Amino Acids (2002) 23: 37–44
DOI 10.1007/s00726-001-0107-5

Amino
Acids
Printed in Austria

Domoic acid neurotoxicity in hippocampal slice cultures

B. Jakobsen[1,2], **A. Tasker**[3], and **J. Zimmer**[1]

[1] Anatomy and Neurobiology, University of Southern Denmark-Odense, Denmark
[2] NsGene A/S, Ballerup, Denmark
[3] Department of Anatomy and Physiology, University of Prince Edward Island, Canada

Received June 29, 2001
Accepted August 6, 2001
Published online June 3, 2002

Summary. The neurotoxicity of domoic acid was studied in 2–3 week old rat hippocampal slice cultures, derived from 7 day old rat pups. Domoic acid 0.1–100 μM was added to the culture medium for 48 hrs, alone or together with the glutamate receptor antagonists NS-102 (5-Nitro-6,7,8,9-tetrahydrobenzo[G]indole-2,3-dione-3-oxime), NBQX (2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F) quinoxaline) or MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d]-cyclohepten-5,10-imine hydrogen maleate), followed by transfer of the cultures to normal medium for additional 48 hrs. Neuronal degeneration in the fascia dentata (FD), CA3 and CA1 hippocampal subfields was monitored and EC_{50} values estimated by densitometric measurements of the cellular uptake of propidium iodide (PI). The CA1 region was most sensitive to domoic acid, with an EC_{50} value of 6 μM domoic acid, estimated from the PI-uptake at 72 hrs. Protective effects of 10 μM NBQX against 3 and 10 μM domoic acid were observed for both dentate granule cells and CA1 and CA3c pyramidal cells. NS102 and MK 801 only displayed protective effects when combined with NBQX. MK801 significantly increased the combined neuroprotective effect of NBQX and NS102 against 10 μM domoic acid in both CA1 and FD, but not in CA3. We conclude, that domoic acid neurotoxicity in CA3 and in hippocampal slice cultures in general primarily involves AMPA/kainate receptors. At high concentrations (10 μM domic acid) NMDA receptors are, however, also involved in the toxicity in CA1 and FD.

Keywords: Domoic acid – NBQX – NS102 – Propidium iodide – Hippocampal slice cultures

Introduction

Domoic acid (DOM) is a naturally occurring amino acid, produced by the red algae, *Chondria armata*, and various subspecies of the plankton *Pseudonitzschia.* By accumulation in filter feeding animals, like shellfish, plankton-produced domoic acid poisoned over one hundred people, with 4 fatal cases, in Canada in 1987 (Perl et al., 1990). The neurological symptoms of the DOM intoxicated cases included seizures, coma and transient or permanent memory loss. Postmortem neuropathological analysis of the fatal cases revealed damage of primarily the hippocampus and amygdala (Teitelbaum et al., 1990), known among other functions to be involved in learning and memory.

DOM is known to activate the kainate subtype of ionotropic glutamate receptors, in particular the receptor subunits GluR5 and GluR6 (Bettler et al., 1992). From studies of cultured cortical neurons and animals it is known that the DOM toxicity is reduced by treatment with the selective GluR6 antagonist NS102 (Johansen et al., 1993; Tasker et al., 1996).

Here we used rat hippocampal slice cultures to study DOM neurotoxicity in the different subregions of hippocampus, and the contribution of different glutamate receptor subunits, by addition of different glutamate receptor antagonists to the culture medium, alone or in combinations with each other and DOM.

The use of organotypic brain slice cultures has several advantages compared to both live animals and dissociated, primary neuronal cultures, by providing easy access to the tissue and cells, and better control of experimental conditions at the same time as the basic organotypic cellular composition and synaptic circuitry is preserved. Hippocampal slice cultures in particular are well characterized with regard to their neuronal and connective organization and electrophysiological properties (Gähwiler et al., 1984; Zimmer and Gähwiler, 1984; Frotscher et al., 1990; Finsen et al., 1992). Several reports have moreover already demonstrated the feasibility of using organotypic brain slice cultures in experimental models of gluta-

mate receptor mediated neurotoxicity (Jakobsen and Zimmer, 2001; Noraberg et al., 1999; Kristensen et al., 1999; Simantov et al., 1999).

Materials and methods

Drugs and chemicals

Domoic acid was obtained from Biovectra DCL Ltd (Charlottetown, Canada) and MK801 from Research Biochemicals Incorporated (Natick, USA). NS-102 was a generous gift from NeuroSearch A/S (Ballerup, Denmark) and NBQX a generous gift from NOVO Nordic A/S (Copenhagen, Denmark).

