5-Hydroxyanthranilic Acid, a Tryptophan Metabolite, Generates Oxidative Stress and Neuronal Death via p38 Activation in Cultured Cerebellar Granule Neurones

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Abstract The essential amino acid tryptophan is primarily metabolised through the kynurenine pathway, some components of which may be neurotoxic. We have now examined the potential toxicity of several kynurenine metabolites in relation to the generation of oxidative stress and activation of cell death signalling pathways in cultured cerebellar granule neurons. 3-Hydroxykynurenine (3HK), 3-hydroxyanthranilic acid (3HAA) and 5-hydroxyanthranilic acid (5HAA) induced cell death which increased with exposure time and compound concentration. The neurotoxic effects of 3HK, 3HAA and 5HAA were prevented by catalase, but not by superoxide dismutase. In addition, Western blot analysis demonstrated p38 activation due to 3HK or 5HAA treatment, although caspase-3 activation was not evident in either case. The results indicate that kynurenine metabolites can be neurotoxic via a caspase-3 independent mechanism, and that the minor metabolite 5HAA is as potent a toxin as the better documented compounds 3HK and 3HAA.

Keywords Cerebellar granule neurones · Kynurenines · Quinolinic acid · 5-Hydroxyanthranilic acid · Neurotoxicity · Oxidative stress · Tryptophan

Introduction

The kynurenine pathway is the primary route for tryptophan metabolism and generates a series of compounds with physiological roles in the regulation of neuronal excitability (see Stone 2001; Stone and Darlington 2002; Moroni 1998), synthesis of the essential co-factor NAD and immune function (Opitz et al. 2007). However, several of these compounds have neurotoxic properties, as reported in neuronal cultures, brain slices and in vivo studies (Wu et al. 2000; Chiarugi et al. 2001; Schwarcz and Pellicciari 2002; Smith et al. 2007). One component of the pathway is quinolinic acid, which has excitatory actions via NMDA receptors (Perkins and Stone 1983) leading to neurotoxicity (Schwarcz et al. 1983; Guidetti and Schwarcz 1999). Two other compounds on the main pathway also produce cell toxicity: 3-hydroxykynurenine (3HK) and 3-hydroxyanthranilic acid (3HAA). Application of 3HAA for 48 h induces a significant degree of apoptosis in monocyte-derived cell lines (U937 and THP-1) (Morita et al. 2001) and in neurones (Okuda et al. 1996; Chiarugi et al. 2001).

There is evidence that oxidative stress may be involved in neuronal death (Kostrzewa et al. 2000), including that caused by 3HK, since reduced viability can be prevented by co-application of catalase or allopurinol (Eastman and Guilarte 1989, 1990; Okuda et al. 1996). Furthermore N-acetylcysteine, an antioxidant particularly effective at scavenging the hydroxyl radical, provided protection against 3HK-induced neurotoxicity (Okuda et al. 1998). The toxicity of 3HAA in macrophages was enhanced by ferrous or manganese ions and attenuated by the antioxidants Trolox, α-tocopherol or allopurinol (Morita et al. 2001), again implying a role for hydroxyl radicals. Even the neurotoxicity produced by quinolinic acid is only partly attributable to activation of NMDA receptors, since the generation of reactive oxygen species (ROS) and oxidative stress (Behan et al. 1999; Santamaria et al. 2001; Guidetti and Schwarcz 1999; Okuda et al. 1996, 1998; St'astny et al. 2004) are also involved.

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The present study was conceived with two main aims. Firstly, it was intended to examine whether a minor metabolite of kynurenine, 5-hydroxyanthranilic acid (5HAA) would also show toxicity towards neurons, and whether it could induce oxidative stress. Secondly, we wished to examine some of the possible molecular changes associated with damage produced by the kynurenine metabolites in order to shed further light on the molecular mechanisms involved.

