiation process has been reported in neuronal cultures of different origins when treated with differentiating agents such as nerve growth factor [25], retinoic acid [24] or TNF-α [22]. These findings suggest that NO production may participate in the differentiation process. A likely possibility is that NO triggers a switch to growth arrest, which is a prerequisite for cells to enter a program of terminal differentiation [25].

Some cells double-labeled for PSA-NCAM and NOS I were also found outside the areas of adult neurogenesis. The presence of a few PSA-NCAM-positive cells in the corpus callosum of adult mouse brains has been reported previously [29]. In postnatal brains, isolated immature newly formed cells have also been found in the corpus callosum, striatum, white matter and neocortex, and they have been interpreted as the result of the radial migration of glial progenitor cells from the SVZ [10,14,15,19].

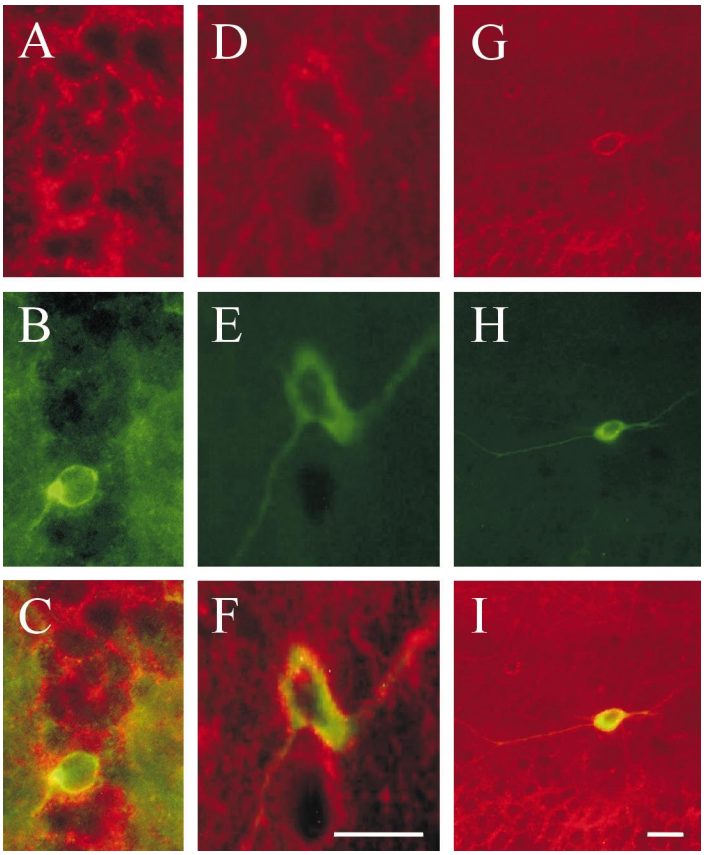

Fig. 4. Colocalization of PSA-NCAM and NOS I in the sites of terminal differentiation. High magnification photomicrographs show mouse brain sections stained with PSA-NCAM (A, D, G) or NOS I (B, E, H) immunofluorescence. In C, F and I, both labels were superimposed. The images show colocalization of PSA-NCAM and NOS I in a periglomerular neuron in the olfactory bulb (A–C), and neurons in the corpus callosum (D–F) and the oriens layer of the dorsal hippocampus (G–I). Calibration bars: 25 μm.

However, the PSA-NCAM/nitrergic cells that we have found presented the morphological and immunohistochemical characteristics of a well-differentiated neuronal phenotype, which suggests that they may result from ectopic migration of neuronal precursors either from the SVZ or the RMS. Intracerebroventricular injections of EGF, resulting in a dramatic increase in the total number of subependymal proliferating cells, induced their migration away from the lateral ventricle wall into adjacent parenchyma [3]. Based on this finding, the possibility exists that, under physiological growth factor stimulation, a small percentage of the cells formed also migrate outside their normal pathway. Expression of NOS I by these cells is then concomitant with their differentiation by a process similar to that occurring in the olfactory bulb. The origin of the few differentiated neurons expressing both nitrergic and precursor markers in the dorsal hippocampus is difficult to assess, since they are located far from the dentate gyrus of the hippocampus, which is also a neurogenic area in the adult brain.

