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UPREGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE MAINTAINS NITRIC OXIDE PRODUCTION IN THE CEREBELLUM OF THIOACETAMIDE CIRRHOTIC RATS

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Abstract—This study examines the expression and cellular distribution pattern of nitric oxide synthase (NOS) isoforms, nitrotyrosine-derived complexes, and the nitric oxide (NO) production in the cerebellum of rats with cirrhosis induced by thioacetamide (TAA). The results showed local changes in the tissue distribution pattern of the NOS isoforms and nitrated proteins in the cerebellum of these animals. Particularly, eNOS immunoreactivity in perivascular glial cells of the white matter was detected only in TAA-treated animals. In addition, although neither neuronal NOS (nNOS) nor inducible NOS (iNOS) cerebellar protein levels appeared to be affected, the endothelial NOS (eNOS) isoform significantly increased its expression, and NO production slightly augmented in TAA-treated rats. These NOS/NO changes may contribute differently to the evolution of the hepatic disease either by maintaining the guanosine monophosphate–NO signal transduction pathways and the physiological cerebellar functions or by inducing oxidative stress and cell damage. This model gives rise to the hypothesis that the upregulation of the eNOS maintains the physiological production of NO, while the iNOS is silenced and the nNOS remains unchanged. The differential NOS-distribution and expression pattern may be one of the mechanisms involved to balance cerebellar NO production in order to minimize TAA toxic injury. These data help elucidate the role of the NOS/NO system in the development and progress of hepatic encephalopathy associated with TAA cirrhosis.

Key words: hepatic encephalopathy, nNOS, iNOS, eNOS, nitrated proteins, nitrates/nitrites.

Hepatic encephalopathy (HE) is a complex syndrome that affects the entire CNS (Adams and Folay, 1953), and one of the main disorders involved in the development of this disease is liver cirrhosis (Butterworth, 2000). The cerebellum, a nervous center where motor orders and responses are modulated (Delgado-García, 2001), undergoes neurodegenerative changes in alcoholic and cirrhotic patients (Kril and Butterworth, 1997). The use of thioacetamide (TAA), a hepatotoxic agent responsible for the development of an experimental model of cirrhosis (Zimmermann et al., 1986; Torres et al., 1998), also induces biochemical and behavioral effects characteristic of HE (Sarhan et al., 1993) with degenerative changes involving both the neuronal and neuroglial populations of the cerebellum (Peeling et al., 1993; Matsushita et al., 1999). TAA is metabolized to toxic metabolites TAA sulfine (sulfoxide) and sulfene (sulfone); both toxic products alter, among other metabolic processes, the urea cycle (Cascales et al., 1979) resulting in a systemic elevation of ammonia that is considered one of the major pathogenic factors in HE (Saran et al., 2004). At the molecular level, HE has been associated with glutamatergic dysfunction, interruption of cellular calcium homeostasis and generation of free radicals and superoxide (Rahman and Hodgson, 2003).

The messenger nitric oxide (NO) is produced in large amounts in the cerebellum (Paakkari and Lindsberg, 1995) by the enzyme NO synthase (NOS). NOS isoforms—neuronal (nNOS), endothelial (eNOS), and inducible (iNOS)—have been found in several cell populations within the cerebellum (Garthwaite and Boulton, 1995; Hartell et al., 2001). NO has been related to many physiological and pathological processes (Szabó, 1996; Hotter et al., 1996; Hazell and Butterworth, 1999), including HE (Schliess and Häussinger, 2001). In this sense, it has been demonstrated that the NO molecule may help protect against HE failure triggered by TAA (Chu et al., 2001). However, the exact molecular mechanisms underlying HE are still unknown (Albrecht and Jones, 1999) and consequently so is the role that NO may have in the progress of the disease (Rao, 2002).

When NO is produced in large amounts, as in response to an inflammatory stimuli, it may react with superoxide to produce peroxynitrite, a powerful oxidant capable of damaging a vast array of cells (Rodrigo et al., 2000, 2001; Peinado et al., 2003). NO directly, or indirectly through peroxynitrite, may also nitrate tyrosine protein residues to form nitrotyrosine complexes, these being good markers of the cumulative exposure to NO. Consequently, nitrotyrosine detection is an efficient tool to study pathological cerebellar changes in NO targets, given that NO derivatives can alter the cerebellar functionality (Alonso et al., 2002; Chung et al., 2002; Serrano et al., 2003).

Therefore, the aim of the present study is to analyze the expression and cellular distribution pattern of NOS isoforms and nitrotyrosine-derived complexes as well as

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Abbreviations: cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; HE, hepatic encephalopathy; iNOS, inducible nitric oxide synthase; IR, immunoreactivity; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NOx, nitrate, nitrite and *S*-nitroso compounds; nTyr, nitrotyrosine; PB, phosphate buffer; PBS, phosphate buffer saline; TAA, thioacetamide.

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NO production in the cerebellum of TAA-induced rats, and thereby help elucidate the possible role of the NOS/NO system in the HE development associated with this cirrhotic model.