Slice cultures

Slice cultures were prepared and grown by the interface method (Stoppini et al., 1991). In brief, 7-day-old Wistar rats (Moellegaard, Denmark) were killed by instant decapitation and their brains removed under aseptic conditions. After isolation, transverse sections of the hippocampus were cut at 350 μm by a McIlwain tissue chopper, transferred to Geys balanced salt solution (GIBCO-BRL, Life Technologies, Denmark) for separation and then placed, as groups of six equally spaced slices, on semiporous Millipore membranes in plastic inserts (Millicell-CM 0.4 μm, 30 mm diameter, Millipore Corporation Bedford, USA), which after placement of the slices were transferred to 6-well culture trays with 1 ml of growth medium in each well. The medium consisted of 25 ml Hanks BSS, 50 ml OPTI-MEM and 25 ml horse serum (all GIBCO-BRL, Life Technologies, Denmark), supplemented by 1 ml 50% D (+) glucose monohydrate (Merck, Germany). The culture trays were placed at 36°C in an incubator with 5% CO_2 and 100% humidity in atmospheric air. After 3 days, the culture medium was replaced with 1 ml serum-free, chemical defined Neurobasal medium, with addition of 2 ml B27 supplement (both GIBCO-BRL, Life Technologies, Denmark) and 500 μl L-glutamine (Sigma-Aldrich, Denmark) per 98 ml Neurobasal medium. For the next 2–3 weeks, the medium was changed twice a week together with regular microscopically inspection of the cultures. Only cultures with intact and well-defined hippocampal neuronal layers were subsequently analyzed.

Propidium iodide uptake

Cellular uptake of the fluorescent dye propidium iodide (PI) (Sigma-Aldrich, Denmark) was used as a quantifiable marker for neuronal degeneration [11]. For PI uptake in 2–3 week old DOM exposed and control hippocampal slice cultures, PI was added to the culture medium to give a final concentration of 2 μM for at least 3 hrs before the basic PI uptake (d0) was recorded by fluorescence microscopy (Olympus IMT-2, 4 X), using standard rhodamine filters and a digital camera (SenSys, Photometrics, USA), connected to a PowerMac with IPLab Spectrum image processing software (Signal Analytics Corporation, USA). The time course and dose response of DOM was then recorded in terms of PI uptake at 24 hrs (d1) and 48 hrs (d2) during DOM exposure and during "recovery" in normal medium at 72 hrs (d3) and 96 hrs (d4) (see Fig. 1) with 2 μM PI present in the medium throughout. Using NIH 1.54 Image Analysis software the dentate granule cell layer and the pyramidal cell layers of CA1, CA3ab and CA3c were outlined and the mean gray-levels of the enclosed areas calculated. To correct for autofluorescence and any spontaneous neurodegeneration in the cultures, the values recorded at d0 were subtracted from the measurements at d1–d4.

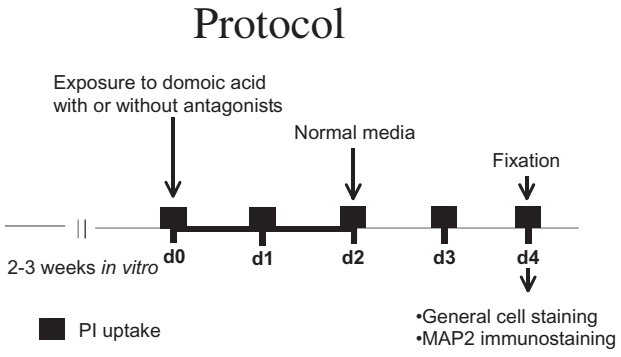

Fig. 1. Experimental protocol for DOM exposure to hippocampal slice cultures. DOM (0.1 μM–100 μM) was applied in the culture medium either alone or in combinations with 10 μM NBQX, 10 μM NS102 and 10 μM MK801 for 48 hrs followed by 48 hrs recovery. Cultures were thereafter processed histologically and stained immunocytochemical for MAP2 or general cell staining with recording of propidium iodide (*PI*) uptake just before and after 24 (*d1*) and 48 hrs (*d2*) of DOM exposure, followed by recordings in the recovery period at 72 hrs (*d3*) and 96 hrs (*d4*)

Estimation of EC50 values. To determine the EC_{50} value of domoic acid for the different hippocampal subregions by PI uptake cultures were exposed to 0.1–100 μM DOM for 48 hrs followed by normal medium for 48 hrs of "recovery". After the last recordings of PI uptake (d4, see above), the cultures were either fixed in phosphate buffered 4% paraformaldehyde (PFA) (pH 7.4) for 30 min, and stained with toluidine blue for general cell staining, or transferred to 20% sucrose for 72 hrs, and frozen and cryostat sectioned at 20 μm for subsequent immunostaining for microtubule associated protein 2 (MAP2) (see below).

Co-exposure to glutamate receptor antagonists. Using the same protocol for PI uptake as above, cultures were exposed to 3 μM or 10 μM DOM in combination with the AMPA/KA antagonists NS102 (10 μM) and/or NBQX (10 μM) and/or the NMDA antagonist MK801 (10 μM) in order to test for the involvement of KA, AMPA and NMDA receptors in DOM toxicity. The two doses of DOM were chosen, because 3 μM DOM was found to be the lowest concentration to induce toxicity, and 10 μM was close to the EC_{50} values of the different subfields at day 3.

Immunohistochemical staining for microtubule associated protein 2 (MAP2)

Cultures exposed to 0.1–100 μM DOM were processed and stained immunocytochemical for MAP2 by immersion fixation in 4% PFA for 0.5 hrs, transfer to 20% sucrose solution for cryoprotection for 72 hrs, embedding in Cryo-embed (AX-LAB, Denmark) and frozen, before cryostat sectioning at 20 μm and mounting on glass slides. The sections were stored at −20°C, before immunohistochemical staining for microtubule associated protein 2 (MAP). The sections were incubated in 10% fetal bovine serum for 30 min, incubated by an antibody directed against MAP2 (Sigma-Aldrich, Denmark, 1 : 1000) overnight at 4°C, washed three times in Tris buffered saline (TBS) +1% Triton, followed by incubation with biotinylated anti-mouse IgG (Sigma-Aldrich, Denmark, 1 : 200) for 1 hr and streptavidin-horseradish peroxidase (Sigma-Aldrich, Denmark, 1 : 70) for 1 hr, again washed three times, and stained with 0.2% 3,3′-diaminobenzidine (Sigma-Aldrich, Denmark) for 5 min. After washing, the sections were dehydrated in ethanol,

cleared in xylene and coverslipped in Dammar-Xylol (Chroma-Gesellschaft, Germany).