Taken together, these findings provide the morphological support for a possible participation of NO in the formation of new neurons after birth, and may be useful for elucidating the in vivo role of NO in the control of neurogenesis in the adult CNS.

Acknowledgements

This work was supported by grants from the Fondo de Investigación Sanitaria (97/2054) and the Comunidad Autónoma de Madrid (08.5/0019/1997). The authors wish to thank Dr. Rougon for kindly providing the anti-PSA-NCAM antibody.

These results were presented in the 6th International Meeting on the Biology of Nitric Oxide, Stockholm 1999, and will be published in the Proceedings.

References

[1] D.S. Bredt, S.H. Snyder, Transient nitric oxide synthase neurons in embryonic cerebral cortical plate, sensory ganglia and olfactory epithelium, Neuron 13 (1994) 301–313.
[2] D.S. Bredt, P.H. Hawing, S.H. Snyder, Localization of nitric oxide

synthase indicating a neural role for nitric oxide, Nature 347 (1990) 768–770.
[3] C.G. Craig, V. Tropepe, C.M. Morshead, B.A. Reynolds, S. Weiss an, D. van der Kooy, In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain, J. Neurosci. 16 (1996) 2649–2658.
[4] F. Doetch, I. Caillé, D.A. Lim, J.M. García-Verdugo, A. Alvarez-Buylla, Subventricular zone astrocytes are neural stem cells in the adult mammalian brain, Cell 97 (1999) 703–716.
[5] P.S. Eriksson, E. Perfilieva, T. Björk-Eriksson, A.-M. Alborn, C. Nordborg, D.A. Peterson, F.H. Gage, Neurogenesis in the adult human hippocampus, Nat. Med. 4 (1998) 1313–1317.
[6] A.G. Estevez, N. Spear, S.M. Manuel, R. Radi, C.E. Henderson, L. Barbeito, J.S. Beckman, Nitric oxide and superoxide contribute to motor neuron apoptosis induced by trophic factor deprivation, J. Neurosci. 18 (1998) 923–931.
[7] C. Estrada, E.R. Matarredona, M. Murillo-Carretero, J.A. Noval, B. Moreno-López, Nitric oxide modulates survival and proliferation of post-natal brain derived subventricular zone neuronal precursors, Abstracts Soc. Neurosci. 25 (1999) 1542.
[8] J. Garthwaite, C.L. Boulton, Nitric oxide signalling in the nervous system, Annu. Rev. Physiol. 57 (1995) 683–706.
[9] G. Giuili, A. Luzi, M. Poyard, G. Guellaën, Expression of mouse brain soluble guanylyl cyclase and NO synthase during ontogeny, Dev. Brain Res. 81 (1994) 269–283.
[10] J.E. Goldman, Lineage, migration, and fate determination of post-natal subventricular zone cells in the mammalian CNS, J. Neuro-oncol. 24 (1995) 161–164.
[11] H. Hu, H. Tomasiewicz, T. Magnuson, U. Rutishauser, The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone, Neuron 16 (1996) 735–743.
[12] G. Keilhoff, B. Seidel, H. Noack, W. Tischmeyer, D. Stanek, G. Wolf, Patterns of nitric oxide synthase at the messenger RNA and protein levels during early rat brain development, Neuroscience 75 (1996) 41193–41201.
[13] B. Kuzin, I. Roberts, N. Peunova, G. Enikolopov, Nitric oxide regulates cell proliferation during drosophila development, Cell 87 (1996) 639–649.
[14] S.W. Levison, C. Chuang, B.J. Abramson, J.E. Goldman, The migrational patterns and development fates of glial precursors in the rat subventricular zone are temporally regulated, Development 119 (1993) 611–622.
[15] S.W. Levison, J.E. Goldman, Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain, Neuron 10 (1993) 201–212.
[16] C. Lois, A. Alvarez-Buylla, Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia, Proc. Natl. Acad. Sci. USA 90 (1993) 2074–2077.
