Journal of Neurochemistry, 2001, **76**, 47–55

Potentiation by histamine of synaptically mediated excitotoxicity in cultured hippocampal neurones: a possible role for mast cells

Stephen D. Skaper, Laura Facci, Wai Jing Kee and Paul J. L. M. Strijbos

Department of Neuroscience Research, SmithKline Beecham Pharmaceuticals, Harlow, UK

Abstract

Excessive glutamatergic neurotransmission, particularly when mediated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, is thought to underlie neuronal death in a number of neurological disorders. Histamine has been reported to potentiate NMDA receptor-mediated events under a variety of conditions. In the present study we have utilized primary hippocampal neurone cultures to investigate the effect of mast cell-derived, as well as exogenously applied, histamine on neurotoxicity evoked by excessive synaptic activity. Exposure of mature cultures for 15 min to an Mg^{2+}-free/glycine-containing buffer to trigger synaptic transmission through NMDA receptors, caused a 30–35% neuronal loss over 24 h. When co-cultured with hippocampal neurones, activated mast cells increased excitotoxic injury to 60%, an effect that was abolished in the presence of histaminase. Similarly, addition of histamine during magnesium deprivation produced a concentration-dependent potentiation (+60%; EC_{50} : 5 μM) of neuronal death which was inhibited by sodium channel blockers and NMDA receptor antagonists, although this effect did not involve known histamine receptors. The histamine effect was further potentiated by acidification of the culture medium. Cultures 'preconditioned' by sublethal (5 min) Mg^{2+} deprivation exhibited less neuronal death than controls when exposed to a more severe insult. NMDA receptor activation and the extracellular regulated kinase cascade were required for preconditioning neuroprotection. The finding that histamine potentiates NMDA receptor-mediated excitotoxicity may have important implications for our understanding of conditions where enhanced glutamatergic neurotransmission is observed in conjunction with tissue acidification, such as cerebral ischaemia and epilepsy.

Keywords: excitotoxicity, extracellular regulated kinase, histamine, mast cells, preconditioning, synaptic transmission.
J. Neurochem. (2001) **76**, 47–55.

Glutamate neurotoxicity, particularly when mediated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors, has been hypothesized to underlie several types of acute brain injury, including cerebral ischaemia, and mechanical brain trauma (Rothman and Olney 1987; Choi 1988). Although the precise cellular mechanisms that lead to neurotoxicity under these conditions remain unclear, evidence suggests that excessive synaptic activity may be involved. Prolonged epileptiform activity is associated with neurotoxicity, and disruption of glutamatergic afferents to the affected region, or application of NMDA receptor antagonists reduces seizure-induced neuronal loss (Clifford *et al*. 1989; Meldrum 1990). *In vitro*, removal of glutamate receptor blockade or elimination of Mg^{2+} from the extracellular medium, in order to alleviate Mg^{2+}-blocking of the NMDA receptor, induces excessive synaptic transmission (Mody *et al*. 1987) and NMDA receptor-mediated neuronal degeneration in hippocampal and cortical cell cultures (Furshpan and Potter 1989; Abele *et al*. 1990; Rose *et al*. 1990; Skaper *et al*. 1991, 1998).

Axonal projections from histaminergic neurones in the anterior hypothalamus widely innervate the CNS, including cortical and hippocampal areas that are rich in the NMDA subtype of glutamate receptors (Schwartz *et al*. 1991). At autaptic synapses of cultured hippocampal neurones, histamine selectively increases the NMDA-mediated

Received April 3, 2000; revised manuscript received June 22, 2000; accepted August 11, 2000.

Address correspondence and reprint requests to Dr S. D. Skaper, Department of Neuroscience Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AW, UK.
E-mail: Stephen_Skaper-1@sbphrd.com

Abbreviations used: CPP, 3-[(R,S)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid; DIC, days in culture; DNQX, 6,7-dinitroquinoxaline-2,3-dione; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMDA, N-methyl-D-aspartate; TTX, tetrodotoxin.

component of excitatory synaptic currents (Bekkers 1993; Vorobjev *et al*. 1993), an effect that appears specific for receptors containing the NMDAR1/NMDAR2 receptor subunits (Williams 1994). Furthermore, histamine increases the magnitude of NMDA-induced whole cell currents in isolated hippocampal neurones (Vorobjev *et al*. 1993), and enhances the peak amplitude and slows the onset of desensitization of NMDA-induced ion currents in cultured cortical neurones (Zwart *et al*. 1996). This effect of histamine appears not to be mediated by classical histamine receptors, suggesting an involvement of atypical histamine receptors or a direct effect of histamine on the NMDA receptor itself (Vorobjev *et al*. 1993). Nevertheless, histamine could play a role in modulating the function of NMDA receptors *in vivo*. Indeed, histamine facilitates the induction of long-term potentiation and can cause a long-lasting enhancement of hippocampal population spikes reminiscent of long-term potentiation (Kostopoulos *et al*. 1988; Brown *et al*. 1994).

It seems plausible that histamine modulation of NMDA receptor-related events may also occur under conditions of neuropathology. Histamine release within the thalamus is thought to contribute to NMDA-mediated excitotoxicity in a thiamine deficiency-induced model of Wernicke's encephalopathy (Langlais and Mair 1990; Langlais *et al*. 1994). Interestingly, the thalamus is enriched in mast cells. As mast cells are a rich source of histamine, these cells may represent a major non-neuronal source of this biogenic amine in the brain (Goldschmidt *et al*. 1984, 1985; Bienenstock *et al*. 1987; Purcell and Atterwill 1995; Silver *et al*. 1996). This raises the question as to whether mast cells can contribute to histamine modulation of neurotransmission and, in the context of the current work, whether mast cells could mediate the potential deleterious actions of histamine on NMDA receptor-dependent excitotoxicity. Using cultured hippocampal neurones, we now show that histamine, including that released from activated mast cells, is capable of directly increasing neuronal death under conditions of enhanced synaptic neurotransmission. We report that hyperexcitability induced by removal of Mg^{2+} from the extracellular medium triggers a histamine-sensitive form of neuronal death that is dependent on NMDA receptors and sodium channel activation, and is sensitive to changes in pH. As localized tissue acidification occurs during epilepsy and cerebral ischaemia, histamine could aggravate excitotoxicity under these conditions.