Results

Toxicity of domoic acid

Densitometric measurements of PI uptake after 24 hrs (d1), 48 hrs (d2) exposure to 0.1–100 μM DOM, and subsequent 24 hrs (d3) and 48 hrs (d4) "recovery" in normal medium (n = 12–30) were used to determine the EC_{50} value in the different subfields of hippocampus (Fig. 2). For each subfield the EC_{50} value was calculated based on the sigmoid curve fit for PI uptake. After 24 hrs of exposure (d1) the EC_{50} values were of in the 20–21 μM range for all subregions: CA1 (19.3 μM) $\geq$ FD (20.2 μM) $\geq$ CA3c (21.2 μM) $\geq$ CA3ab (21.9 μM), After 48 hrs of exposure (d2) the EC_{50} values were in the 11–12 μM range: CA1 (10.8 μM) $\geq$ CA3ab (11.8 μM) $\geq$ CA3c (12.0 μM) $\geq$ FD (12.1 μM). The lowest EC_{50} values were obtained for all the subregions at d3, with CA1 as the most susceptible subfield: CA1 (6.3 μM) $>$ FD (9.6 μM) $\geq$ CA3c (9.9 μM) $\geq$ CA3ab (10.6 μM) (Fig. 2). Photomicrographs of the PI uptake at d3 in control cultures and cultures exposed to 3 and 10 μM DOM, demonstrate the increased PI uptake and sensitivity of the CA1 region at this time point (Fig. 3). Following exposure to 3 μM DOM the PI uptake was primarily increased in CA1, whereas after 10 μM DOM all neuronal cell layers show increased uptake. At d4 the EC_{50} values increased again (probably due to loss of PI stained cells from d3 to d4), with CA1 still being the most susceptible subfield. At this time, the differences between individual subfields had increased: CA1 (9.4 μM) $>$ CA3ab (11.4 μM) $>$ CA3c (16.5 μM) $>$ FD (24.6 μM) (Fig. 2).

Nissl staining performed on cultures at d4, after 48 hrs of DOM exposure and 48 hrs of recovery,