EXPERIMENTAL PROCEDURES

Animals and cirrhotic experimental model

A total of 20 adult (200–250 g; 4-month-old) male Wistar rats were kept under standard conditions of light and temperature and allowed *ad libitum* access to a commercial rat chow. Half of these animals were given TAA in the drinking water (300 mg/ml for 97 days) to trigger hepatic cirrhosis (Torres et al., 1998), while the other half served as controls. To verify that the cirrhotic process and the neuronal injury characteristic of hepatogenic encephalopathy (Sarhan et al., 1993; Peeling et al., 1993) had occurred, the livers and brains from TAA-treated rats were histopathologically examined. All the experiments followed E.U. guidelines on the use of animals for biochemical research (86/609/EU) minimizing the number of animals and their suffering.

Immunohistochemistry

A total of 10 rats (five control and five TAA) were deeply anesthetized (15 mg/100 g of body weight, i.p.; Ketolar; Parke Davis, Madrid, Spain) and perfused through the left ventricle with 50 ml

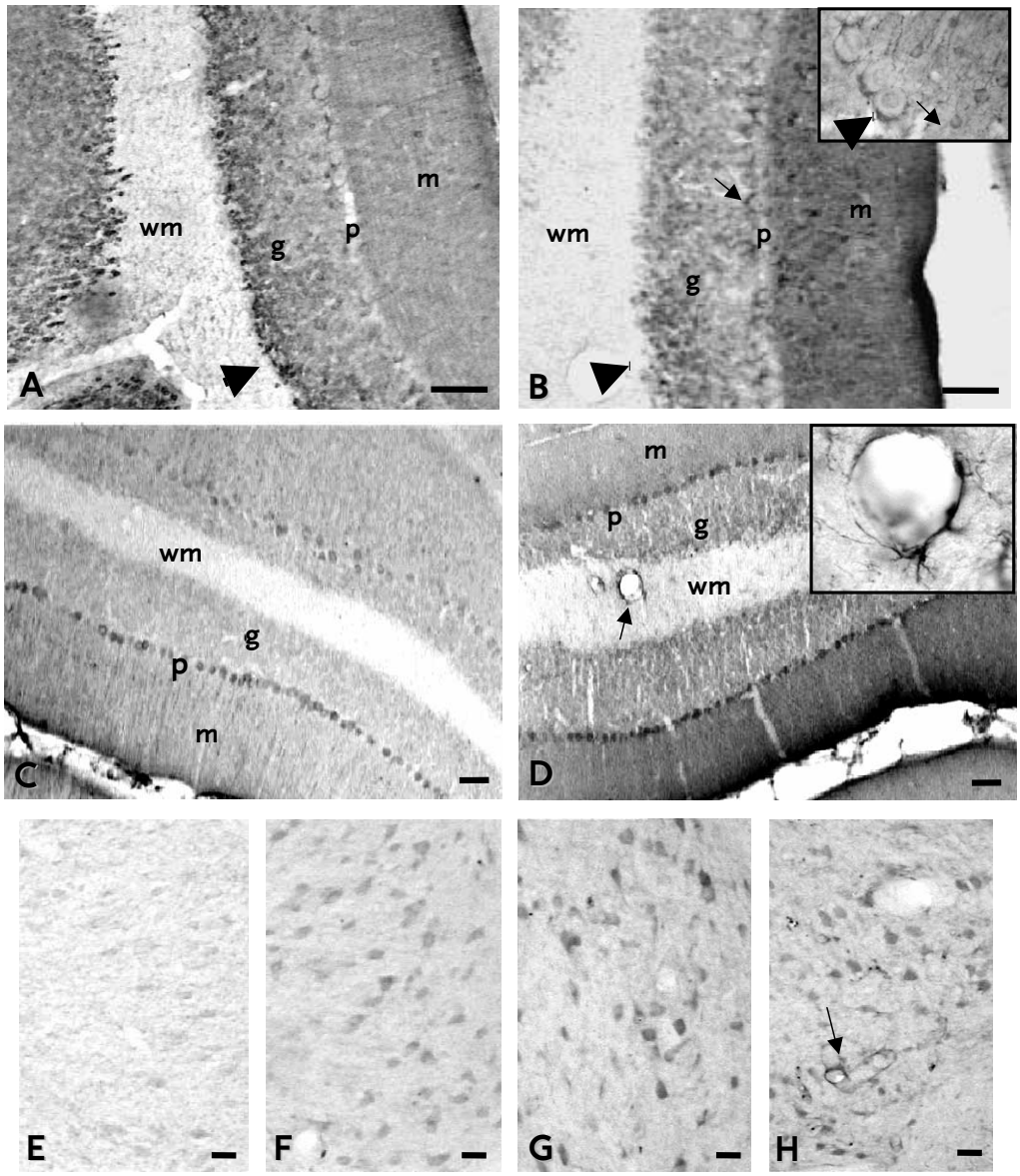

Fig. 1. NOS isoforms IR (NOS-IR) in the cerebellum of control (A, C, E, and G) and rats with TAA-induced cirrhosis (TAA; B, D, F, and H); nNOS-IR in the cerebellar cortex of control (A) and TAA (B) groups; inset in B shows a detail of nNOS-IR basket (thick arrow) and stellate (thin arrow) cells; eNOS-IR in the cerebellar cortex of control (C) and TAA (D) groups; see eNOS-IR in the white matter of the TAA group (arrow). Inset in D shows a detail of the eNOS-IR glial structures surrounding the blood vessels; iNOS-IR in the dentate nucleus of control (E) and TAA group (F). eNOS-IR in the dentate nucleus of control (G) and TAA group (H). g, granular layer; m, molecular layer; p, Purkinje cells layer; wm, white matter. Scale bar=100 μm (A, B); C, D, 50 μm; E–H, 20 μm.

of 0.01 M phosphate buffer saline (PBS), pH 7.4, as a vascular rinse, followed by 500 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Each cerebellum was then removed and postfixed in the same fixative for 4 h at room temperature. The organ was again rinsed and cryoprotected by immersion overnight at 4 °C, in 0.1 M PB containing 30% sucrose.

Immunocytochemical procedures were performed as previously described (Uttenthal et al., 1998; Martínez-Lara et al., 2003). Briefly, serial 40-μm-thick rostro-caudal sections were made using a vibratome VT10005 (Leica Microsystems, Heidelberg, Germany). Free-floating sections were incubated for 4 h in PBS containing 0.1% Triton X-100, and then overnight at 4 °C in one of the following: nNOS (1:3000; this antiserum was a gift from Dr. V. Riveros-Moreno, Wellcome Research Laboratories, Beckenham, UK; Riveros-Moreno