Amino Acids (1998) 15: 77–88

Amino
Acids

Effects of taurine depletion on cell migration and NCAM expression in cultures of dissociated mouse cerebellum and N2A cells

T. E. Maar[1], T.M. Lund[1], G. Gegelashvili[1], R. Hartmann-Petersen[2], J. Moran[3], H. Pasantes-Morales[3], V. Berezin[2], E. Bock[2], and A. Schousboe[1]

[1] PharmaBiotec Research Center, Department of Pharmacology, Royal Danish School of Pharmacy, Copenhagen, Denmark
[2] Center for Medical Biotechnology, Institute of Molecular Pathology, Protein Laboratory, Panum Institute, University of Copenhagen, Denmark
[3] Institute of Cellular Physiology, Department of Neurosciences, National University of Mexico, Mexico City, Mexico

Accepted February 11, 1998

Summary. Cultures of dissociated cerebellum from 5- to 6-day-old mice as well as of the N2A neuronal cell line were exposed to guanidino ethane sulfonate (GES, 2–5 mM) to reduce the cellular taurine content. Control cultures were kept in culture medium or medium containing 2–5 mM GES plus 2–5 mM taurine to restore the intracellular taurine content. Taurine depletion led to changes in the expression of certain splice variants of NCAM mRNA such as the AAG and the VASE containing forms, while no differences were seen in the expression of the three forms of NCAM protein. In the N2A cells taurine depletion led to a decreased migration rate of the cells. The results suggest that the reduced migration rate of neurons caused by taurine depletion may be correlated to changes in expression of certain adhesion molecules such as NCAM. Moreover, taurine appears to be involved in regulation of transcription processes.

Keywords: Amino acids – Taurine – Neurons – NCAM – Migration – Transcription

Introduction

Formation of the neuronal network in cerebellum and in particular the synaptic contacts between granule cell parallel fibers and Purkinje cell dendritic trees involves migration of the granule cells during early postnatal development (Rakic, 1971; Rakic et al., 1994). This migration process requires an intricate interplay between cell surface molecules which are expressed on both neurons and glial cells (Lindner et al., 1983; Tomaselli et al., 1986, 1988;

Persohn and Schachner, 1987; Edmondson et al., 1988; Hatten and Mason, 1990). In addition to this, it has been shown by Sturman and colleagues in a series of elegant experiments that depletion of taurine in cats brought about by keeping the animals on a taurine free diet (Hayes et al., 1975) will lead to severe neurologic symptoms and to arrest of normal migration of the granule cells (Sturman et al., 1985a,b). A similar effect of taurine depletion has been reported in microwell cultures of dissociated early postnatal mouse cerebellum where migration of neuroblasts most likely representing granule cells was severely retarded in taurine depleted cultures (Maar et al., 1995). Normal migration rates in these cultures could be restored by addition of taurine to the culture medium (Maar et al., 1995). Recently, it has been shown using analogous culture systems not only from cerebellum but also from cerebral cortex or hippocampus that an antibody to the neural cell adhesion molecule (NCAM, cf. Gegelashvili and Bock, 1996) interferes with formation of cell aggregates and neurites (Maar et al., 1997). NCAM, the expression of which is developmentally regulated (Lyons et al., 1992) is believed to play important roles in the shaping of the central nervous system by regulation of cell migration, motility and adhesion (Gegelashvili and Bock, 1996). NCAM is a common designation of the group of membrane-associated glycoproteins which are the products of a single gene consisting of 25 exons. Differential use of exons 15 and 18 results in the production of three major isoforms: NCAM-A (ca. 180 kDa), NCAM-B (ca. 140 kDa), and NCAM-C (ca. 120 kDa) (Walsh and Doherty, 1991; Gegelashvili and Bock, 1996). Alternative splicing of other exons, including the 30 nucleotide-long exon VASE or the exons aAAG, ensures further heterogeneity within these isoforms. NCAM-A and -B, the major neuronal isoforms, are encoded for by 7.4 kb and 6.7 kb mRNAs, respectively. The third isoform, NCAM-C, that is attached to the plasma membrane *via* a glycosyl-phosphatidyl-inositol (GPI) anchor, is encoded for by differentially polyadenylated 5.2 kb and 2.9 kb mRNA species and is only rarely detected in neurons, especially in cerebellar granule neurons (Gegelashvili et al., 1993a). These mRNA species are, however, expressed in astrocytes (Gegelashvili et al., 1993b). Insertion of the VASE exon is known to inhibit neuronal sprouting (Doherty et al., 1992) probably by changing the structure of Ig-homology domain IV, and the expression of this splice variant occurs at high levels late in development and in the adult animal where neuronal functions are consolidated. Glycosylation of NCAM is another means of developmental regulation of the NCAM molecule, and along with alternative pre-mRNA splicing and post-translational modifications a gallery of NCAM molecules is generated. In this context NCAM knock out mice have been demonstrated to exhibit impaired migration of neurons in defined regions of the brain including the olfactory bulb (Cremer et al., 1994), an effect which can also be obtained by removal of polysialic acid during development (Ono et al., 1994). Such a diversity of NCAM serves the fine tuning of cell adhesion and signal transduction, two basic activities that underlie the morphoregulatory properties of NCAM and its higher biological functions. However, little is known about epigenetic cues regulating the expression of the NCAM diversity. Several hormones, growth factors, and