Materials and methods

Cell culture

Hippocampi were isolated from embryonic Sprague–Dawley rats (gestational age 17.5 days; Charles River, Margate, Kent, UK), incubated with 0.08% (w/v) trypsin, and dissociated in Neurobasal medium containing 10% heat-inactivated fetal calf serum (Skaper *et al*. 1990). Cells were pelleted by centrifugation (200 g, 5 min) and resuspended in Neurobasal medium containing B27 supplements (with antioxidants), 25 μM glutamate, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin. The cell suspension was plated onto poly-D-lysine (10 μg/mL) coated 48-well culture plates (Nunc, Roskilde, Denmark), at a density of 4.5×10^4 cells per cm^2. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2−95% air. After 5 days, one-half of the medium was replaced with an equal volume of maintenance medium (plating medium but containing B27 supplements without antioxidants, and lacking glutamate). Additional medium exchanges (0.5 volume) were performed every 3–4 days thereafter. Cells were used between 14 and 16 days in culture (DIC). During this period, neurones developed extensive neuritic networks, and formed functional synapses (data not shown).

Mast cells were collected from the peritoneal lavage of male Sprague–Dawley rats (250–300 g; Charles River) and were isolated over a bovine serum albumin gradient to $>90\%$ purity, as judged by toluidine blue and safranin staining (Mousli *et al*. 1989; Facci *et al*. 1995).

Neurotoxicity assays

Cultures were washed once with Locke's solution (pH 7.0–7.4) (Skaper *et al*. 1990) with or without 1 mM $MgCl_2$. The Mg^{2+}-free buffer contained 0.1 μM glycine. Drug treatments were carried out for 15–30 min (25°C) in a final volume of 0.5 mL. In the case of mast cell-neurone co-cultures, transwell inserts (3-μm pore size, 9-mm diameter; Nunc) were seeded with 5×10^4 mast cells in RPMI-1640 medium and placed in 24-well plates overnight. Inserts with mast cells were then placed into wells with hippocampal cells. Inserts contained 0.2 mL Locke's solution (± $MgCl_2$). Mast cell activation was achieved using an antigenic stimulus (0.3 μg/mL anti-DNP IgE/0.1 μg/mL DNP-albumin) (Facci *et al*. 1995). The mast cell-containing inserts were removed at the end of the Mg^{2+}-free incubation. After this time all cell monolayers were washed with complete Locke's solution and returned to their original culture medium for 24 h. Cytotoxicity was evident during the 24 h after the insult. Viable neurones had phase-bright somata of round-to-oval shape, with smooth, intact neurites. Neurones were considered nonviable when they exhibited neurite fragmentation and somatic swelling and vacuolation. Cell survival was quantified 24 h after the insult by a colorimetric reaction with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Mosmann 1983; Manthorpe *et al*. 1986; Skaper *et al*. 1990). Absolute MTT values obtained were normalized and expressed as a percentage of sham-treated sister cultures (defined as 100%). Control experiments showed that the loss of viable neurones assessed in this manner was proportional to the number of neurones damaged, as estimated by trypan blue staining.

Mitogen-activated protein (MAP) kinase phosphorylation

Hippocampal neurones (4×10^5 per well, six-well plates) at 15 DIC were incubated for 5 min in Mg^{2+}-free Locke's solution (pH 7.0) without or with 0.1 μM glycine and 30 μM histamine (37°C). Cell monolayers were then washed with ice-cold phosphate-buffered saline (pH 7.4) and immediately lysed [0.1% Triton

X-100, 20 mM HEPES, 50 mM NaF, 25 mM NaCl, 2 mM EDTA, 20 mM β-glycerophosphate, 0.5 mM dithiothreitol and 1 mM sodium orthovanadate, pH 8.0, and complete inhibitor cocktail (Boehringer Mannheim, Lewes, UK)]. Cell lysates (10 μg) were resolved by electrophoresis on 10% polyacrylamide minigels and transferred to polyvinylidene difluoride membranes. Blots were probed with a antiphospho p44/42 polyclonal antibody. Bound antibody was detected using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, UK). The blots were then stripped with 100 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate and 62.5 mM Tris-HCl, pH 6.7. Stripping efficacy was assessed by incubation with secondary antibody and verified by a lack of signal from the stripped blot. The blots were subsequently reprobed with anti-p44/42 antibody.

Statistics

Data were analysed by one-way ANOVA with Student–Newman–Keuls *post hoc* test for differences between groups.

Materials

Neurobasal medium, B27 supplements and fetal calf serum were purchased from Life Technologies (Paisley, UK); all other tissue culture reagents, tetrodotoxin (TTX), monoclonal anti-DNP IgE (clone SPE-7), DNP-human serum albumin (30–40 mol of DNP/mol albumin) and MTT were from Sigma (Dorset, UK); 3-((*R,S*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 6,7-dinitroquinoxaline-2,3-dione (DNQX), riluzole, MK-801 and histamine receptor antagonists and agonists were from Tocris Cookson Ltd (Bristol, UK). MAP kinase antibodies were from Promega (Southampton, UK); polyacrylamide gels were from BioRad (Hemel Hempstead, UK).

Results

Pharmacology of hippocampal neurone injury triggered by removal of extracellular Mg^{2+}

Exposure of cultured hippocampal neurones to an Mg^{2+}-free, glycine-containing buffer (pH 7.4) for 15–30 min produced neuronal death 24 h later (Fig. 1). Immediately following the insult period, approximately 30% of neurones developed a darkened and swollen cell body. This form of injury appeared to involve secondary release of glutamate acting on NMDA receptors as the NMDA antagonists CPP and MK-801 abolished cell death, whereas the kainate/AMPA receptor antagonist DNQX was without effect (Fig. 1). Furthermore, blockade of voltage-gated Na^+ channels by TTX or riluzole (Song *et al*. 1997) also confered neuroprotection (data not shown; Fig. 1).