et al., 1995), iNOS (1:2500; Uttenthal et al., 1998; Rodrigo et al., 2001), eNOS (1:150; Transduction), or nitrotyrosine (1:300; Uttenthal et al., 1998; Rodrigo et al., 2001) antisera diluted in PBS containing 0.2% Triton X-100. After being washed, the sections were processed by the avidin–biotin peroxidase complex procedure (Vector Laboratories, Burlingame, CA, USA) following the manufacturer's instructions. The peroxidase activity was demonstrated by the nickel-enhanced diaminobenzidine reaction (Shu et al., 1988). Control procedures were performed on adjacent sections of the same tissues. No immunolabeling was detected when the primary antibody was omitted or replaced with an equivalent concentration either of pre-immune serum or of normal rabbit serum.

NADPH-diaphorase histochemistry

NADPH-diaphorase histochemical staining was used as a complementary method for the indirect visualization of NOS activity by light microscopy. Free-floating sections from the same animals used in immunohistochemistry studies were incubated for 4 h in PBS containing 0.1% Triton X-100. After several washes in 0.1 M Tris–HCl pH 7.4 buffer, they were incubated in the dark, for 45 min at 37 °C, in 0.1 M Tris–HCl, pH 7.4, containing 1 mM β-NADPH and 2 mM NBT (in 70% dimethylformamide). The sections were then washed twice with 0.1 M Tris–HCl, pH 7.4, quickly dehydrated in a graded ethanol series, cleared, and mounted in DPX (Fluka, Madrid, Spain). Control reactions were carried out, omitting or replacing β-NADPH with an equivalent amount of either α-NADPH or 0.1 M Tris–HCl.

NO measurements and Western-blot analysis

For biochemical studies, 10 rats (five control and five TAA) were killed by cervical dislocation and the brains immediately removed. The cerebellum was dissected, rinsed in saline solution and stored at −80 °C until used.

NO production was estimated by means of the nitrate, nitrite and *S*-nitroso compounds concentrations in cerebellar tissue (NOx). Samples were homogenized in 1/2/2 (w/v/v) deproteinization solutions (10% $ZnSO_4$ and 0.5N NaOH) and centrifuged for 5 min at 14,000 r.p.m. The resulting supernatants were removed for chemiluminescence analysis in a NO analyzer (Sievers NOA 280i). NOx concentrations were calculated by comparison with standard solutions of sodium nitrate (Braman and Hendrix, 1989).

For Western-blot analysis, samples were homogenized in 1/3 (w/v) of 30 mM Tris–HCl, pH 7.4 containing 0.5 mM DTT, 1 mM EDTA, 1% SDS and protease inhibitors. The resulting homogenates were centrifuged for 1 h at 100,000×*g*. All the operations were performed at 0–4 °C. Protein concentrations in the supernatants were determined by the Bradford method (Bradford, 1976).