Materials and Methods

Cerebellar Granule Neuronal (CGN) Cultures

Cultures from 8 day-old Sprague-Dawley rat pups, by which age CGNs are known to express NMDA receptors (Trenkner 1998), were prepared as used routinely in our laboratory (Fatokun et al. 2007). Briefly, cerebella were excised into sterile buffer solution containing 250 mg D(+)-glucose, 300 mg bovine serum albumin and 1 ml of 3.82% w/v $MgSO_4$, made up to 100 ml with phosphate-buffered saline. Following removal of the meninges, the cerebella were finely chopped, transferred into a 0.25% trypsin solution and incubated at 37°C for 20 min. Soybean trypsin inhibitor (SBTI) and DNAse solution were added prior to centrifugation. The supernatant was discarded and the cell pellet re-suspended and triturated through flame-polished pipettes three times before 2 ml of Eagle's minimum essential medium (MEM) at 37°C was added and a final trituration step undertaken. Viability was at least 95% as assessed by Trypan Blue staining. For 96-well plates used in viability assays, cell density was adjusted to 1×10^6 cells per ml with MEM supplemented with 10% foetal calf serum, 2 mM glutamine, 50 µg/ml gentamicin and 25 mM KCl. In obtaining protein samples for Western blot analysis, cultures were plated at 1.5×10^6 cells per ml in 100 mm dishes. All plates and dishes were coated with poly-D-lysine (PDL) prior to seeding to enhance adhesion. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cytosine arabinoside (10 µM) was added after 24 h to eliminate dividing non-neuronal cells. Neuronal morphology was monitored with an Olympus DP50 inverted phase-contrast microscope fitted with a digital camera system for image capture by DPSoft software (Olympus UK Ltd., Southall, UK).

Source of Reagents

Minimum Essential Medium, L-glutamine (200 mM solution), gentamicin 50 mg/ml, 1× phosphate-buffered saline and DEPC-treated water were obtained from Gibco-Invitrogen, Paisley UK. Bovine serum albumin; trypsin [porcine pancreas] powder; SBTI; DNase I powder; poly-D-lysine; Trypan Blue dye; heat-inactivated foetal calf serum; cytosine arabinoside and the kynurenine metabolites 3-hydroxyanthranilic acid (3HAA), 5-hydroxyanthranilic acid (5HAA) and 3-hydroxykynurenine (3HK) were obtained from Sigma–Aldrich, Poole, Dorset. Potassium chloride was purchased from BDH Laboratory Supplies, and magnesium sulphate from FSA Laboratory Supplies.

Fluorescein Diacetate (FDA) Assay

Control and treated neuronal viabilities were assessed by the well-established fluorescein diacetate (FDA) assay in which only living cells take up and cleave the non-fluorescent esterified fluorescein (Sigma F7378) to produce the fluorescein which fluoresces (Rotman and Papermaster 1996). Culture wells were incubated for 10 min at 37°C with MEM containing 5 µg/ml FDA and 1% ethanol prior to determining fluorescence emission at 538 nm following excitation at 485 nm with a Fluoroskan plate reader from which viability was calculated and normalised to control cell readings.

Western Blot Analysis

Western Blotting analysis was undertaken using the Novex Mini-cell Blot system (Invitrogen, Paisley, UK). Protein samples (20–30 µg) from control and treated cultures were prepared in RIPA buffer [containing 50 mM Tris, 150 mM NaCl, 0.5% Triton X-100, 0.1% SDS, 1% IGEPAL (Sigma 17771) and protease inhibitors]. These were added to a lithium dodecyl sulphate loading buffer (cat no. NP0007—Invitrogen) and reducing agent (cat no. NP0004—Invitrogen) and heated at 70°C for 10 min prior to centrifugation at 2000 rpm at 4°C for 1 min. Samples were loaded, together with a lane of protein markers, onto commercially available 12% SDS-polyacrylamide gels (cat no. NP0341—Invitrogen). The gel tank was filled with MOPS running buffer (cat no. NP0001—Invitrogen) and run at 150 V for 90 min. Proteins were subsequently electroblotted onto methanol-soaked PVDF membranes (cat no. LC2005) at 30 V for 1 h (and at a current of 400 mAmp) using NuPage transfer buffer (cat no. NP0006). At the end of this period, Ponceau staining (cat no. P7170 Sigma) was applied to confirm constant transfer across sample lanes. The membrane was blocked from non-specific binding by immersion in Tris-buffered saline (TBS) solution containing 5% milk and 0.05% Tween-20 for 60 min, before overnight incubation at 4°C with selected primary antibodies. When blotting for phosphorylated proteins, 5% IgG-free BSA solution was used instead of milk solution. Next day, the membranes were washed three times to remove unbound primary antibody, and an appropriate dilution of secondary antibody in TBS solution containing 5% milk and 0.05%

Tween-20 was applied for 60 min. The membrane was then rinsed five times in TBS for 15 min to remove all unbound secondary antibody. Enhanced chemiluminescence of target proteins was achieved using enzymatic chemiluminescence (ECL) solution (cat no. RPN2132, Amersham, Little Chalfont, UK) applied to the membrane for 5 min prior to exposure to film in darkroom conditions. 'Housekeeping' proteins (actin or glyceraldehyde phosphate dehydrogenase [GAPDH]) were monitored to ensure equal loading of samples.