[17] C. Lois, A. Alvarez-Buylla, Long-distance neuronal migration in the adult mammalian brain, Science 264 (1994) 1145–1148.
[18] C. Lois, J.M. García-Verdugo, A. Alvarez-Buylla, Chain migration of neuronal precursors, Science 271 (1996) 978–981.
[19] M.B. Luskin, K.W. McDermott, Divergent lineages for oligodendrocytes and astrocytes originating in the forebrain subventricular zone, Glia 11 (1994) 211–226.
[20] C.M. Morshead, B.A. Reynolds, C.G. Craig, M.W. McBurney, W.A. Staines, D. Morassutti, S. Weiss, D. van der Kooy, Neural stem cells in the adult mammalian forebrain, Neuron 13 (1994) 1071–1082.
[21] C.M. Morshead, C.G. Craig, D. van der Kooy, In vivo clonal analyses reveal the properties of endogenous neural stem cell proliferation in the adult mammalian forebrain, Development 125 (1998) 2251–2261.
[22] E. Obregón, M.C. Punzón, J. González-Nicolás, E. Fernandez-Cruz, M. Fresno, M.A. Muñoz-Fernández, Induction of adhesion/differentiation of human neuroblastoma cells by tumor necrosis factor-alpha requires the expression of an inducible nitric oxide synthase, Eur. J. Neurosci. 9 (1997) 1184–1193.
[23] P. Olgivie, K. Schilling, M.L. Billingsley, H.H. Schmidt, Induction and variants of neuronal nitric oxide synthase type I during synaptogenesis, FASEB J. 9 (1995) 799–806.
[24] T. Ogura, K. Nakayama, H. Fujisawa, H. Esumi, Neuronal nitric oxide expression in neuronal cell differentiation, Neurosci. Lett. 204 (1996) 89–92.
[25] N. Peunova, G. Enikolopov, Nitric oxide triggers a switch to growth arrest during differentiation of neuronal cells, Nature 375 (1995) 68–73.
[26] J. Rodrigo, D.R. Springall, O. Uttenthal, M.L. Bentura, F. Abadia-Molina, V. Riveros- Moreno, R. Martínez-Murillo, J.M. Polak, S. Moncada, Localization of nitric oxide synthase in the adult rat brain, Phil. Trans. R. Soc. Lond. B 345 (1994) 175–221.
[27] A.J. Roskams, D.S. Bredt, T.M. Dawson, G.V. Ronnett, Nitric oxide mediates the formation of synaptic connections in developing and regenerating olfactory receptor neurons, Neuron 13 (1994) 289–299.
[28] G. Rougon, C. Dubois, N. Buckley, J.L. Magnani, W. Zollinger, A monoclonal antibody against meningococcus group B polysaccharides distinguishes embryonic from adult N-CAM, J. Cell Biol. 103 (1986) 2429–2437.
[29] P. Rousselot, C. Lois, A. Alvarez-Buylla, Embryonic (PSA) N-CAM reveals chains of migrating neuroblasts between the lateral ventricle and the olfactory bulb of adult mice, J. Comp. Neurol. 351 (1995) 51–61.
[30] M. Santacana, L.O. Uttenthal, M.L. Bentura, A.P. Fernández, J. Serrano, J. Martinez de Velasco, D. Alonso, R. Martinez-Murillo, J. Rodrigo, Expression of neuronal nitric oxide synthase during embryonic development of the rat cerebral cortex, Dev. Brain Res. 111 (1998) 205–222.
[31] M. Tamatani, S. Ogawa, Y. Niitsu, M. Tohyama, Involvement of Bcl-2 family and caspase-3-like protease in NO-mediated neuronal apoptosis, J. Neurochem. 71 (1998) 1588–1596.
[32] A.M. Vincent, K. Maiese, Nitric oxide induction of neuronal endonuclease activity in programmed cell death, Exp. Cell Res. 246 (1999) 290–300.
[33] J. Wood, J. Garthwaite, Models of the diffusional spread of nitric oxide: implications for neural nitric oxide signaling and its pharmacological properties, Neuropharmacology 33 (1994) 1235–1244.
[34] H.H. Wu, C.V. Williams, S.C. McLoon, Involvement of nitric oxide in the elimination of a transient retinotectal projection in development, Science 265 (1994) 1593–1596.