Equal amounts of the denatured proteins per lane (iNOS, eNOS, nitrotyrosine: 60 μg; nNOS: 40 μg), were loaded and separated on a 7.5%/10% (NOS/nitrotyrosine) SDS-polyacrylamide gel (Mini Protean II; BioRad), as described by Laemmli (1970). Afterwards, proteins were transferred to a PVDF membrane (Immobilon P; Millipore). The membranes were blocked with 5% powdered non-fat milk in 25 mM Tris–HCl, pH 7.6; 137 mM NaCl, 2.6 mM KCl, 0.1% Tween-20, and incubated overnight at 4 °C with diluted monoclonal anti-iNOS and anti-eNOS (1/1000), rabbit polyclonal anti-nNOS (1:3000) and anti-nitrotyrosine (1:1000) in blocking solution. Bound antibody was revealed by means of an enhanced chemiluminescence kit (Amersham) according to the manufacturer's instructions. The relative amount of the proteins in each sample was quantified by densitometric scanning. After immunodetection, membranes were probed with anti α-tubulin (Sigma) as a loading control.

Statistics

Data are expressed as means±S.D. Student's *t*-test was performed to evaluate significant differences between groups. Differences were considered significant at $P<0.05$.

RESULTS

NOS isoform immunocytochemistry

Immunoreactivity (IR) of nNOS (nNOS-IR) in the cerebellar cortex is presented in Fig. 1A, B. Both control (Fig. 1A) and TAA-cirrhotic rats (Fig. 1B) showed nNOS-IR stellate and basket cells; however, basket-cell bodies and their processes surrounding Purkinje cell showed higher IR in TAA-cirrhotic than in control rats (insert in Fig. 1B). In addition, in the molecular layer the stellate-cell population appeared to have not only high IR but also a greater number of nNOS-IR cells in the TAA group (Fig. 1B). The granular layer showed nNOS-IR granular cells, particularly in the vicinity of the white matter of both control and TAA-treated animals. In the cerebellar cortex, iNOS-IR was absent in control and TAA-cirrhotic rats (results not shown). In both groups, eNOS-IR was located in Purkinje cell bodies and vessels endothelial cells (Fig. 1C, D). In addition, immunopositive structures in the white matter were detected only in the TAA group (Fig. 1D); the morphology and location of these structures imply that these are astrocytes with eNOS-IR processes surrounding the blood vessels (insert in Fig. 1D).

The deep cerebellar nuclei (Fig. 1E–H; dentate, fastigial and interposed) showed some nNOS-IR neurons, but no visible differences were found between the TAA and control groups (results not shown). In relation to iNOS-IR (Fig. 1E, F), a faint staining in some neurons and glia (which morphologically could be microglial cells) was detected only in the TAA-treated rats (Fig. 1F). Finally, eNOS-IR perivascular glia and vessels in both control and TAA cirrhotic animals were found (Fig. 1G, H), although in the cerebellar nuclei of the treated animals the stained structures outnumbered those found in control (Fig H).

NADPH-diaphorase histochemistry

NADPH diaphorase staining (Fig. 2A–D), is strongly detected in granular, stellate and basket cells of the cortex, in neurons of the three cerebellar nuclei, and in the endothelial cells of both control and TAA-treated animals; however, specific stained glial structures around the vessels were evident only