cytokines have been shown to affect the expression of different isoforms of NCAM *in vitro* (for review, see Gegelashvili and Bock, 1996). However, a possible influence of neurotransmitters, especially of amino acid transmitters and related molecules, on NCAM expression is so far poorly elucidated. Since taurine may be essential for neuronal migration it may be assumed that taurine depletion could have an effect on the expression of NCAM in neurons which, in turn, might lead to a severe disturbance of e.g. cell migration. The present study was undertaken to gain additional information about the mechanisms by which taurine depletion can influence neuronal development. Thus, using the taurine uptake inhibitor guanidino ethane sulfonate (GES) to deplete the cells in cerebellar cultures and a neuronal cell line for taurine (Huxtable et al., 1979; Moran and Pasantes-Morales, 1991; Maar et al., 1995), and antibodies and specific oligonucleotide probes to NCAM, it was investigated whether expression of NCAM protein and mRNA splice variants was influenced in such cultures. The results demonstrate a correlation between a low intracellular taurine content and a change in the NCAM mRNA expression pattern, which implies that taurine may be able to influence neuronal transcriptional processes during development.

Experimental procedures

Primary cultures of dissociated cerebellum

Cultures of dissociated cerebellum were prepared essentially as described by Trenkner and Sidman (1977) but since large amounts of tissue were required for Northern blot analysis the method was adapted to a slight modification of that described by Schousboe et al. (1989). Briefly, cell suspensions of mouse cerebellum obtained from 5- to 6-day-old mice (Animal quarter, Panum Institute, Univ. Copenhagen) were prepared by chopping the tissue, followed by trypsinization and subsequent trituration after addition of a trypsin inhibitor. This cerebellar cell suspension was centrifuged, resuspended and adjusted to 5×10^6 cells/ml in a slightly modified DMEM with additional glucose (25 mM) and KCl (19 mM), and supplemented with 10% horse serum, 0.2 mM glutamine, 0.1 IU/l insulin and 100 IU/ml penicillin and taurine (2.0 mM) or GES (2.0 mM) as specified. Cells were plated in NUNC 80 cm^2 T-flasks at a density of 4×10^6 cells/ml. Cultures were incubated for 3–5 days at 37°C in a humidified atmosphere containing 5% CO_2 before harvesting. These cultures consist of a mixture of neurons (mostly granule cells) and astroglial cells (Maar et al., 1995).

Cell lines

The neuronal N2A cell line was used in addition to the primary cultures. N2A cultures used for mRNA extraction were grown in T-75 flasks in the presence or absence of GES (2.0 or 5.0 mM), GES (2.0 or 5.0 mM) + taurine (2.0 or 5.0 mM) for 5 days. DMEM media were supplemented as described above (primary cultures) and exchanged once after three days. Cultures were maintained for 5 days before harvesting for mRNA extraction.