Mast cell-derived histamine potentiates synaptically mediated excitotoxicity

Mast cells occur in many organs and tissues, including the CNS of several species, including humans (Dropp 1976; Theoharides 1990). When mast cells are activated, they undergo compound exocytosis, releasing histamine and other secretagogues (Kaminer *et al*. 1995). To determine whether mast cell-released histamine could contribute to the excitotoxic pathway in the present model, hippocampal neurones were incubated with peritoneal mast cells, previously cultured on a tissue culture insert. Mast cells were activated with antigen to crosslink high-affinity IgE receptors (Galli 1993) during a 30-min exposure period to Mg^{2+}-free conditions. Subsequently, the inserts were then removed, and neuronal survival assessed 24 h later. The presence of activated mast cells increased excitotoxic cell death by a further 30% (Table 1). Importantly, enzymatic digestion of medium histamine by diamine oxidase (histaminase) eliminated the potentiating effect of activated

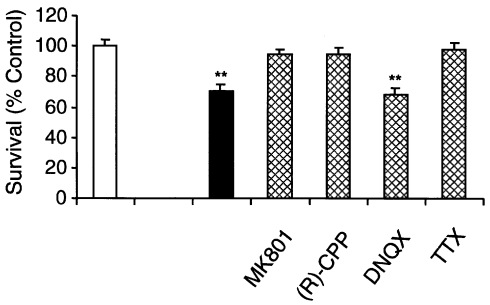

Fig. 1 Exposure of hippocampal cells to a Mg^{2+}-free environment produces neuronal cell death 24 h later: pharmacological characterization. Hippocampal neurones (14–16 DIC) were incubated 15 min (25°C) in Mg^{2+}-free/glycine-containing Locke's solution (pH 7.4) (■) with MK-801, 1–10 μM; (*R*)-CPP, 10 μM; DNQX, 10 μM; TTX, 1 μM (▨, drug). Neuronal survival was quantified 24 h later by MTT assay. Values are means ± SD (three experiments). $^{**}p < 0.01$ vs. control (+ Mg^{2+}) (□).

Table 1 Mast cell-released histamine potentiates synaptically mediated excitotoxicity in hippocampal neurones

Culture conditions	% Neuronal survival
Control (+ Mg^{2+})	100
Mg^{2+}-free + glycine	
Alone	68 ± 6
Histamine (30 μM)	37 ± 2[a]
Mast cells	74 ± 3
Mast cells + antigen	42 ± 3[a]
Mast cells + antigen + histaminase	65 ± 4

Hippocampal neurones (14 DIC) were incubated 30 min (25°C) in Mg^{2+}-free Locke's solution (pH 7.0), supplemented with: glycine (0.1 μM) + histamine (30 μM); or with inserts containing peritoneal mast cells (5×10^4) stimulated with antigen. Histaminase was added to 20 μg/mL. Neuronal survival was quantified 24 h later by MTT assay. Values are means ± SD (three experiments). Survival is expressed relative to Mg^{2+}-containing buffer (100%). [a]$p < 0.001$ versus Mg^{2+}-free plus glycine alone.

mast cells on excitotoxicity (Table 1). In this co-culture system, the histamine content of the culture medium increased to 10 μM in the presence of activated mast cells (data not shown).

Exogenous histamine potentiates injury induced by NMDA-mediated synaptic neurotransmission

When hippocampal neurones were incubated in Mg^{2+}-free, glycine-containing Locke's solution (pH 7.4) with histamine, a significant potentiation of cell death was observed. Neuronal death increased from 30% (in the absence of histamine) to 55% (with 30 μM histamine). The histamine effect was concentration-dependent, with an EC_{50} of approximately 5 μM. A similar dose-response curve has been reported for histamine enhancement of NMDA-mediated synaptic transmission in hippocampal neurones (Bekkers 1993; Vorobjev *et al*. 1993; Yanovsky *et al*. 1995). Histamine potentiation of neuronal injury under conditions of hyperexcitability was antagonized by TTX, MK-801 and CPP, but not DNQX; additionally, histamine did not induce injury in the presence of Mg^{2+} (data not shown). The time course of cellular death was not altered by histamine. Lowering the pH of the Mg^{2+}-free buffer from pH 7.4 to pH 7.0 sensitized the neuronal cultures to the potentiating effect of histamine.

Involvement of histamine receptors or binding sites

The three known subtypes of the histamine receptor (H_1, H_2, and H_3) are all expressed in the brain (Schwartz *et al*. 1986, 1991). To investigate a role for these receptors in the potentiating effect of histamine on excitotoxicity, the effects of various histamine receptor antagonists were assessed. Addition of mepyramine or triprolidine (H_1; 10 μM, $n = 3$), cimetidine or tiotidine (H_2; 10 μM, $n = 3$) and thioperamide or clobenpropit (H_3; 10 μM, $n = 3$), applied together with histamine (30 μM), failed to affect the potentiating effect of histamine on excitotoxicity. Furthermore, the histamine agonists 2-(3-bromophenyl)-histamine (H_1; 1–30 μM, $n = 3$), dimaprit (H_2; 1–30 μM, $n = 3$), and imetit (H_3; 1–10 μM, $n = 3$) did not reproduce the histamine-evoked potentiation of excitotoxicity under Mg^{2+}-free conditions (pH 7.0).

Synaptically mediated excitotoxicity is a function of seeding density and culture age

Hippocampal cells were seeded at different densities, and assessed for their sensitivity to Mg^{2+} removal-induced toxicity after 14 days in culture. The extent of neurotoxicity was determined by the seeding density (Fig. 2). Neuronal death in the higher density cultures (23–45 $\times$ 10^3 cells/cm^2) was significantly greater than in lower density cultures (2.9–5.6 $\times$ 10^3 cells/cm^2). At the lowest plating density (2.9 $\times$ 10^3 cells/cm^2) toxicity in response to Mg^{2+} removal was no longer evident. However, hippocampal neurones at this low density were still responsive to the excitotoxic action of exogenously added glutamate (Fig. 2). Culture age was also an important determinant for neurotoxicity. Hippocampal neurones at 7 DIC, unlike those at 14 DIC, were unaffected by exposure to Mg^{2+}-free, glycine-and histamine-supplemented solution (Table 2), although these young neurones degenerated rapidly following a brief incubation with glutamate. The latter form of toxicity was NMDA receptor-mediated, as judged by the protective effects of MK-801 but, in this case, not by TTX (Table 2). Moreover, addition of histamine failed to increase neuronal sensitivity to exogenous glutamate neurotoxicity in these younger cultures (Table 3). These observations are

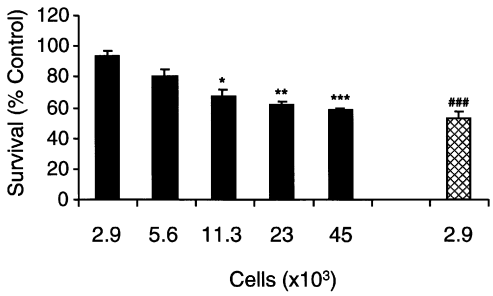

Fig. 2 Hippocampal injury induced by synaptic transmission is cell density dependent. Hippocampal neurones were plated at the indicated densities. At 14 DIC neurones were incubated 15 min (25°C) in Mg^{2+}-free/glycine-containing Locke's solution (pH 7.0) with 30 μM histamine (■). Replicate cultures plated at 2.9 $\times$ 10^3 cells/cm^2 were incubated alternatively with 100 μM glutamate in Mg^{2+}-free Locke's solution (15 min, 25°C) (▨). Neuronal survival in all cultures was quantified 24 h later by MTT assay. Values are means ± SD (three experiments). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. 2.9 $\times$ 10^3 cells/cm^2; ###$p < 0.001$ vs. at 2.9 $\times$ 10^3 cells/cm^2 in Mg^{2+}-free/glycine-containing Locke's solution with histamine.