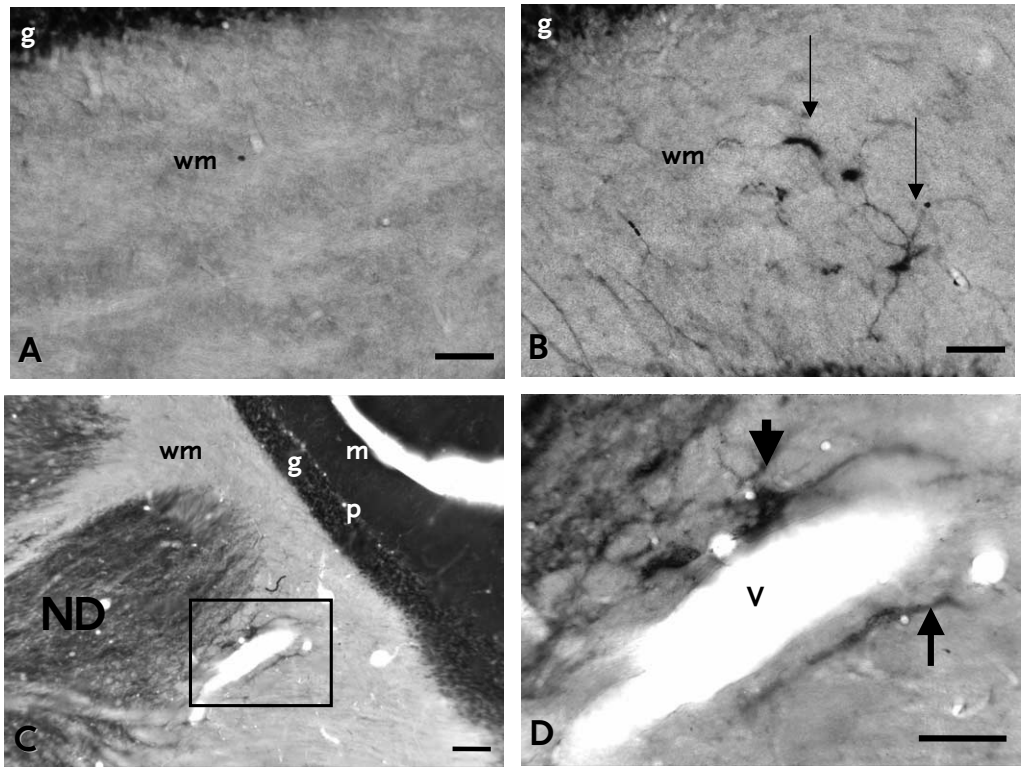

Fig. 2. NADPH diaphorase histochemistry in the cerebellum of control (A) and rats with TAA-induced cirrhosis (B–D). See the staining in perivascular glia in the white matter of the TAA group (arrows). Square in C is represented in D; g, granular layer; m, molecular layer; ND, dentate nucleus; p, Purkinje cells layer; wm, white matter; V, vessel. Scale bar=50 μm (A, B, D); C, 10 μm.

in the white matter of the TAA group. These latter stained structures showed the same features as those seen with eNOS immunocytochemistry (Fig. 2B–D).

Nitrotyrosine immunocytochemistry

In the cerebellar cortex of both TAA and control groups, nitrotyrosine (nTyr) IR appeared in Purkinje cells and in some neurons from molecular and granular layers (Fig. 3A, B). In Purkinje cells the immunoreactive product, a mark of the protein nitration, was located mainly at the nuclear level and in the apical processes (inset in Fig. 3A). Scattered nTyr-IR glial cells were also observed throughout the entire thickness of the cortex and in the white matter (Fig. 3C, D). The morphology and location of these glial cells point to their being astrocytes, showing nTyr-IR feed-end processes surrounding the nearest vessels (insert in Fig. 3B). As can be seen in Fig. 3C, D, the IR of these glial cells in the white matter of the cirrhotic rats was more patent and the immunopositive cells were more numerous than in control.

Both experimental groups showed immunolabeled glial cells in cerebellar nuclei with the same characteristics as those found in the cerebellar cortex (Fig. 3E, F) but no immunoreaction was detected in neurons either in control or in TAA-treated rats (Fig. 3E, F).

NOS isoforms and nitrated-protein expression

To quantify the response of NOS isoforms in the cerebellum after TAA administration, we performed Western-blot analysis using the nNOS, eNOS and iNOS antibodies on denatured homogenates from control and TAA-cirrhotic groups. The nNOS and eNOS antisera revealed a positive band, each at the appropriate molecular weight (150 and 140 kDa, respectively; Fig. 4). The analysis of iNOS expression showed a faint 130 kDa band in all the samples (not shown).

As depicted in Fig. 4, TAA treatment did not alter nNOS expression, while eNOS significantly increased ($P<0.05$).

We detected four nTyr immunoreactive bands (Fig. 5) corresponding to protein of 57.6, 50, 42, and 24 kDa. No significant changes were found in bulk-nitrated proteins with the TAA treatment.

Determination of NOx

To complete the immunocytochemical analysis and protein expression of NOS isoforms and nitrated proteins, NO production was evaluated by determining the NOx level. As reflected in Fig. 6, by comparing the NOx level in the control and cirrhotic animals, we detected an increase, although not significant, in the TAA cerebellum.

DISCUSSION

The results presented here reveal slight changes in the tissue and cellular distribution pattern of the nNOS, iNOS and nitrated proteins in the cerebellum of rats with TAA-induced cirrhosis. In addition, cirrhotic animals showed strong eNOS IR and NADPH-diaphorase activity in