Preparation of mRNA and Northern blot analysis

Primary cultures as well as cell lines were washed twice in PBS without calcium or magnesium, lysed in the flasks using a homogenization buffer (6 M LiCl, 3 M urea) and

then further homogenized mechanically (Ultra Thorax). Total RNA was obtained from homogenates by three extractions, two using phenol:chloroform 5:1 followed by another with phenol:chloroform:isoamylalcohol 25:24:1. A poly(A+)RNA-enriched fraction was obtaind by one cycle of oligo (dT)-cellulose chromatography. Samples (10μg) were denatured in 50% formamide/6% formaldehyde (v/v) by heating at 60°C for 15 min and subsequently submitted to electrophoresis through 0.8% agarose/6% formaldehyde gels in MOPS buffer (pH 7.2). After electrophoresis, RNA was blotted onto nitrocellulose filters (Schleicher and Schull, Dassel, Germany) in 20 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na-citrate, pH 7.0) utilizing a Vacu-Aid vacuum blotting system (Hybaid, Teddington, UK).

DNA oligonucleotide probes (30–42 bases) specific for exons 7 (E7) and VASE (EVASE), or exon combinations 7/8 (E7/8) and 12/a/AAG/13 (E12aAAG13), were essentially of the same composition as described previously (Anderson et al., 1990). The probe for mouse/rat *c*-fos (30-mer) was complementary to nt 1390–1419 in a mouse gene sequence (GeneBank EMBL accession nr. J00370). The probes were labeled with [^{32}P]dATP (NEN, DuPont, USA) using a DNA tailing kit (Boehringer Mannheim, Germany). Hybridization of labeled probes (2–5 pmol) was performed overnight in a buffer containing 4 × SSC, 0.1% SDS, 0.1% Denhardt's solution (Sigma, St. Louis, USA) at the estimated temperatures (60–67°C). After hybridization, filters were washed in 1 × SSC at the hybridization temperature, sealed in plastic, exposed to radiosensitive phosphor screens, and, after 2 days, scanned. Obtained images were evaluated using ImageQuant software (Phosphorimager SI, Molecular Dynamics, USA).

Western blot analysis

Cerebellar cultures were kept *in vitro* for five days, then washed twice in PBS without calcium or magnesium (Gibco, BRL) and lysed in the microwell plates using lysing buffer. Protein content was measured in triplicates by the method of Lowry et al. (1951). Equal amounts of protein samples were separated by sodium-dodecyl polyacrylamide gel-electrophoresis (SDS-PAGE), and subsequently transferred onto Immobilon P membranes (Millipore) by semidry blotting (JKA Biosciences, Denmark). NCAM bands were visualized by a polyclonal rabbit NCAM antibody and a secondary swine anti-rabbit alkaline phosphatase conjugated antibody (DAKO A/S, Denmark).

Motility measurements

The motility of N2A on plastic was evaluated using time-lapse video recording and computer-assisted image analysis. Ten microscopic fields each containing approximately 15 single cells, were recorded automatically spaced by 5 min in time, and the resulting images were processed using the PRIMA image analysis system (Protein Laboratory, University of Copenhagen, Denmark). The migration rate of all single cells from the three independent experiments was calculated from the displacement of cells during five min intervals.

Results

NCAM Protein expression

Cultures of dissociated postnatal mouse cerebellum were used for Western blot analysis of NCAM expression. Figure 1 shows that the cultures regardless of the culture condition (plain culture medium, taurine, GES or taurine plus

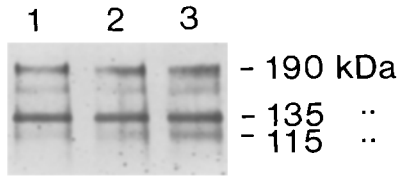

Fig. 1. Western blot analysis of expression of NCAM isoforms in cultures of dissociated mouse cerebellum grown for 3 days in plain culture medium (*1*), medium plus 2.0 mM GES (*2*) or medium plus 2.0 mM GES and 2.0 mM taurine (*3*). Cultures were grown and extracted for analysis as detailed in Experimental procedures. SDS-PAGE and subsequent Western blotting were performed as described in Experimental procedures employing an NCAM antibody conjugated to alkaline phosphatase prepared at the Protein Laboratory. The position of molecular weight markers are indicated at the right. The bands at 190, 135 and 115 kDa correspond to NCAM-A, -B and -C, respectively