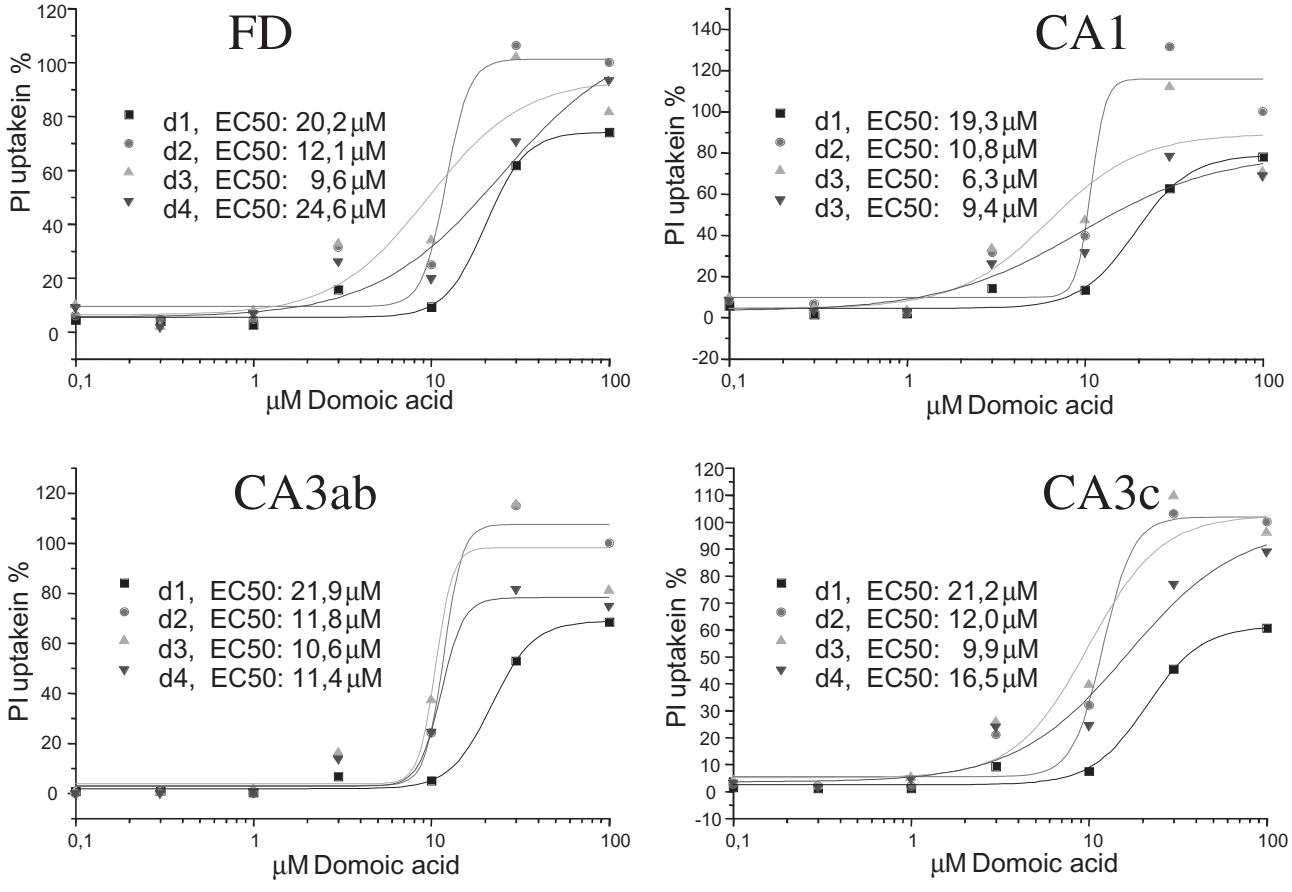

Fig. 2. Dose-response relationships between increasing concentrations of DOM and PI uptake in the different hippocampal subfields at day 1 (*d1*), 2 (*d2*), 3 (*d3*) and 4 (*d4*). *FD*, Dentate granule cells; *CA1*, CA1 pyramidal cells; *CA3ab* and *CA3c*, CA3ab and CA3c pyramidal cells respectively. The maximal plateau levels of PI uptake at day 2 were set to 100%

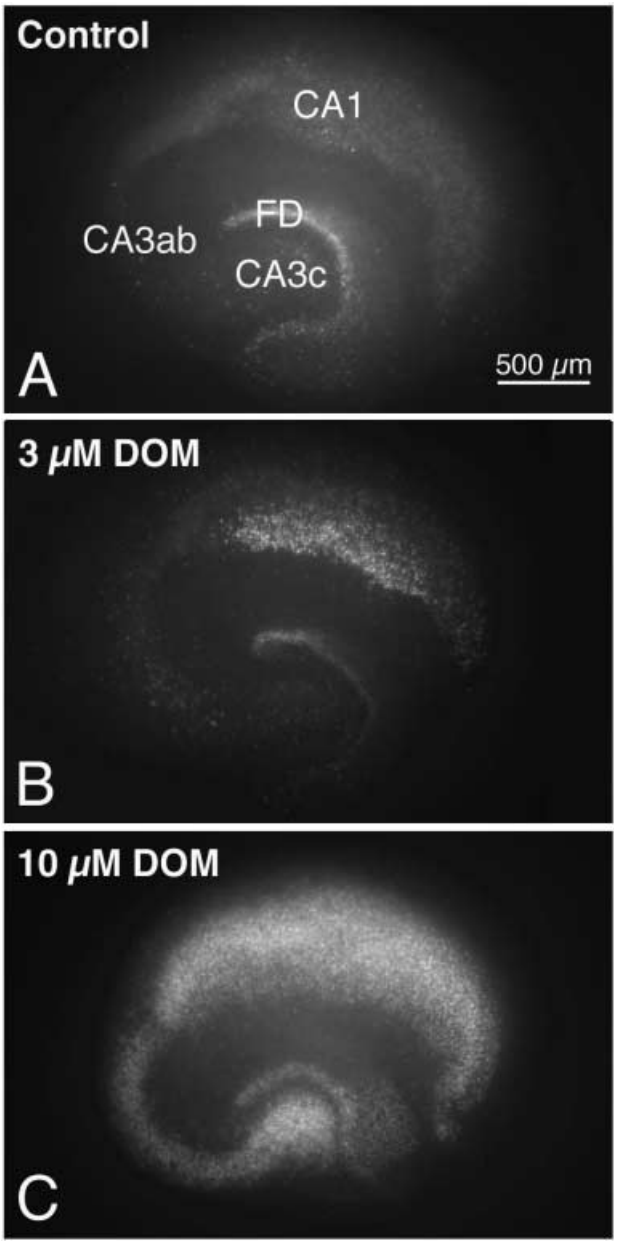

Fig. 3. Digital fluorescence micrographs of hippocampal slice cultures showing propidium iodide (PI) uptake at time-point d3 (see Fig. 1). – **A** Control culture; – **B** Culture exposed to 3 μM DOM; – **C** Culture exposed to 10 μM DOM. Note increased PI uptake in the CA1 subfield after 3 μM DOM