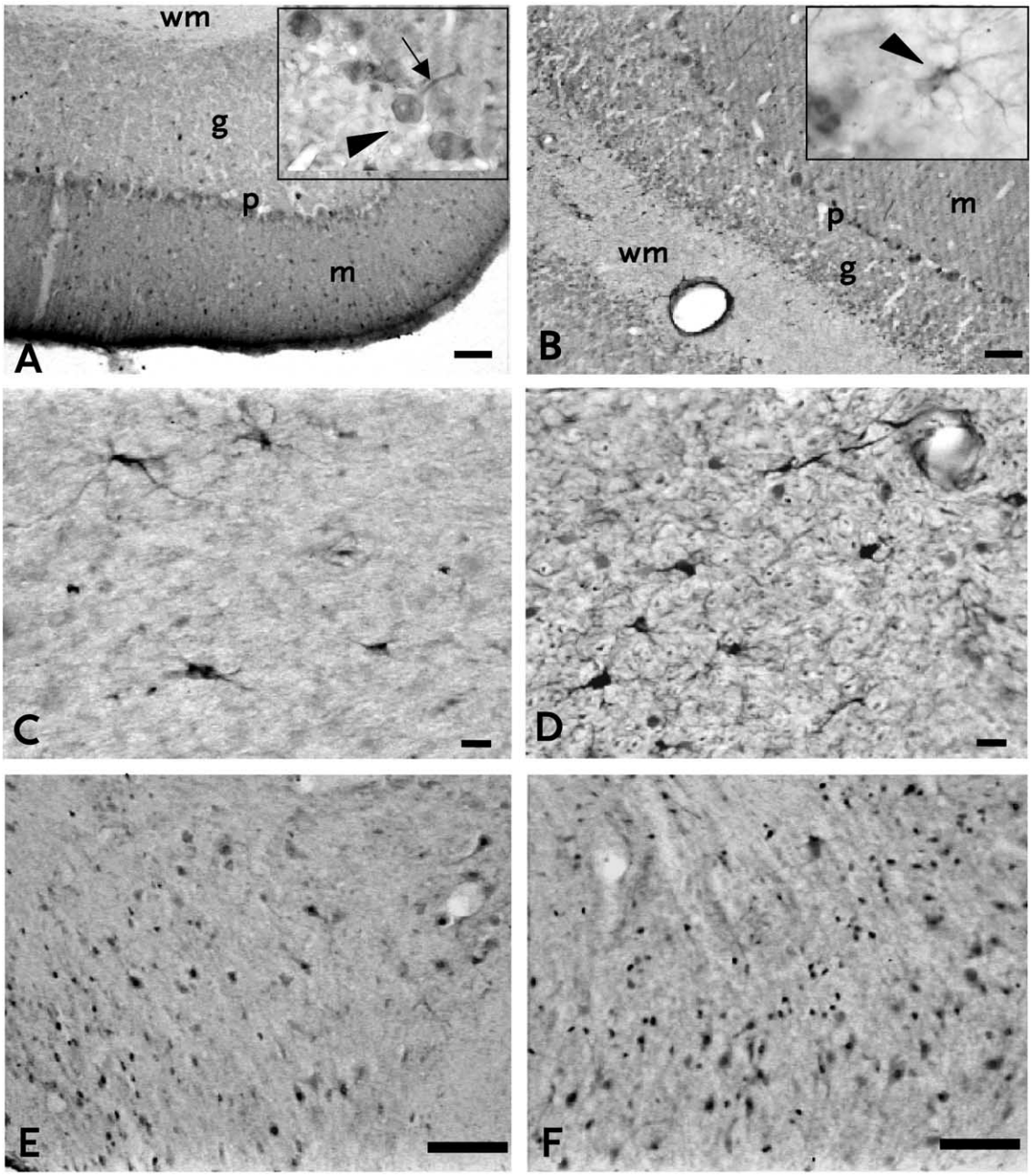

Fig. 3. nTyr-IR in the cerebellum of control (A, C and E) and rats with TAA-induced cirrhosis (TAA; B, D and F); nTyr-IR in the cerebellar cortex of control (A) and in TAA (B); insers in A and B show details of nTyr-IR in the nucleus and apical process of the Purkinje cells (thin arrow) and in scattered glial cells throughout the cortex (thick arrow) respectively; nTyr-IR glial cells in the white matter of control (C) and TAA (D) groups; noted that immunoreactive glial cells in TAA group (D) outnumbered those from control groups (C); nTyr-IR in the dentate nucleus of control (E) and TAA (F) groups. g, granular layer; m, molecular layer; p, Purkinje cells layer; wm, white matter. Scale bar=50 μm (A, B); C, D, 10 μm; E–H, 100 μm.

perivascular glial cells. Moreover, although neither nNOS nor iNOS cerebellar protein levels appeared to be significantly affected, the eNOS isoform augmented its expression and a trend of increased NO production took place in cirrhotic rats under a prolonged treatment (97 days) with the hepatotoxic TAA.

These results support current data on the involvement of NO in the HE syndrome with respect to its vasodilator properties (Schliess and Häussinger, 2001; Rao, 2002). In this sense, studies in TAA-cirrhotic animals have demonstrated that the administration of L-NAME, an inhibitor of NO synthesis, upsets the HE development by raising the levels of endotoxin and tumor-necrosis factor-α, ultimately leading to fulminant hepatic failure with increased mortality (Chu et al., 2001). Hyperammonemia, considered one of the main factors responsible for HE, alters glutamate–NO–cyclic guanosine monophosphate (cGMP) pathways, although the exact relationship between the molecular bases