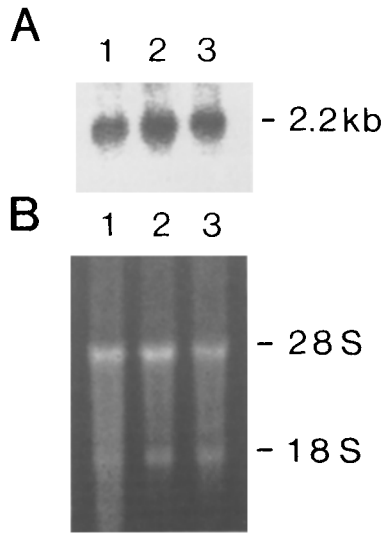

Fig. 2. Northern blot analysis of expression of c-*fos* mRNA in cultures of dissociated mouse cerebellum grown for 4 days in plain culture medium (*1*), medium plus 2.0 mM GES (*2*) or medium plus 2.0 mM GES and 2.0 mM taurine (*3*). Cultures were grown and extracted for mRNA as detailed in Experimental procedures. Panel **A** shows the hybridization with a c-*fos* oligonucleotide probe and panel **B** shows ethidium bromide staining of the gels which were loaded with 10 μg RNA per lane

GES) expressed the 3 isoforms of NCAM and that this expression was unaffected by the presence of taurine or GES in the media.

NCAM mRNA expression

In order to assess any diverse effects of the addition of GES alone or together with taurine a Northern blot analysis of *c*-fos expression was performed. Figure 2 shows that exposure of the cells to 2.0 mM GES alone or together

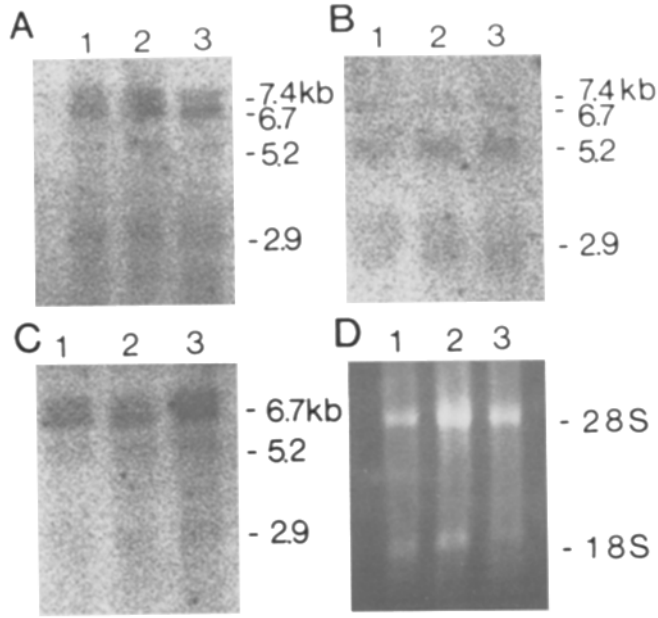

Fig. 3. Northern blot analysis of expression of NCAM mRNA splice variants, E7 (**A**), AAG (**B**) and VASE (**C**) in cultures of dissociated mouse cerebellum grown and extracted for mRNA as detailed in Experimental procedures. Panel **D** shows ethidium bromide staining of the gels loaded with 10 μg mRNA each. Lane *1* represents an extract from cells grown in plain culture medium, Lane *2* an extract from cells grown in the presence of 2.0 mM GES and lane *3* an extract of cells grown in a medium containing 2.0 mM GES plus 2.0 mM taurine. Bands corresponding to 7.4 and 6.7 kb encode for NCAM-A and -B isoforms whereas bands at 5.2 and 2.9 kb encode for NCAM-C

with 2.0 mM taurine led to an upregulation of *c*-fos mRNA expression by 10–40% compared to control cultures.