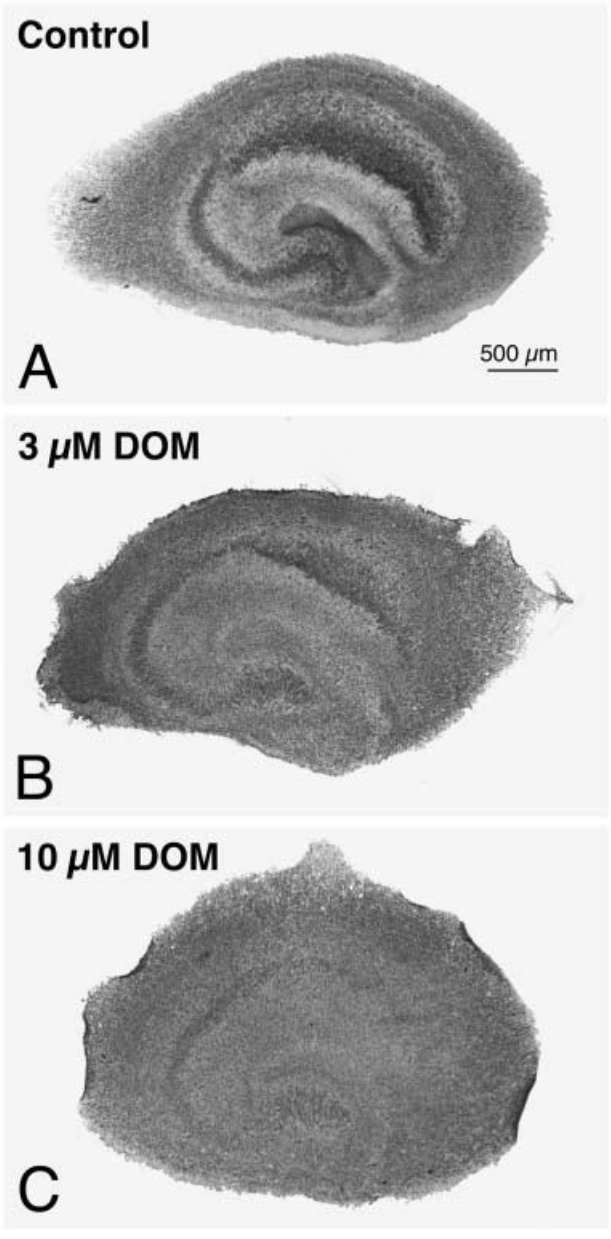

Fig. 4. Digital micrographs of Nissl stained hippocampal slice cultures. – **A** Control culture; – **B** Culture exposed to 3 μM DOM for 48 hrs followed by 24 hrs recovery; – **C** Culture exposed to 10 μM DOM for 48 hrs followed by 48 hrs recovery

showed a clear loss of cell staining after exposure to 3 μM DOM and almost total loss of stain after exposure to 10 μM DOM (Fig. 4). The difference in cell loss between the individual hippocampal subfields was not as evident in Nissl stainings of as in PI uptake.

Effects of glutamate receptor antagonists. The effects of AMPA/KA and NMDA antagonists on DOM toxicity as expressed by PI uptake in the different subregions of hippocampus is shown in Fig. 5 (n = 9–23). In the *dentate granule cells* of fascia dentata (Panel A) the PI uptake mediated by 3 μM DOM was reduced by co-incubation with 10 μM NBQX, while 10 μM of the NMDA antagonist MK801 increased the DOM toxicity. The toxicity of 10 μM DOM on the dentate granule cells was only significantly reduced