Table 2 Effect of culture age on synaptically mediated excitotoxicity in hippocampal neurones

Culture condition (Mg^{2+}-free)	% Neuronal survival
Control (+ Mg^{2+})	100
Glycine + histamine (7 DIC)	101.7 ± 4.1
Glutamate (7 DIC)	53.7 ± 2.9
Glutamate + MK-801 (7 DIC)	103.5 ± 4.7
Glutamate + glycine/histamine (7 DIC)	57.7 ± 1.8
Glycine + histamine (14 DIC)	49.1 ± 2.9

Hippocampal neurones (7 or 14 DIC) were incubated 15 min (25°C) in Mg^{2+}-free Locke's solution (pH 7.0), supplemented with: glycine (0.1 μM) and histamine (30 μM); glutamate (100 μM); MK-801 (10 μM). Neuronal survival was quantified 24 h later by MTT assay. Values are means ± SD (three experiments). Survival is expressed relative to that obtained in Mg^{2+}-containing buffer (100%) at either 7 or 14 days.

Table 3 Histamine does not potentiate exogenous glutamate neurotoxicity in hippocampal neurones

	% Neuronal survival	
Glutamate (μM)	– histamine	+ histamine
0	100 ± 2.0	97.2 ± 4.7
10	79.7 ± 5.8	69.7 ± 2.9
30	67.5 ± 2.6	68.0 ± 1.0
100	56.1 ± 3.6	52.9 ± 4.4

Hippocampal neurones (7 DIC) were incubated 15 min (25°C) in Mg^{2+}-free Locke's solution (pH 7.0) with the indicated concentrations of glutamate without or with addition of 30 μM histamine and 0.1 μM glycine (' + *histamine*'). Neuronal survival was quantified 24 h later by MTT assay. Values are means ± SD (three experiments).

consistent with the described synaptic maturation of hippocampal neurones *in vitro* (Segal 1983; Abele *et al*. 1990).

Induction of tolerance in hippocampal neurones: excitotoxic preconditioning

Sublethal ischaemic exposure *in vitro* (Sakaki *et al*. 1995; Bruer *et al*. 1997; Grabb and Choi 1999) and *in vivo* (Kitagawa *et al*. 1990; Glazier *et al*. 1994; Chen *et al*. 1996) renders neurones resistant to a subsequent more severe insult. To explore whether preconditioning could also be observed in the present model of excitotoxic injury, mature hippocampal neurones were subjected to a sublethal (5 min) incubation in Mg^{2+}-free, glycine- and histamine-supplemented buffer. Following a recovery period of either 2 or 24 h, the cultures were subjected to a lethal (15 min) incubation in Mg^{2+}-free solution. Significant preconditioning-induced protection against excitotoxicity was observed when a 24-h preconditioning period was utilized. (Table 4). This effect was not seen with a shorter, 2 h preconditioning period. Neuroprotection was blocked if MK-801 was applied during the preconditioning period (Table 4). Stimulation of glutamate receptors and influx of Ca^{2+} can lead to the phosphorylation of p44/42 MAP kinase (Kurino *et al*. 1995; Xia *et al*. 1996). Activation of the extracellular regulated kinase was required for preconditioning. A highly selective and potent inhibitor of MAPK kinase (MEK), U0126 (Favata *et al*. 1998) reduced the protective effect of preconditioning when included during the 5-min Mg^{2+}-free period (Table 4). Similar results were obtained with the structurally unrelated MEK inhibitor PD 098059 (data not shown). The preconditioning stimulus induced an increase in the phosphorylation levels of Erk, which was completely blocked by U0126 (Fig. 3).

Table 4 Preconditioning protects against synaptically mediated excitotoxicity in hippocampal neurones

Culture condition	% Neuronal survival
Control (+ Mg^{2+})	100
Mg^{2+}-free (5 min)	95 ± 3
Mg^{2+}-free (15 min)	49 ± 4
Mg^{2+}-free (5 min) followed by:	
Mg^{2+}-free (15 min) after 2 h	45 ± 7
Mg^{2+}-free (15 min) after 24 h	79 ± 5[a]
Mg^{2+}-free (5 min) + MK-801 (10 μM), followed by:	
Mg^{2+}-free (15 min) after 24 h	50 ± 6
Mg^{2+}-free (5 min) + U0126 (10 μM), followed by:	
Mg^{2+}-free (15 min) after 24 h	63 ± 5[b]

Hippocampal neurones (14–16 DIC) were initially incubated for 5 min (25°C) in Mg^{2+}-free Locke's solution (pH 7.0), supplemented with: glycine (0.1 μM) and histamine (30 μM) ('Mg^{2+}-free'). Cultures were then returned to their original medium for 2 h or 24 h, at which time they were subjected to a 15-min Mg^{2+}-free incubation. Additional cultures were subjected to a single 5-min or 15-min Mg^{2+}-free incubation and then returned to their original medium for 24 h. Neuronal survival was quantified by MTT assay, 24 h following the final Mg^{2+}-free incubation in all treatment groups. Values are means ± SD (three experiments). Survival is expressed relative to Mg^{2+}-containing buffer (100%). [a]$p < 0.01$ versus Mg^{2+}-free (15 min); [b]$p < 0.05$ versus Mg^{2+}-free (15 min).