Using a mixture of oligonucleotide probes hybridizing specifically to adjacent portions of exons 7 and 8, as well as to exons VASE and AAG, it was shown that treatment of the cells with GES leading to depletion of the cellular taurine pool resulted in a small upregulation of total NCAM mRNA as shown also by the increased hybridization signal using the general NCAM probe hybridizing to exon 7 (Fig. 3A). A similar result was obtained using the neuronal cell line N2A. As seen in Fig. 4, the Northern blot analysis of NCAM mRNA extracted from the N2A cells using the E7 probe showed that mRNA from cells grown in a medium containing GES (2.0 mM) plus taurine (2.0 mM) exhibited a much more pronounced hybridization signal than mRNA from cells maintained in GES (2.0 mM) alone. In addition to the determination of total NCAM mRNA using the E7 oligonucleotide probe for hybridization, Northern blots of the extracts of cerebellar cell cultures were hybridized with probes identifying the AAG and VASE splice variants. Figure 3B,C show that the hybridization signals for the two probes at 6.7 kb were down-regulated when the cells had been grown in the presence of GES (2.0 mM)

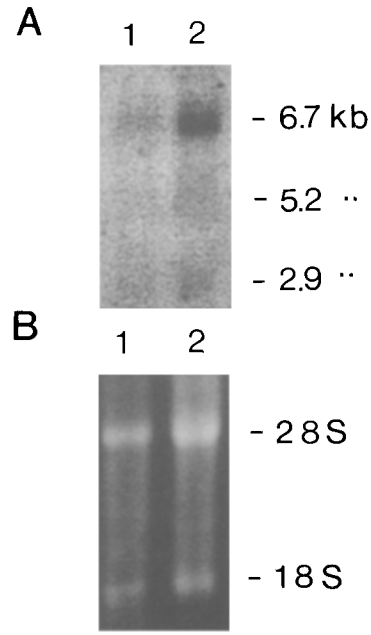

Fig. 4. Northern blot analysis of expression of NCAM mRNA in N2A cells grown in a medium containing 2.0 mM GES plus 2.0 mM taurine (*1*) or 2.0 mM GES (*2*) using the E7 oligonucleotide probe for hybridization (**A**). Panel **B** shows the corresponding ethidium bromide staining of the gels loaded with 10 μg RNA per lane

and that this could be reversed when taurine was present in the culture medium. Due to the fact that the gel loaded with mRNA from the GES treated cultures had more mRNA than those representing the controls or GES plus taurine treated cells (Fig. 3D), the down-regulation of the expression of the AAG (Fig. 3B) and VASE (Fig. 3C) splice variants was more pronounced than it may appear from the blots directly. A semiquantitative estimate of the intensity of the respective signals based on optical scanning of the gels has been provided in Table 1. It is seen that while the E7 probe showed a more intense signal for the GES treated cultures both at 5.2 and 6.7 kb, the down-regulation of the AAG and VASE signals were only observed at 6.7 kb.

Migration of cells

Cell migration and the effect of taurine depletion was studied in the N2A neuronal cell line and compared to similar results obtained previously using microwell cultures of dissociated early postnatal mouse cerebellum (Maar et al., 1995). As seen from Table 2, the N2A cells migrated with a reduced velocity when they were grown in GES, an effect which was even more pronounced in the granule cells of the cerebellar microwell cultures. In both cases, the effect of taurine depletion resulting from the treatment with GES was fully reversed by addition of taurine to the culture medium. Hence, GES

Table 1. Quantification of Northern blots showing mRNA extracted from cerebellar cultures and probed for the NCAM mRNA species E7, VASE and AAG

mRNA species	Intensity ratio*		
	2.9kb	5.2kb	6.7kb
E7	1.15	2.07	1.38
VASE	1.13	1.00	0.59
AAG	1.07	1.02	0.24

Cultures of dissociated cerebellum were prepared as detailed in Experimental procedures. Subsequently, mRNA was extracted and used for electrophoresis and Northern blotting as detailed in Experimental procedures. The experiment was repeated twice with similar results. The values originate from the experiment shown in Fig. 3. *) The intensity ratio is defined as the intensity of the band from cells treated with GES (2.0mM) divided by the averaged intensities of bands from controls and cells cultured with GES (2.0mM) plus taurine (2.0mM).