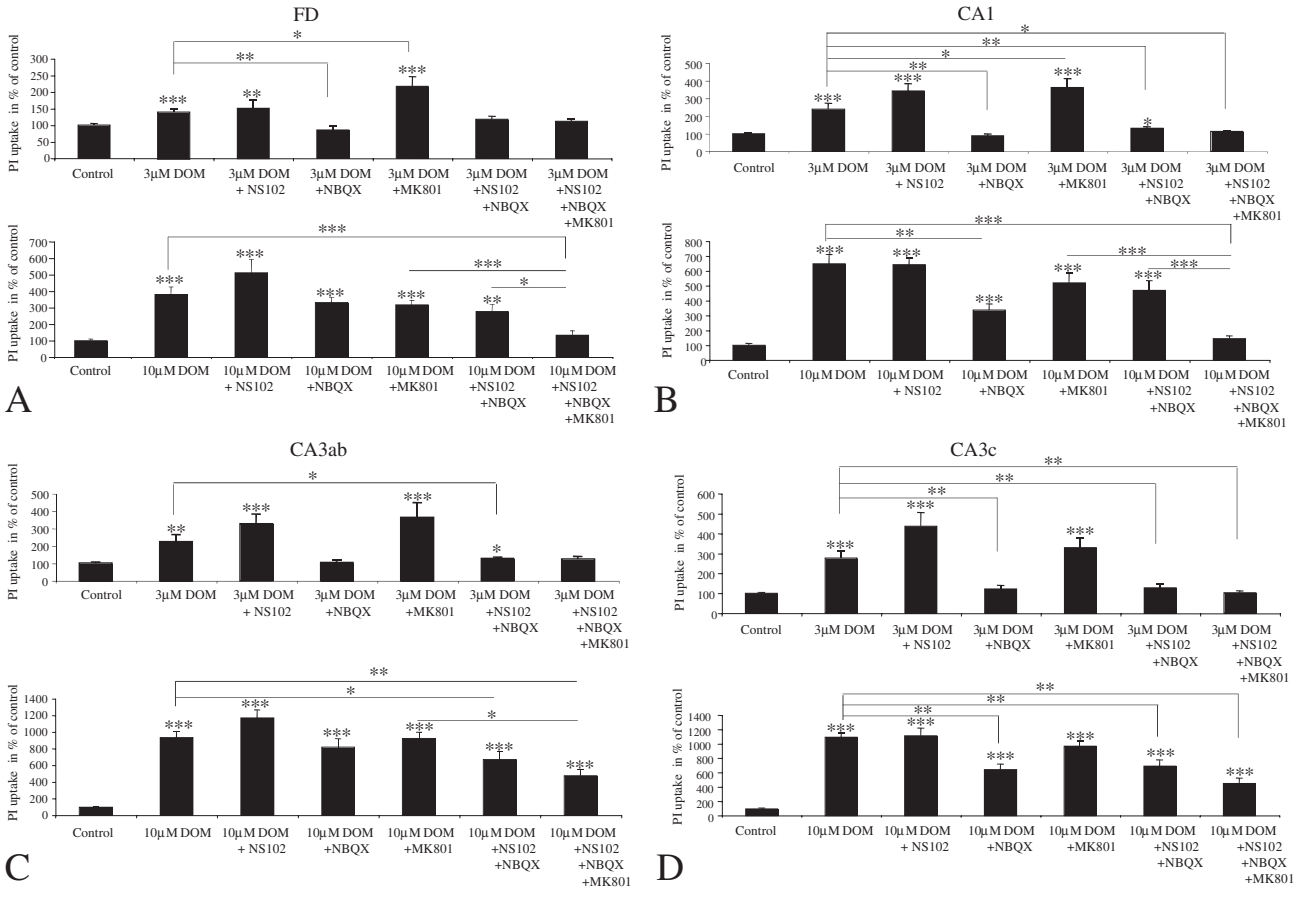

Fig. 5. Densitometric measurements of propidium iodide uptake at time-point d3 in the dentate granule cells in fascia dentate (FD) (**A**) and the hippocampal CA1 (**B**), CA3ab (**C**) and CA3c pyramidal cell layers (**D**). Measurements were performed on: Untreated cultures (Control), cultures exposed to 3 or 10 μM DOM alone (3 μM DOM or 10 μM DOM), and 3 or 10 μM DOM in combination with 10 μM NS102 (3 or 10 μM DOM + NS102), 10 μM NBQX (3 or 10 μM DOM + NBQX), 10 μM MK801 (3 or 10 μM DOM + MK801) or in combination with 10 μM NS102 plus 10 μM NBQX (3 or 10 μM DOM + NS102 + NBQX) or 10 μM NS102 plus 10 μM NBQX plus 10 μM MK801 (3 or 10 μM DOM + NS102 + NBQX + MK801). The measurements of PI uptake are presented as the percentage of the mean PI uptake in untreated control cultures (100%) ± S.E.M. Statistical significance was assessed using 2-tailed Student's t-test. *Asterisks* above individual columns indicate significant difference from control. *Asterisks* above lines indicate significant difference between indicated groups (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$)

when 10 μM of NS102, NBQX and MK801 was applied in combination. Addition of 10 μM MK801 to the AMPA/KA antagonists (10 μM NS102 and 10 μM NBQX) resulted in a significant reduction in PI uptake compared to 10 μM NS102 plus 10 μM NBQX (Fig. 5A).

In *CA1* (Panel B) the toxic effect of both 3 and 10 μM DOM was reduced by 10 μM NBQX, as well as a cocktail of NS102, NBQX and MK801 in 10 μM doses. Addition of 10 μM MK801 alone increased the toxicity of 3 μM DOM, as seen for dentate granule cells. As for dentate granule cells further addition of 10 μM MK801 to the two AMPA/KA antagonists (10 μM NS102 and 10 μM NBQX) significantly reduced the PI uptake compared to DOM exposed cultures co-treated with the combination of 10 μM NS102 and 10 μM NBQX alone (Fig. 5B).

In *CA3* including both CA3ab and CA3c, the toxicity of 3 μM and 10 μM DOM was antagonized by a mixture of 10 μM NS102 and 10 μM NBQX (Figs. 5C and 5D). In the case of 10 μM DOM a combination 10 μM NS102 + 10 μM NBQX +10 μM MK801 also reduced the PI uptake compared to cultures exposed to DOM alone. The addition of MK801 did, however, not enhance the protective effect of the AMPA/KA antagonists, as it was seen in FD and CA1.