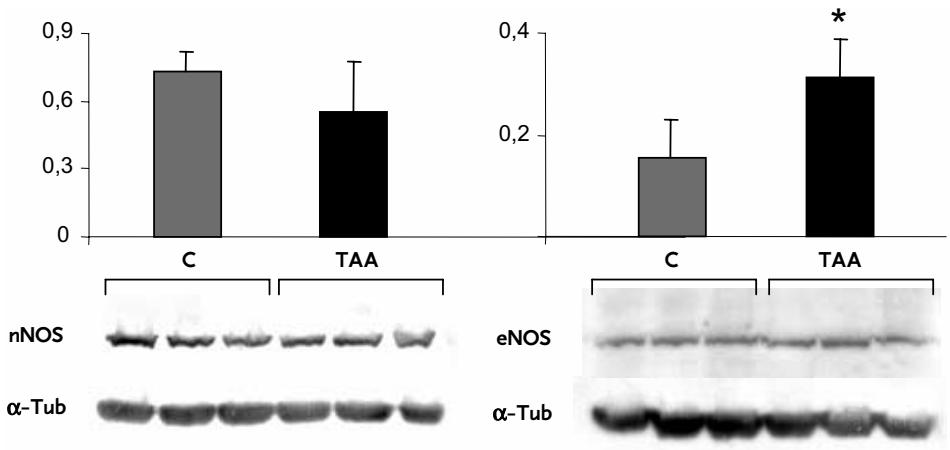

Fig. 4. Representative Western blot analysis of nNOS and eNOS expression in the cerebellum of control (C) and TAA-treated (TAA) rats. Top panel: densitometric quantification of the nNOS and eNOS protein in the different experimental groups. Results are average values of five experimental animals in each group. Bottom panel: representative autoradiographies of the corresponding nNOS and eNOS band in the cerebellum of control and TAA-treated animals; α-Tubulin immunodetection was also included as a protein-loading control. * Protein expression significantly greater than in control group ($P<0.05$).

of this important modulator pathway and neurological alterations remains poorly understood (Hermenegildo et al., 1998; Monfort et al., 2001). Consequently, to shed light on this matter, we first investigated whether cirrhosis induces changes in the expression and cellular distribution of NOS isoforms in the cerebellum, which has the highest level of NO in the CNS (Bredt et al., 1991; Siles et al., 2002).

Initially, our immunocytochemical and histochemical results suggested that NO production from nNOS granular, basket and stellate cells, previously described by other authors (Bredt et al., 1990; Southam et al., 1992; Rodrigo et al., 1994), could be augmented in cirrhotic rats, as demonstrated by the stronger staining detected in these cell types. However, the biochemical approaches showed unchanged nNOS expression in the cirrhotic animals. Other studies in experimental chronic liver failure revealed significant alterations in the expression of key components of the NO-cGMP signal-transduction pathway. Such evidence includes increased expression and activities of nNOS (Rao et al., 1997) and of the α-subunit of the soluble guanilate cyclase (sGC) although the effect of NO on sCG activation depends on the brain area analyzed. Consistently, Corbalan et al. (2002) have reported cGC activation by NO to be increased in the frontal cortex but reduced in

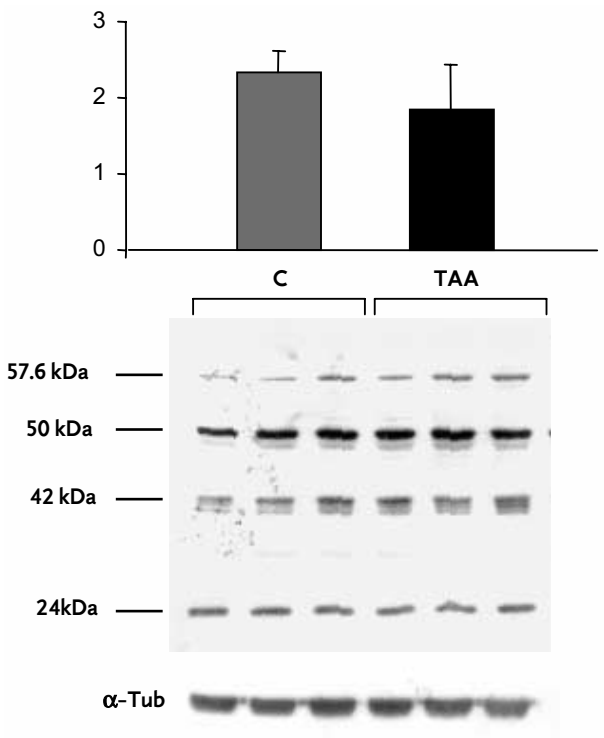

Fig. 5. Western-blot analysis of the nTyr-modified proteins in the cerebellum of control (C) and TAA-treated rats. Top panel: densitometric quantifications of bulk nTyr-modified protein expression in the different experimental groups. Results are average values of five experimental animals in each group. Bottom panel: representative autoradiography of the nTyr immunoreactive bands (57.6, 50, 42 and 24 kDa) in the cerebellum of control and TAA-treated rats; α-tubulin immunodetection was also included as a protein-loading control.