Discussion

In the present studies we have utilized a novel tissue culture model to induce neurotoxicity. This model involves removing the endogenous blockade by magnesium of the NMDA receptor, which triggers a self-perpetuating cycle of excitotoxicity, which is of relatively slow onset, and is critically dependent on NMDA receptors and activation of

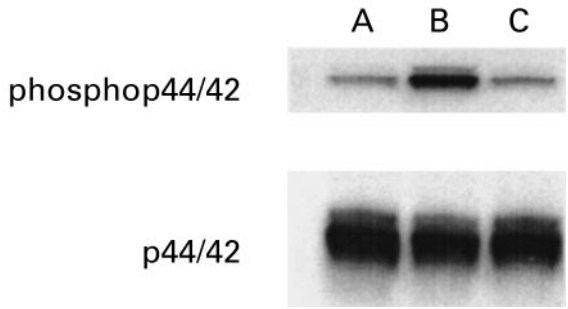

Fig. 3 Synaptic preconditioning induces Erk phosphorylation in DIV 15 hippocampal neurones. Western blot analysis of tyrosine-phosphorylated Erk (42 and 44 kDa). Preconditioning [5 min incubation in Mg^{2+}-free Locke's solution (pH 7.0) with: glycine (0.1 μM) and histamine (30 μM)] induces Erk phosphorylation (B) over control (A). Preconditioning-induced Erk phosphorylation is blocked by the MEK inhibitor U0126 (10 μM) (C).

TTX-sensitive sodium channels. This type of cell death is similar to that described in our previous studies, where we applied NMDA for a brief period to activate the neurotoxic pathway (Strijbos *et al*. 1996; Strijbos 1998). In analogy with these earlier studies, neuronal death was mediated by NMDA receptors and sensitive to sodium channel blockade. However, our previous work also demonstrated that this form of excitotoxicity was associated with secondary glutamate release, and, ultimately, formation of neurotoxic nitric oxide (Strijbos *et al*. 1996; Strijbos 1998). It is unknown whether secondary glutamate release occurs in our current studies; preliminary experiments suggest that nitric oxide does not mediate this form of neurotoxicity (our unpublished data).

We demonstrate that under Mg^{2+}-free, glycine-supplemented conditions (mast cell-derived) histamine can directly enhance the sensitivity of hippocampal neurones to synaptically mediated excitotoxicity involving NMDA receptors. This receptor activation presumably occurs at synaptic sites and may be akin to events occurring in status epilepticus, in which neurones are apparently killed as a result of massive waves of excitatory synaptic activity (Sloviter 1987; Furshpan and Potter 1989). Histamine has been described to potently modulate the gating of NMDA channels in the hippocampus (Bekkers 1993; Vorobjev *et al*. 1993), and to enhance peak amplitude and slow the onset of desensitization of NMDA-induced ion currents in cultured cortical neurones (Zwart *et al*. 1996). The dose-response curve for histamine potentiation of hippocampal cell death under conditions of hyperexcitability was similar to that reported for histamine modulation of NMDA receptors in hippocampal cells (Bekkers 1993; Vorobjev *et al*. 1993). Histamine can cause long-lasting enhancement of hippocampal population spikes (Kostopoulos *et al*. 1988) and can facilitate the induction of long-term potentiation (Brown *et al*. 1994) in rodent brain slices. Our findings suggest that in addition to histamine modulation of physiological processes, histamine may also participate in the pathophysiology of disorders that involve synaptically mediated excitotoxicity, e.g. in stroke where extracellular K^+ increases and neurones depolarize, resulting in increased glutamate release and relief of the Mg^{2+} block of NMDA receptors.

The histaminergic system originates from the posterior hypothalamus and innervates the whole CNS with widely arborizing projections (Panula *et al*. 1989; Wada *et al*. 1991). The modulating actions of histamine in the brain occur through activation of three types of receptors: H_1 receptor-mediated excitation, H_2 receptor-mediated potentiation of excitation through block of a Ca^{2+}-activated K^+ conductance, and H_3 receptor-mediated reduction of transmitter release (Prell and Green 1986; Schwartz *et al*. 1986; Haas 1992). Histamine, acting via H_1 and H_2 receptors, has been reported to also increase the evoked release of glutamate from hippocampal synaptosomes (Rodriguez *et al*. 1997). A novel potentiating effect of histamine on NMDA-mediated synaptic transmission in hippocampal cultures has previously been reported (Bekkers 1993; Vorobjev *et al*. 1993). The results described here demonstrate that histamine is also capable of enhancing NMDA-mediated synaptic activity leading to excitotoxic cell death. Similar to the potentiating effect of histamine on NMDA currents (Bekkers 1993; Vorobjev *et al*. 1993), the effects of histamine on synaptic transmission-induced death occurred in the absence of an interaction with archetypal histamine receptors, as its actions were not mimicked by histamine H_1, H_2 and H_3 receptor agonists or blocked by their respective receptor antagonists. This may suggest the involvement of an atypical histamine receptor or a direct effect of histamine on the NMDA receptor.

Mast cells occur within the CNS of many species (Dropp 1976; Theoharides 1990; Silver *et al*. 1996). Up to 50% of whole brain histamine levels and 90% of the thalamic histamine is attributable to the presence of these cells (Goldschmidt *et al*. 1985). Isolated brain mast cells secrete histamine (Cocchiara *et al*. 1999), which could modulate neuronal events. When peritoneal mast cells were co-applied with the hippocampal cultures and stimulated with antigen under Mg^{2+}-free conditions, a greater degree of neuronal death was observed which relied critically on mast cell-derived histamine. The numbers of mast cells applied to the neuronal co-culture are very large compared to what the hippocampus might see *in vivo* (Goldschmidt *et al*. 1985); however, the described apposition between mast cells and neurones *in vivo* (Rozniecki *et al*. 1999) may result in much higher and localized histamine levels. Histamine release has been proposed to be involved in the pathogenesis of thiamine deficiency-induced neuronal necrosis within the thalamus (Langlais *et al*. 1994). In the latter study, α-fluoromethylhistidine, an irreversible inhibitor of histamine synthesis protected against neuronal loss, suggesting that histamine release may contribute significantly to excitotoxic neuronal death in Wernicke's encephalopathy (Langlais *et al*. 1994). The distribution of mast cells and histamine terminals within the thalamus (Hough 1988; Panula *et al*. 1989) bears a striking resemblance to the pattern of thiamine deficiency-induced lesions in the same brain area, proposing that mast cells as well as neuronal histamine could contribute to this form of neurotoxicity. The long-lasting increase in neuronal histamine release induced by cerebral ischaemia (Adachi *et al*. 1992) could also play a role in excitotoxic brain injury.