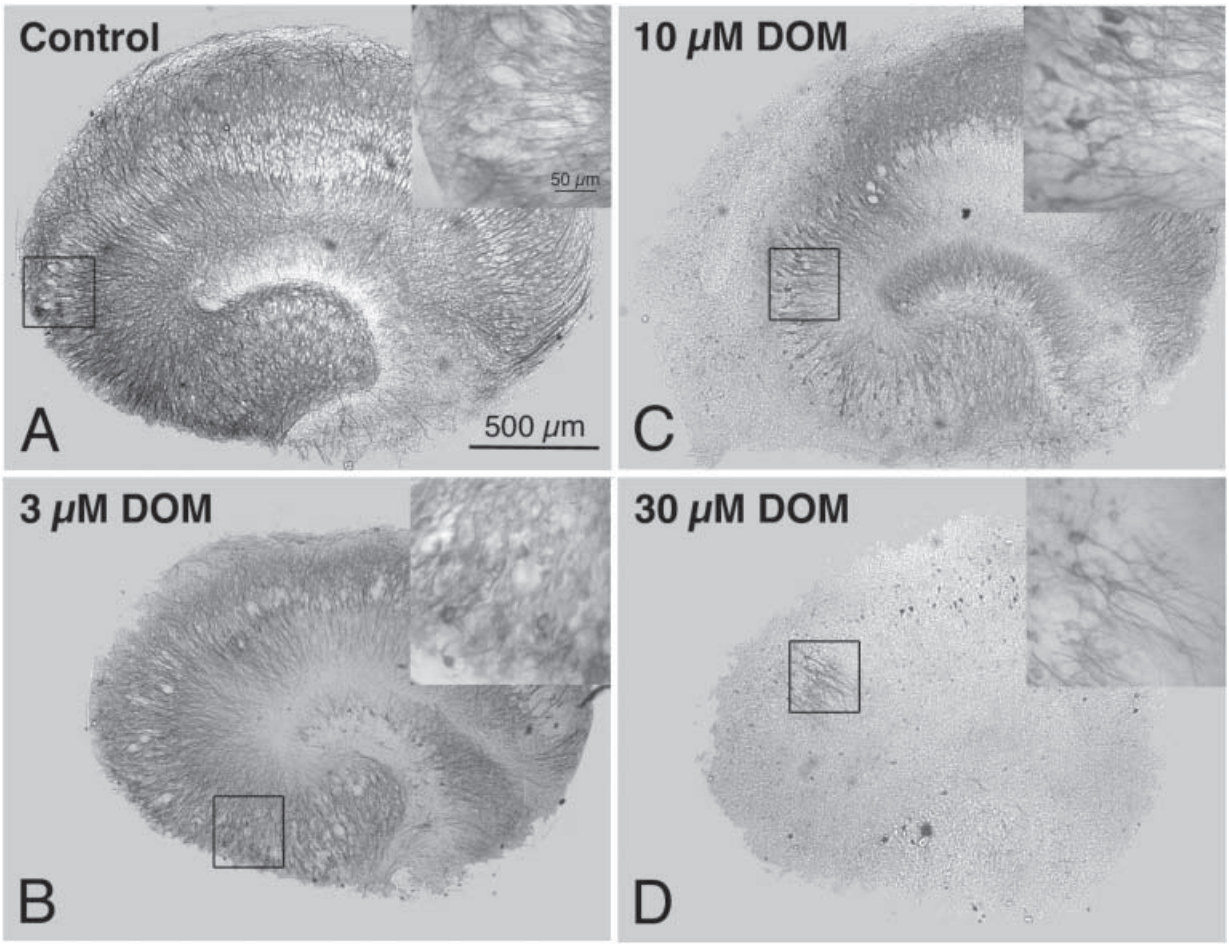

Fig. 6. Digital photomicrographs of MAP2 immunostaining of sections from control cultures (**A**) and cultures exposed to 3 μM (**B**), 10 μM (**C**) and 30 μM DOM (**D**) The *inserts* show higher magnification of *framed areas*. In control cultures (**A**) dendritic fields show denser staining than cell body layers. After 3 and 10 μM DOM treatment (**B** and **C**), immunoreactive staining appears in the cell bodies. Loss of immunoreactiviy in the dendrites appears at 10 μM DOM (**C**). In culture exposed to 30 μM DOM (D), there was a complete loss of MAP2 immunoreactivity, except for some immunoreactivity in the CA2 subfield

MAP2 staining

The microtubule-associated protein MAP2 is a cytoskeletal protein primarily localized to dendrites, and a marker of the structural integrity of the neurons. Control cultures displayed intense MAP2 staining in dendrites with a weaker staining of the cell bodies (Fig. 6A). After 48 hrs exposure to 3 μM DOM and 48 hrs "recovery", MAP2 staining had increased in the neuronal cell bodies, but appeared unchanged in the dendrites (Fig. 6B). Exposure to 10 μM DOM further increased the cell body staining, at the same time as a loss of MAP2 immunoreactivity had appeared in the dendrites (Fig. 6C). Administration of 30 μM DOM resulted in a total loss of MAP2 immunoreactivity in all subfields, except in some cultures with persistence of some immunoreactivity in the *CA2* subfield. (Fig. 6D) (n = 12–18).

Discussion

Our results unequivocally demonstrate that DOM is toxic to hippocampal neurons grown in slice culture with CA1 pyramidal cell layer being the most susceptible, displaying the lowest EC_{50} values. Although all previous studies of DOM toxicity also have shown loss of CA1 neurons, most studies in vivo have demonstrated greater toxicity in CA3 than in CA1 (Tryphonas et al., 1990; Strain and Tasker, 1991). This has been attributed to the higher expression of kainate receptors in the CA3 subfield (Wisden and Seeburg,

1993). Kainate receptors are also present in hippocampal slice cultures with highest density in the CA3 region, according to both H^3-Kainate bindings studies and *in situ* hybridization studies of the kainate receptor subunits GluR6 (unpublished results) and KA1 and KA2 (Gerfin-Moser et al., 1995). These observations, combined with the present finding that the kainate antagonist NS102 did not protect against DOM toxicity when applied alone (see Fig. 5), imply that DOM toxicity in hippocampal slice cultures primarily is mediated by AMPA rather than kainate receptors. This conclusion is supported by our finding that the toxicity of 3 μM DOM could be completely antagonized by 10 μM of the AMPA receptor antagonist NBQX (Fig. 5). At the higher dose of DOM (10 μM) a more complex picture emerged. Except for NBQX alone in CAI pyramidal cells, of both in dentate granule cells and CA1 pyramidal cells the AMPA/KA antagonists NS102 and NBQX, either each of them alone or in combination, was unable to significantly antagonize DOM toxicity (Fig. 5). Similarly, the NMDA antagonist MK801, did not produce any significant reduction in the toxicity of 10 μM DOM. When all three antagonists were applied together they did, however, totally block DOM toxicity. This observation not only demonstrates that NMDA receptors are involved in DOM toxicity in fascia dentata and CA1 at high concentrations, but strongly implies that high dose DOM toxicity in granule and CA1 pyramidal cells results largely from AMPA receptor-mediated glutamate release in hippocampal slice cultures. As such the data are consistent with previous reports *in vitro* (Novelli et al., 1992; Berman and Murray, 1997) and *in vivo* (Tasker and Strain, 1998) of involvement of NMDA receptors in DOM-mediated toxicity.