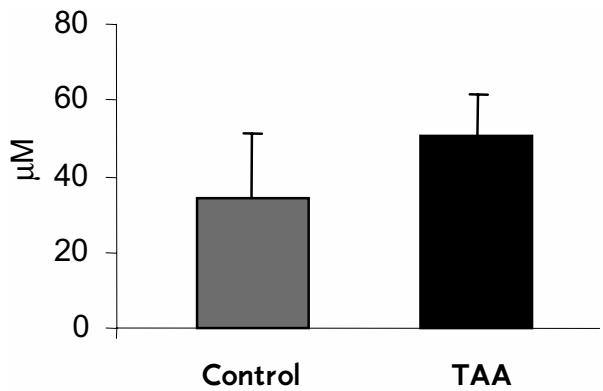

Fig. 6. NOx levels in the cerebellum of control and TAA-treated (TAA) rats. Results are average values (μM) of five experimental animals in each groups.

the cerebellum of cirrhotic patients. Similar results have been reported in different models of hyperammonemia (Zwiller et al., 1985; Monfort et al., 2001), indicating that ammonia must be the main factor responsible for such alterations in the cerebellum. In light of these reports, our apparent contradictory results may be interpreted as regional variations that may offset one another to maintain a balance in protein expression or activity and consequently in the NO supply by this isoform in TAA-cirrhotic animals.

With regard to iNOS, our Western-blot probes scarcely detected the 130 kDa band in any group; however, once again, the immunocytochemical results could support, at least in the cerebellar nuclei, a slight change in the cellular expression of this isoform induced by the TAA treatment. No reports are available in relation to the iNOS isoform in the brain of cirrhotic animals, although, studies performed on liver homogenates of TAA-treated rats and in the liver of cirrhotic patients report increased iNOS activity and iNOS-mRNA expression (Díez-Fernández et al., 1997; McNaughton et al., 2002). Moreover, the selective iNOS inhibitor aminoguanidine improves mortality in a TAA rat-model of induced liver failure but not the less selective inhibitor L-NAME (Rahman and Hodgson, 2003) which causes an even worse mortality rate in these TAA-treated rats (Chu et al., 2001). Our HE TAA-cirrhotic model did not apparently significantly alter iNOS expression in the whole cerebellum but it triggered some perceptible regional histological changes of this isoform.

Regarding eNOS, our immunocytochemical studies reveal that this NOS isoform had the distribution pattern most altered by TAA treatment, showing abundant perivascular glial positive cells in white matter only in cirrhotic animals. In addition, increased expression of the eNOS protein level accompanied the immunoreactive change. The cell location of the eNOS expression in the brain is controversial; thus, apart from the endothelium of the blood vessels, eNOS-like protein was detected in pyramidal neurons of the hippocampus (Dinerman et al., 1994). Also, eNOS has been found in astrocytes, and several groups reported that eNOS was detected preferentially in astrocytes rather than in neurons (Barna et al., 1996; Gabbott and Bacon, 1996). Endothelial cells and astrocytes are two major components of the blood–brain barrier for brain defense. It has been proposed (Duchini, 1996) that endothelial cells are directly or indirectly responsible for the brain pathology in HE and that this damage is mediated by specific cytokines and NO. Interactions between cytokines and CNS endothelial cells may trigger a cascade of events including enhanced blood–barrier permeability, brain edema, astrocyte alterations, and gliosis. Astrocytes, which are the only cells in the mammalian brain to contain the necessary metabolic machinery for ammonia removal, manifest alterations in the expression of many key proteins during chronic liver failure (Rao, 2002). These molecular changes could play an important role in the pathophysiology of HE.

Nitrated complexes in neuronal populations were scarcely affected in cirrhotic rats, either in the cortex or in cerebellar nuclei. Nevertheless, our data point to astrocytes as the main cell population with increased nTyr IR after TAA treatment. In fact, these glial cells play a key part in HE (Matsushita et al., 1999) because they undergo hyperplasic and hypertrophic changes in several brain regions (i.e. cerebellum, cerebral cortex, basal ganglia, hippocampus or hypothalamus) of cirrhotic individuals (Matsushita et al., 1999; Butterworth, 2000). These remodeling changes, by triggering neuroprotective mechanisms, involve new expression of proteins such as GFAP (Matsushita et al., 1999; Butterworth, 2000) that could be the targets of NO. In this sense, our data, showing a 50 kDa nitrated protein that could correspond to GFAP, appear to support the possible modulator role of NO in astrocytosis TAA-induced process.

On the other hand, changes in nTyr-IR in neuronal population may be differently explained based on the neuronal damage reported in HE (Butterworth, 2000). Thus, Peeling et al. (1993) have demonstrated neurodegeneration in Purkinje cells, layer III pyramidal neurons of the cerebral cortex and neurons from CA4 area. In this case, a local hyperproduction of NO affects proteins and may thereby contribute to regional damage from oxidative stress, as described in other pathological situations such as ischemia (Rodrigo et al., 2001).

In short, the data presented here indicate that HE induced by TAA-caused hepatic cirrhosis triggers changes in NO regulation, which may differentially contribute to the evolution of the hepatic disease. On one hand, NO may have a beneficial effect, helping to maintain GMP-NO pathways and the physiological cerebellar functions. On the other hand, excessive NO production could contribute to the oxidative stress, inducing cellular damage. In our model, a local upregulation of the eNOS constitutive isoforms, which maintains NO production while iNOS and nNOS remain stable, may be a mechanism to balance NO production in the cerebellum and to minimize TAA toxic injury.

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