Table 2. Effect of taurine depletion on migration (μm/h) of N2A cells and cerebellar granule neurons

Condition	Migration (μm/h)	
	N2A cells	Granule cells
Control	52.1 ± 2.1	30.8 ± 2.6
GES	43.5 ± 1.6*	16.3 ± 1.0*
GES + taurine	51.2 ± 2.2	29.3 ± 2.4

N2A cells were grown as detailed in Experimental procedures in the control culture medium or in the presence of GES (5.0mM) alone or together with taurine (5.0mM). After 5 days in culture, the migration of cells was estimated as specified in Experimental procedures. Cerebellar granule cells were cultured in microwells (Maar et al., 1995) in plain culture medium (control) or in the presence of GES (2.0mM) or GES (2.0mM) plus taurine (2.0mM) for 72h. Migration of cerebellar granule cells was determined as described by Maar et al. (1995) and the data were recalculated from the previously published results. Asterisks indicate statistically significant differences from the control ($P < 0.001$, Student's t-test) and results are averages ± SEM of 350 (N2A) and 10–30 (granule cells) experiments.

had apparently no effect of its own but the action can be ascribed to the ability of GES to lower the intracellular level of taurine.

Discussion

The present observation that taurine depletion in the neuronal cell line N2A brought about by culturing the cells in the presence of the taurine transport inhibitor GES led to a decreased rate of cell migration is in agreement with the previous demonstration of a similar phenomenon in cerebellar granule

cell cultures (Maar et al., 1995). The finding confirms and extends the demonstration that taurine depletion in cats severely affects migration of cerebellar granule neurons during early postnatal development (Sturman et al., 1985a,b). The finding that this effect of taurine depletion correlates with an alteration in the expression of certain splice variants of NCAM mRNA suggests that this effect on cell migration may be related to differences in cell-cell surface interactions brought about by altered expression of cell adhesion molecules as previously suggested (Hatten and Mason, 1990; Maar et al., 1995, 1997). The finding that treatment of the cells with GES led to an altered expression of *c*-fos should, however, be taken as an indication that some indirect inverse effects of GES may at least partly explain the effects on NCAM expression since induction of *c*-fos may be related to cell stress (Griffiths et al., 1997). It has, however, previously been shown that treatment of cerebellar granule cell cultures with GES had no effect on cell viability and release of the neurotransmitter glutamate (Maar et al., 1995).

Signal transduction pathway(s) affected by taurine, as well as mechanisms of its interference with cell-to-cell adhesion, particularly those mediated by NCAM, are poorly understood. Thus, taurine may prevent neutrophil adhesion, most likely due to inhibition of lipid peroxidation and neutrophil activation (Son et al., 1996). However, this mechanism is unlikely to take place in neuronal cultures. Taurine depletion by GES stimulates phosphorylation of 44- and 20-kDa proteins present in the mitochondrial fractions of the rat heart and retina, respectively (Lombardini, 1995; Lombardini and Props, 1996). Kinase/phosphatases mediating the taurine-induced effects have not yet been identified, although chelerythrine, an inhibitor of protein kinase C, appeared to affect the phosphorylation state of the same proteins (Lombardini, 1995; Lombardini and Props, 1996). As a molecule that is capable of regulating the osmotic status of brain cells (Pasantes-Morales and Schousboe, 1997), taurine may affect the mitogen-activated protein kinase (MAPK) cascade, a suspected pathway for the osmosensing signal transduction and subsequent expression of a variety of genes, including early response genes (Itoh et al., 1994; Burg et al., 1997). Interestingly, the similar MAP kinase cascade may be regulated by ciliary neurotrophic factor (CNTF) (Inoue et al., 1996), a cytokine that mimics some of the effects of taurine in retina (Fuhrman et al., 1995). However, just few bioactive substances, including cytokines and growth factors, have been shown to alter the overall NCAM levels or the ratios of different splice forms (for review, see Gegelashvili and Bock, 1996). The cAMP-dependent up-regulation of NCAM-B, particularly of the VASE-containing variants, have been observed in cultured astrocytes (Gegelashvili et al., 1993b). However, it is yet unclear whether taurine interferes with a similar pathway in cerebellar granule neurons. In any case, the altered ratio of the VASE containing vs. VASE-negative NCAM isoforms may significantly modify both the signal transducing abilities and adhesive efficacy of NCAM (Chen et al., 1994; Doherty et al., 1996). Therefore, such a shift in the NCAM composition may well account for the taurine/GES-induced changes in the migration of granule neurons *in vitro* (Moran et al., 1996; Trenkner et al., 1996), although the role of the taurine-sensitive

posttranslational modifications of NCAM (e.g. phosphorylation) may not be ruled out. In the light of the revealed taurine/GES-sensitive phosphorylation events, the latter possibility may be worthwhile examining experimentally.