The total block of 10 μM DOM toxicity in fascia dentata and CA1 obtained by adding 10 μM MK801 to 10 μM of NS102 and NBQX was not observed in CA3. Here inclusion of MK801 did not reduce the PI uptake any further than the mixture of 10 μM S102 and 10 μM NBQX (Fig. 5). This argues against an involvement of NMDA receptors in high dose DOM-mediated toxicity in CA3, and provide the first evidence of differential mechanisms of DOM toxicity in different hippocampal subfields.

We conclude that DOM is toxic to CA1 and CA3 pyramidal cells and dentate granule cells in hippocampal slice cultures, and that the toxicity appears to be mediated primarily by AMPA receptors, either alone (CA3) or in combination with NMDA receptors (fascia dentata and CA1). The findings underscore the potential use of hippocampal slice cultures in mechanistic studies of excitotoxicity.

Acknowledgements

The support from the Danish State Biotechnology Program (Neuroscience Center, PharmaBiotec II and III) and the Danish MRC is gratefully acknowledged. The excellent technical help of Inge Nielsen, Randi Godskesen, Dorte Lyholmer and Dorte Frederiksen is also gratefully acknowledged.

References

Berman FW, Murray TF (1997) Domoic acid neurotoxicity in cultured cerebellar granule neurons is mediated predominantly by NMDA receptors that are activated as a consequence of excitatory amino acid release. J Neurochem 69 2: 693–703

Bettler B et al (1992) Cloning of a putative glutamate receptor: a low affinity kainate-binding subunit. Neuron 8 2: 257–265

Finsen BR et al (1992) Somatostatin and neuropeptide Y in organotypic slice cultures of the rat hippocampus: an immunocytochemical and in situ hybridization study. Neuroscience 47/1: 105–113

Frotscher M, Heimrich B, Schwegler H (1990) Plasticity of identified neurons in slice cultures of hippocampus: a combined Golgi/electron microscopic and immunocytochemical study. Prog Brain Res 83: 323–339

Gähwiler BH (1984) Development of the hippocampus in vitro: cell types, synapses and receptors. Neuroscience 11 4: 751–760

Gerfin-Moser A et al (1995) Alterations in glutamate but not GABAA receptor subunit expression as a consequence of epileptiform activity in vitro. Neuroscience 67 4: 849–865

Jakobsen B, Zimmer J (2001) Modulation of AMPA excitotoxicity in hippocampal slice cultures. NeuroReport 12 16: 3593–7

Johansen TH et al (1993) A novel non-NMDA receptor antagonist shows selective displacement of low-affinity [3H]kainate binding. Eur J Pharmacol 246 3: 195–204

Kristensen BW et al (1999) Excitotoxic effects of non-NMDA receptor agonists in organotypic corticostriatal slice cultures. Brain Res 841/1–2: 143–159

Noraberg J, Kristensen BW, Zimmer J (1999) Markers for neuronal degeneration in organotypic slice cultures. Brain Res Protoc 3/3: 278–290

Novelli A et al (1992) Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. Brain Res 577/1: 41–48

Perl TM et al (1990) An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. N Engl J Med 322/25: 1775–1780

Simantov R et al (1999) Antisense knockdown of glutamate transporters alters the subfield selectivity of kainate-induced cell death in rat hippocampal slice cultures. J Neurochem 73/5: 1828–1835

Stoppini L, Buchs PA, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37/2: 173–182

Strain SM, Tasker RA (1991) Hippocampal damage produced by systemic injections of domoic acid in mice. Neuroscience 44/2: 343–352

Tasker RA, Strain SM, Drejer J (1996) Selective reduction in domoic acid toxicity in vivo by a novel non-N-methyl-D-aspartate receptor antagonist. Can J Physiol Pharmacol 74/9: 1047–1054

Tasker RA, Strain SM (1998) Synergism between NMDA and domoic acid in a murine model of behavioural neurotoxicity. Neurotoxicology 19/4–5: 593–597

Teitelbaum J et al (1990) Neurological sequelae of domoic acid intoxication. Can Dis Wkly Rep 16 [Suppl 1E]: 9–12

Tryphonas L et al (1990) Acute neurotoxicity of domoic acid in the rat. Toxicol Pathol 18/1(Pt 1): 1–9

Wisden W, Seeburg PH (1993) A complex mosaic of high-affinity kainate receptors in rat brain. J Neurosci 13/8: 3582–3598

Zimmer J, Gähwiler BH (1984) Cellular and connective organization of slice cultures of the rat hippocampus and fascia dentata. J Comp Neurol 228/3: 432–446

Authors' address: Dr. Birthe Jakobsen, Anatomy & Neurobiology, University of Southern Denmark, Winslowparken 21, DK-5000 Odense C, Denmark, Fax: +45 6590 6321,
E-mail: B.Jakobsen@imbmed.sdu.dk