Histamine enhancement of hippocampal neurone injury under conditions of synaptic activity-mediated excitotoxicity appeared to be potentiated by extracellular acidity. A pH-dependent facilitation of synaptic transmission by histamine in hippocampus *in vitro* has been reported previously (Saybasili *et al*. 1995; Yanovsky *et al*. 1995). Increased

synaptic activity causes local acidification (Somjen 1984), such that extracellular pH may be transiently lowered during normal brain function (Buzsaki *et al*. 1987) and, more pertinently, during hypoxia/ischaemia or epileptiform activity. Under these conditions, histamine could act to maintain transmission or to aggravate excitotoxicity. Conversely, alkaline transients occur during normal synaptic release of glutamate (Somjen 1984), and a depressant action of histamine under these conditions may confer neuroprotection.

Hippocampal neurones preconditioned by a sublethal exposure to Mg^{2+}-free, glycine- and histamine-containing buffer were rendered resistant to injury induced by a subsequent longer exposure to this insult. Preconditioning-induced ischaemic tolerance has been observed both *in vitro* (Sakaki *et al*. 1995; Bruer *et al*. 1997; Grabb and Choi 1999) and *in vivo* (Kitagawa *et al*. 1990; Glazier *et al*. 1994; Chen *et al*. 1996). The basis of ischaemic tolerance has not been fully defined, although a role for NMDA receptor activation has been suggested (Kato *et al*. 1992; Grabb and Choi 1999). In the present study, NMDA receptor activation and the extracellular regulated kinase signalling were required for preconditioning-induced neuroprotection. Activation of the Ras/extracellular regulated kinase cascade was required for oxygen-glucose deprivation preconditioning in cultured cortical neurones (Gonzalez-Zulueta *et al*. 2000; but see Tauskela *et al*. 1999). Previous studies have reported MAPK activation in the hippocampus after sublethal ischaemia (ShamLoo *et al*. 1999) and in myocardial preconditioning (Maulik *et al*. 1996). As with ischaemic tolerance (Kitagawa *et al*. 1990; Kato *et al*. 1991; Grabb and Choi 1999), we observed that tolerance developed slowly over a number of hours after the preconditioning episode, suggesting that changes in gene expression may be involved, perhaps as a consequence of MAPK activation. Indeed, expression of c-Jun increases in hippocampal CA1 neurones after preconditioning ischaemia (Sommer *et al*. 1995).

Conclusion

The molecular mechanisms underlying NMDA-mediated excitotoxic injury remain to be fully characterized. Several neurological insults linked to an excessive release of glutamate and neuronal death, including hypoxia/ischaemia, hypoglycemia, and kainate-induced seizures result in tyrosine kinase activation, including p44/42 MAPK (Gass *et al*. 1993; Hu and Wieloch 1994; Kim *et al*. 1994; Kurihara *et al*. 1994). p44/42 MAPK may have a role in excitotoxic injury mediated by synaptic mechanisms (Murray *et al*. 1998; Probert *et al*. 1998; Skaper *et al*. 1999) but not excitotoxicity after direct application of glutamate (Probert *et al*. 1998; our unpublished data). Seizure-induced phosphorylation of p44/42 MAP kinase in presynaptic and postsynaptic structures (Murray *et al*. 1998) indicates possible roles for p44/42 MAP kinase in the release of transmitters and/or postsynaptic signalling leading to neuronal injury. For example, *in vitro* ischaemia is associated with MAP kinase-dependent synapsin I phosphorylation (Probert *et al*. 1998), which can lead to increased synaptic vesicle mobility and availability for synaptic release. Cell culture models of synaptically mediated excitotoxicity will prove useful for pathway analysis of injury processes and to identify novel neuroprotective agents.

References

Abele A. E., Scholz K. P., Scholz W. K. and Miller R. J. (1990) Excitotoxiciy inducd by enhanced excitatory neurotransmission in cultured hippocampal pyramidal neurons. *Neuron* **4**, 413–419.

Adachi N., Itoh Y., Oishi R. and Saeki K. (1992) Direct evidence for continuous histamine release in the striatum of conscious freely moving rats produced by middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* **12**, 477–483.

Bekkers J. M. (1993) Enhancement by histamine of NMDA-mediated synaptic transmission in the hippocampus. *Science* **261**, 104–106.

Bienenstock J., Tomioka M., Matsuda H., Stead R. H., Quinonez G., Simon G. T., Coughlin M. D. and Denburg J. A. (1987) The role of mast cells in inflammatory processes: evidence for nerve mast cell interactions. *Int. Arch. Allergy Appl. Immunol.* **82**, 238–243.

Brown R. E., Fedorov N. B., Haas H. L. and Reymann K. G. (1994) Histaminergic modulation of synaptic plasticity in area CA1 of rat hippocampal slices. *Neuropharmacology* **34**, 181–190.

Bruer U., Weih M. K., Isaev N. K., Meisel A., Ruscher K., Bergk A., Trendelenburg G., Wiegand F., Victorov I. V. and Dirnagl U. (1997) Induction of tolerance in rat cortical neurons: hypoxic preconditioning. *FEBS Lett.* **414**, 117–121.

Buzsaki G., Haas H. L. and Henderson E. G. (1987) LTP induced by physiologically relevant stimulus patterns. *Brain Res.* **435**, 331–333.

Chen J., Graham S. H., Zhu R. L. and Simon R. P. (1996) Stress proteins and tolerance to focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **16**, 566–577.

Choi D. W. (1988) Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**, 623–634.

Clifford D. B., Zorumski C. F. and Onley J. W. (1989) Ketamine and MK-801 prevent degeneration of thalamic neurons induced by focal cortical seizures. *Exp. Neurol.* **105**, 272–279.

Cocchiara R., Albeggiani G., Lampiasi N., Boniovanni A., Azzolina A. and Geraci D. (1999) Histamine and tumor necrosis factor alpha production from purified rat brain mast cells mediated by substance P. *Neuroreport* **10**, 575–583.

Dropp J. J. (1976) Mast cells in mammalian brain. *Acta Anat.* **94**, 1–21.

Facci L., Dal Toso R., Romanello S., Buriani A., Skaper S. D. and Leon A. (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. USA* **92**, 3376–3380.

Favata M. F., Horiuchi K. Y., Manos E. J., Daulerio A. J., Stradley D. A., Feeser W. S., Van Dyk D. E., Pitts W. J., Earl R. A., Hobbs F., Copeland R. A., Magolda R. L., Scherle P. A. and Trzaskos J. M. (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem.* **273**, 18623–18632.

Furshpan E. J. and Potter D. D. (1989) Seizure-like activity and cellular damage in rat hippocampal neurons in cell culture. *Neuron* **3**, 199–207.