Acknowledgements

The expert secretarial and technical assistance of Ms Hanne Danø, and Ms Tina Haar and Inge Damgaard, respectively, is gratefully acknowledged. The work was supported by grants from the Danish State Biotechnology Programs and the Lundbeck Foundation.

References

Andersson AM, Gaardsvoll H, Giladi E, Dahl B, Bock E (1990) Characterization of rat brain NCAM mRNA using DNA oligonucleotide probes. FEBS Lett 263: 385–388

Burg MB, Kwon ED, Kultz D (1997) Regulation of gene expression by hypertonicity. Annu Rev Physiol 59: 437–455

Chen A, Haines S, Maxson K, Akeson RA (1994) VASE exon expression alters NCAM-mediated cell-cell interactions. J Neurosci Res 38: 483–492

Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kramer P, Scheff S, Barthels D, Rajewsky K, Wille W (1994) Inactivation of the NCAM gene in mice results in reduction of the olfactory bulb and deficits in spatial learning. Nature 367: 455–459

Doherty P, Moolenaar CECK, Ashton SV, Michalides RJAM, Walsh FS (1992) Use of the VASE exon downregulates the neurite growth promoting activity of NCAM 140. Nature 356: 791–793

Doherty P, Smith P, Walsh FS (1996) Shared cell adhesion molecule (CAM) homology domains point to CAMs signalling via FGF receptors. Perspect Dev Neurobiol 4: 157–168

Edmondson JC, Liem RKH, Kuster JE, Hatten ME (1988) Astrotactin: a novel neuronal cell surface antigen that mediates neuron-astroglial interactions in cerebellar microcultures. J Cell Biol 106: 505–517

Fuhrman S, Kirsch M, Hofmann HD (1995) Ciliary neurotrophic factor promotes chick photoreceptor development in vitro. Development 121: 2695–2706

Gegelashvili G, Bock E (1996) Cell recognition molecules of immunoglobulin superfamily in the nervous system. In: Lee AG (ed) Biomembranes, vol 3. JAI Press Inc, Greenwich, London, pp 33–75

Gegelashvili G, Andersson A-M, Schousboe A, Bock E (1993a) Characterization of NCAM diversity in cultured neurons. FEBS Lett 324: 337–340

Gegelashvili G, Andersson A-M, Schousboe A, Bock E (1993b) Cyclic AMP regulates NCAM expression and phosphorylation in cultured mouse astrocytes. Eur J Cell Biol 62: 343–351

Hatten ME, Mason CA (1990) Mechanisms of glial-guided neuronal migration *in vitro* and *in vivo*. Experientia 46: 907–916

Hayes KC, Carey RE, Schmidt SY (1975) Reginal degeneration associated with taurine deficiency in the cat. Science 188: 949–951

Huxtable RJ, Laird HE, Lippincott SE (1979) The transport of taurine in the heart and the rapid depletion of tissue taurine content by guanidino ethane sulfonate. J Pharmacol Exp Ther 211: 465–471

Inoue M, Nakayama C, Noguchi H (1996) Activating mechanism for CNTF and related cytokines. Mol Neurobiol 12: 195–209

Itoh T, Yamauchi A, Miyai A, Yokoyama K, Kamada T, Ueda N, Fujiiwara Y (1994) Mitogen-activated protein kinase and its activator are regulated by hypertonic stress in Madin-Darby canine kidney cells. J Clin Invest 93:2387–2392

Lindner J, Rathjen FG, Schachner M (1983) L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum. Nature 305: 427–430

Lombardini JB (1995) Paradoxical stimulatory effect of the kinase inhibitor chelerythrine on the phosphorylation of a approximately 20K Mr protein present in the mitochondrial fraction of rat retina. Brain Res 673: 194–198