Galli S. J. (1993) New concepts about the mast cell. *N. Engl. J. Med.* **328**, 257–265.

Gass P., Kiessling M. and Bading H. (1993) Regionally selective stimulation of mitogen activated protein (MAP) kinase tyrosine phosphorylation after generalized seizures in the rat. *Neurosci. Lett.* **162**, 39–42.

Glazier S. S., O'Rourke D. M., Graham D. I. and Welsh F. A. (1994) Induction of ischemic tolerance following brief focal ischemia in rat brain. *J. Cereb. Blood Flow Metab.* **14**, 545–553.

Goldschmidt R. C., Hough L. B. and Glick S. D. (1985) Rat brain mast cells: contribution to brain histamine levels. *J. Neurochem.* **44**, 1943–1947.

Goldschmidt R. C., Hough L. B., Glick S. D. and Padawer J. (1984) Mast cells in rat thalamus: nuclear localization, sex differences and left-right asymmetry. *Brain Res.* **323**, 209–217.

Gonzalez-Zulueta M., Feldman A. B., Klesse L. J., Kalb R. G., Dillman J. F., Parada L. F., Dawson T. M. and Dawson V. L. (2000) Requirement for nitric oxide activation of $p21^{ras}$/extracellular regulated kinase in neuronal ischemic preconditioning. *Proc. Natl. Acad. Sci. USA* **97**, 436–441.

Grabb M. C. and Choi D. W. (1999) Ischemic tolerance in murine cortical cell culture: critical role for NMDA receptors. *J. Neurosci.* **19**, 1657–1662.

Haas H. L. (1992) Electrophysiology of histamine receptors, in: *The Histamine Receptor* (Schwartz J. C. and Haas H. L., eds), pp. 161–177. Wiley Liss, New York.

Hough L. B. (1988) Cellular localization and possible functions for brain histamine: recent progress. *Prog. Neurobiol.* **30**, 469–505.

Hu B.-R. and Wieloch T. (1994) Tyrosine phosphorylation and activation of mitogen-activated protein kinase in the rat brain following transient cerebral ischemia. *J. Neurochem.* **62**, 1357–1367.

Kaminer M. S., Murphey G. F., Zweiman B. and Lavker R. M. (1995) Connective tissue mast cells exhibit time-dependent degranulation heterogeneity. *Clin. Diagn. Lab. Immunol.* **2**, 297–301.

Kato H., Liu Y., Araki T. and Kogure K. (1991) Temporal profile of the effects of pretreatment with brief cerebral ischemia on the neuronal damage following secondary ischemic insult in the gerbil: cumulative damage and protective effects. *Brain Res.* **553**, 238–242.

Kato H., Liu Y., Araki T. and Kogure K. (1992) MK-801, but not anisomycin, inhibits the induction of tolerance to ischemia in the gerbil hippocampus. *Neurosci. Lett.* **139**, 118–121.

Kim Y. S., Hong K. S., Seong Y. S., Park J. B., Kuroda S., Kishi K., Kaibuchi K. and Takai Y. (1994) Phosphorylation and activation of mitogen-activated protein kinase by kainic acid-induced seizure in rat hippocampus. *Biochem. Biophys. Res. Comm.* **202**, 1163–1168.

Kitagawa K., Matsumoto M., Tagaya M., Hata R., Ueda H., Niiobe M., Handa N., Fukunaga R., Kimura K., Mikoshiba K. and Kamada T. (1990) 'Ischemic tolerance' phenomenon found in the brain. *Brain Res.* **528**, 21–24.

Kostopoulos G., Psarropoulou C. and Haas H. L. (1988) Membrane properties, response to amines and to tetanic stimulation of hippocampal neurons in the genetically epileptic mutant mouse tottering. *Exp. Brain Res.* **72**, 45–50.

Kurihara J., Hu B.-R. and Wieloch T. (1994) Changes in the tyrosine phosphorylation of mitogen-activated protein kinase in the rat hippocampus during and following severe hypoglycemia. *J. Neurochem.* **63**, 2346–2348.

Kurino M., Fukunaga K., Ushio Y. and Miyamoto E. (1995) Activation of mitogen-activated protein kinase in cultured rat hippocampal neurons by stimulation of glutamate receptors. *J. Neurochem.* **65**, 1282–1289.

Langlais P. J. and Mair R. G. (1990) Protective effects of the glutamate antagonist MK-801 on pyrithiamine-induced lesions and amino acid changes in rat brain. *J. Neurosci.* **10**, 1664–1674.

Langlais P. J., Zhang S. X., Weilersbacher G., Hough L. B. and Barke K. E. (1994) Histamine-mediated neuronal death in a rat model of Wernicke's encephalopathy. *J. Neurosci. Res.* **38**, 565–574.

Manthorpe M., Fagnani R., Skaper S. D. and Varon S. (1986) An automated colorimetric assay for neurotrophic factors. *Dev. Brain Res.* **25**, 191–198.

Maulik N., Watanabe M., Zu Y. L., Huang C. K., Cordis G. A., Schley J. A. and Das D. K. (1996) Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts. *FEBS Lett.* **396**, 233–237.

Meldrum B. S. (1990) Anatomy, physiology and pathology of epilepsy. *Lancet* **336**, 231–234.

Mody I., Lambert J. D. C. and Heinemann U. (1987) Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J. Neurophysiol.* **57**, 869–888.

Mosmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63.

Mousli M., Bronner C., Bueb J. L., Tschirhart E., Gies J. P. and Landry Y. (1989) Activation of rat peritoneal mast cells by substance P and mastoparan. *J. Pharmacol. Exp. Ther.* **250**, 329–335.

Murray B., Alessandrini A., Cole A. J., Yee A. G. and Furshpan E. J. (1998) Inhibition of the p44/42 MAP kinase pathway protects hippocampal neurons in a cell-culture model of seizure activity. *Proc. Natl. Acad. Sci. USA* **95**, 11975–11980.

Panula P., Pirvola U., Auvinen S. and Airaksinen M. N. (1989) Histamine-immunoreactive nerve fibers in the brain. *Neuroscience* **28**, 585–610.

Prell G. D. and Green J. P. (1986) Histamine as a neuroregulator. *Annu. Rev. Neurosci.* **9**, 209–254.

Probert A. W., Nath R., Basu S. S., Borosky S. A., Boxer P. A., MacKenzie R. G., Greengard P. and Czernik A. J. (1998) The MEK inhibitor, PD-98059, reduces phosphorylation of site 4/5 Synapsin 1 and protects against oxygen/glucose deprivation-mediated neuronal injury. *Soc. Neurosci. Abstract* **24**, 1569.