Lombardini JB, Props C (1996) Effects of kinase inhibitors and taurine analogues on the phosphorylation of specific proteins in mitochondrial fractions of rat heart and retina. Adv Exp Med Biol 403: 343–350

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275

Lyons GE, Moore R, Yahara O, Buckingham ME, Walsh FS (1992) Expression of NCAM isoforms during skeletal myogenesis in the mouse embryo. Dev Dynamics 194: 94–104

Moran J, Pasantes-Morales H (1991) Taurine-deficient cultured cerebellar astrocytes and granule neurons obtained by treatment with guanidino ethane sulfonate. J Neurosci Res 29: 533–537

Moran J, Maar T, Gegelashvili G, Bock E, Schousboe A, Pasantes-Morales H (1996) Taurine deficiency and neuronal migration. Adv Exp Med Biol 403: 519–526

Maar T, Moran J, Schousboe A, Pasantes-Morales H (1995) Taurine deficiency in dissociated mouse cerebellar cultures affects neuronal migration. Int J Devl Neuroscience 13: 491–502

Maar TE, Rønn LCB, Bock E, Berezin V, Moran J, Pasantes-Morales H, Schousboe A (1997) Characterization of microwell cultures of dissociated brain tissue for studies of cell-cell interactions. J Neurosci Res 47: 163–172

Ono K, Tomasiewitz H, Magnussen T, Rutishauser U (1994) N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. Neuron 13: 595–609

Pasantes-Morales H, Schousboe A (1997) Role of taurine in osmoregulation in brain cells: mechanisms and functional implications. Amino Acids 12: 281–292

Persohn E, Schachner M (1987) Immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of the mouse cerebellum. J Cell Biol 105: 569–576

Rakic P (1971) Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electron microscopy study in *Macacus rhesus*. J Comp Neurol 141: 283–312

Rakic P, Cameron RS, Komuro H (1994) Recognition, adhesion, transmembrane signaling and cell motility in guided neuronal migration. Curr Opin Neurobiol 4: 63–69

Schousboe A, Meier E, Drejer J, Hertz L (1989) Preparation of primary cultures of mouse (rat) cerebellar granule cells. In: Shahar A, De Vellis J, Vernadakis A, Haber B (eds) A dissection and tissue culture manual of the nervous system. Alan R Liss, New York, pp 203–206

Son M, Kim HK, Kim WB, Yang J, Kim BK (1996) Protective effect of taurine on indomethacin-induced gastric mucosal injury. Adv Exp Med Biol 403: 147–155

Sturman JA, Moretz RC, French JH, Wiesniewski HM (1985a) Taurine deficiency in the developing cat: persistence of the cerebellar granule cell layer. J Neurosci Res 13: 405–416

Sturman JA, Moretz RC, French JH, Wiesniewski HM (1985b) Postnatal taurine deficiency in the kitten results in a persistence of the cerebellar external granule cell layer: correction by taurine feeding. J Neurosci Res 13: 521–528

Tomaselli KJ, Reichardt LF, Bixby JL (1986) Distinct molecular interactions mediate neuronal process outgrowth on non-neuronal cell surfaces and extracellular matrices. J Cell Biol 103: 2659–2672

Tomaselli KJ, Neugebauer KM, Bixby JL, Lilien J, Reichardt LF (1988) N-Cadherin and integrins: two receptor systems that mediate neuronal process outgrowth on astrocyte surfaces. Neuron 1: 33–43

Trenkner E, Sidman RL (1977) Histogenesis of mouse cerebellum in microwell cultures, cell reaggregation and migration, fiber and synapse formation. J Cell Biol 75: 915–940
Trenkner E, el-Idrissi A, Harris C (1996) Balanced interaction of growth factors and taurine regulates energy metabolism, neuronal survival, and function of cultured mouse cerebellar cells under depolarizing conditions. Adv Exp Med Biol 403: 507–517
Walsh FS, Doherty P (1991) Structure and function of the gene for neural cell adhesion molecule. Semin Neurosci 3: 271–284

Authors' address: Prof. Arne Schousboe, D.Sc., PharmaBiotec Res. Center, The Neurobiology Unit, Department of Pharmacology, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark, Fax +45 35370633

Received December 29, 1997