Purcell W. M. and Atterwill C. K. (1995) Mast cells in neuroimmune function: neurotoxicological and neuropharmacological perspectives. *Neurochem. Res.* **20**, 521–532.

Rodriguez F. J., Lluch M., Dot J., Blanco I. and Rodriguez-Alvarez J. (1997) Histamine modulation of glutamate release from hippocampal synaptosomes. *Eur. J. Pharmacol.* **323**, 283–286.

Rose K., Christine C. W. and Choi D. W. (1990) Magnesium removal induces paroxysmal neuronal firing and NMDA receptor-mediated neuronal degeneration in cortical cultures. *Neurosci. Lett.* **115**, 313–317.

Rothman S. M. and Olney J. W. (1987) Excitotoxicity and the NMDA receptor. *Trends Neurosci.* **10**, 299–302.

Roznieckl J. J., Dimitriadou V., Lambracht-Hall M., Pang X. and Theoharides T. C. (1999) Morphological and functional demonstration of rat dura mater mast cell–neuron interactions in vitro and in vivo. *Brain Res.* **849**, 1–15.

Sakaki T., Yamada K., Otsuki H., Yuguchi T., Kohmura E. and Hayakawa T. (1995) Brief exposure to hypoxia induces bFGF mRNA and protein and protects rat cortical neurons from prolonged hypoxic stress. *Neurosci. Res.* **23**, 289–296.

Saybasili H., Stevens D. R. and Haas H. L. (1995) pH-dependent modulation of N-methyl-D-aspartate receptor-mediated synaptic currents by histamine in rat hippocampus in vitro. *Neurosci. Lett.* **199**, 225–227.

Schwartz J.-C., Arrang J.-M., Garbarg M. and Korner M. (1986) properties and roles of the three subclasses of histamine receptors in brain. *J. Exp. Biol.* **124**, 203–224.

Schwartz J.-C., Arrang J.-M., Garbarg M., Pollard H. and Ruat M. (1991) Histaminergic transmission in the mammalian brain. *Physiol. Rev.* **71**, 1–51.

Segal M. (1983) Rat hippocampal neurons in culture: responses to electrical and chemical stimuli. *J. Neurophysiol.* **54**, 1363–1374.

Shamloo M., Rytter A. and Wieloch T. (1999) Activation of the extracellular signal-regulated protein kinase cascade in the hippocampal CA1 region in a rat model of global cerebral ischemic preconditioning. *Neuroscience* **93**, 81–88.

Silver R., Silverman A. J., Vitkovic L. and Lederhendler I. (1996) Mast cells in the brain: evidence and functional significance. *Trends Neurosci.* **19**, 25–31.

Skaper S. D., Ancona B., Facci L., Franceschini D. and Giusti P. (1998) Melatonin prevents the delayed death of hippocampal neurons by enhanced excitatory neurotransmission and the nitridergic pathway. *FASEB J.* **12**, 725–731.

Skaper S. D., Facci L., Milani L., Leon A. and Toffano G. (1990) Culture and use of primary and clonal neural cells, in: *Methods in Neurosciences*, **Vol. 2** (Conn P. M., ed.), pp. 17–33. Academic Press, San Diego.

Skaper S. D., Leon A. and Facci L. (1991) Ganglioside GM1 prevents death induced by excessive excitatory neurotransmission in cultured hippocampal pyramidal neurons. *Neurosci. Lett.* **126**, 98–101.

Skaper S. D., Murkitt K. L., Facci L., Strijbos P. J. L. M. and Walsh F. S. (1999) Potentiation of network activity-induced hippocampal neuron injury by histamine: a role for the p44/42 MAP kinase pathway. *Soc. Neurosci. Abstract.* **25**, 289.

Sloviter R. S. (1987) Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science* **235**, 73–76.

Somjen G. G. (1984) Acidification of interstitial fluid in hippocampal formation caused by seizures and by spreading depression. *Brain Res.* **311**, 186–188.

Sommer C., Gass P. and Kiessling M. (1995) Selective c-JUN expression in CA1 neurons of the gerbil hippocampus during and after acquisition of an ischemia-tolerant state. *Brain Pathol.* **5**, 135–144.

Song J. H., Huang C. S., Nagata K., Yeh J. Z. and Narahashi T. (1997) Differential action of riluzole on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels. *J. Pharmacol. Exp. Ther.* **282**, 707–714.

Strijbos P. J. L. M. (1998) Nitric oxide in cerebral ischemic neurodegeneration and excitotoxicity. *Crit. Rev. Neurobiol.* **12**, 223–243.

Strijbos P. J. L. M., Leach N. and Garthwaite J. (1996) Vicious cycle involving Na^+ channels, glutamate release, and NMDA receptors mediates delayed neurodegeneration through nitric oxide formation. *J. Neurosci.* **16**, 5004–5013.

Tauskela J. S., Chakravarthy B. R., Murray C. L., Wang Y., Comas T., Hogan M., Hakim A. and Morley P. (1999) Evidence from cultured rat cortical neurons of differences in the mechanism of ischemic preconditioning of brain and heart. *Brain Res.* **827**, 143–151.

Theoharides T. C. (1990) Mast cells: the immune gate to the brain. *Life Sci.* **46**, 607–617.

Vorobjev V. S., Sharonova I. N., Walsh I. B. and Haas H. L. (1993) Histamine potentiates *N*-methyl-D-aspartate responses in acutely isolated hippocampal neurons. *Neuron* **11**, 837–844.

Wada H., Inagaki N., Yamatodani A. and Watanabe T. (1991) Is the histaminergic neuron system a regulatory center for whole-brain activity? *Trends Neurosci.* **14**, 415–418.

Williams K. (1994) Subunit specific potentiation of recombinant *N*-methyl-D-aspartate receptors by histamine. *Mol. Pharmacol.* **46**, 531–541.

Xia Z., Dudek H., Miranti C. K. and Greenberg M. E. (1996) Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J. Neurosci.* **16**, 5425–5436.

Yanovsky Y., Reymann K. and Haas H. L. (1995) pH-dependent facilitation of synaptic transmission by histamine in the CA1 region of mouse hippocampus. *Eur. J. Neurosci.* **7**, 2017–2020.

Zwart R., Blank T. and Spiess J. (1996) Histamine slows the onset of desensitization of rat cortical NMDA receptors. *Neuroreport* **7**, 2206–2210.