Statistics

All results are presented as mean ± 1 SEM. The statistical significance of differences was assessed using ANOVA followed by Tukey's test, with the level of significance taken as $P < 0.05$.

Results

Viability Studies

Treatment with concentrations of 10 μM to 1 mM of 3HK (Fig. 1a), 3HAA (data not shown) or 5HAA (Fig. 2a) for periods of 1, 5 or 9 h caused a reduction in the viability of cerebellar granule neurons (CGNs) which increased with concentration. The results indicated that 5HAA is a neurotoxin with the potency similar to that of 3HK and 3HAA. Microscopic observation revealed that the reduced viability was associated with deterioration in the morphological appearances of the neurons. The regular shaped cell bodies seen in control cultures (Fig. 3a) were replaced by shrunken, irregular somata which showed few neuritic outgrowths after incubation with 3HK (Fig. 3b) or 5HAA (Fig. 3c).

The inclusion of catalase caused a highly significant reduction in the neurotoxic effects of all three compounds, including 3HK (Fig. 1b) and 5HAA (Fig. 2b), whereas superoxide dismutase (SOD) failed to provide protection against any of the compounds (Figs. 1b and 2b, respectively). This protection was again reflected in the microscopic observations of the cells (Fig. 3d, f). Co-application of denatured SOD or denatured catalase did not modify the toxicity of any of the compounds (data not shown), demonstrating that the protective effect of catalase was entirely due to its enzymatic function and implicating ROS formation directly in the toxicity induced by these compounds.

In order to examine the role of protein synthesis in the mechanism of cell death, cycloheximide was included in some cultures treated with the kynurenine metabolites. This compound did not prevent the neurotoxic effects of any of the three compounds (Figs. 3e, 4).

Western Blots

Protein samples from CGNs exposed to 100 μM 3HK or 100 μM 5HAA for 0.5–24 h were used for Western blotting as described above. The blots revealed an increase in phosphorylation of p38 MAPK (p38 mitogen-activated protein kinase pathway) following both 3HK (Fig. 1c, e) and 5HAA (Fig. 2c, e) treatments that were evident only after 30 min and remained up-regulated for up to 9 h exposures before declining to the initial levels. However, no increase in expression of activated caspase-3 was observed with either 3-HK (Fig. 1d, f) or 5HAA (Fig. 2d, f). Indeed, the blots indicated a trend for a decrease of caspase-3 expression, although there was some limited positive staining for activated caspase-3 in untreated control samples, suggesting that a degree of apoptosis was occurring in these unstimulated cultures.

Discussion

A previous study in cultured striatal neurones showed that 3HK produced an accumulation of intracellular peroxides and a significant reduction in cell viability which was prevented by catalase, desferrioxamine (a chelator of iron) or allopurinol (an inhibitor of xanthine oxidase), but not SOD (Okuda et al. 1996). Subsequent study showed protection also by ascorbic acid, trimethylphosphine oxide (TMPO), α-tocopherol and trolox, which prevented neurotoxicity induced by 3HK at 1 and 10 μM, although not 100 μM. N-acteylcysteine, which is a more effective scavenger of hydroxyl radicals than of peroxide, was effective against all three concentrations of 3HK. The oxidative stress in striatal neurones was probably induced within the cytoplasm, since it was also prevented by blocking uptake of 3HK via the large amino acid transporter (Okuda et al. 1998).

Cerebellar granule neurones are also damaged by 3HK, although the mechanism is distinctly different. The co-application of catalase still significantly reduced the neurotoxicity of 3HK, but with SOD the levels of cell death were increased. Furthermore, blockade of the large neutral amino acid transporter was not protective in CGNs, and neither was damage attenuated by allopurinol or NMDA antagonists (Wei et al. 2000).

Both 3HK and 3HAA are capable of generating H_2O_2 and superoxide in phosphate buffer solution, and of reducing Cu^{2+} and Fe^{3+}, in an environment with a physiological pH (Goldstein et al. 2000). These effects were not produced by tryptophan, kynurenine, kynurenic acid,
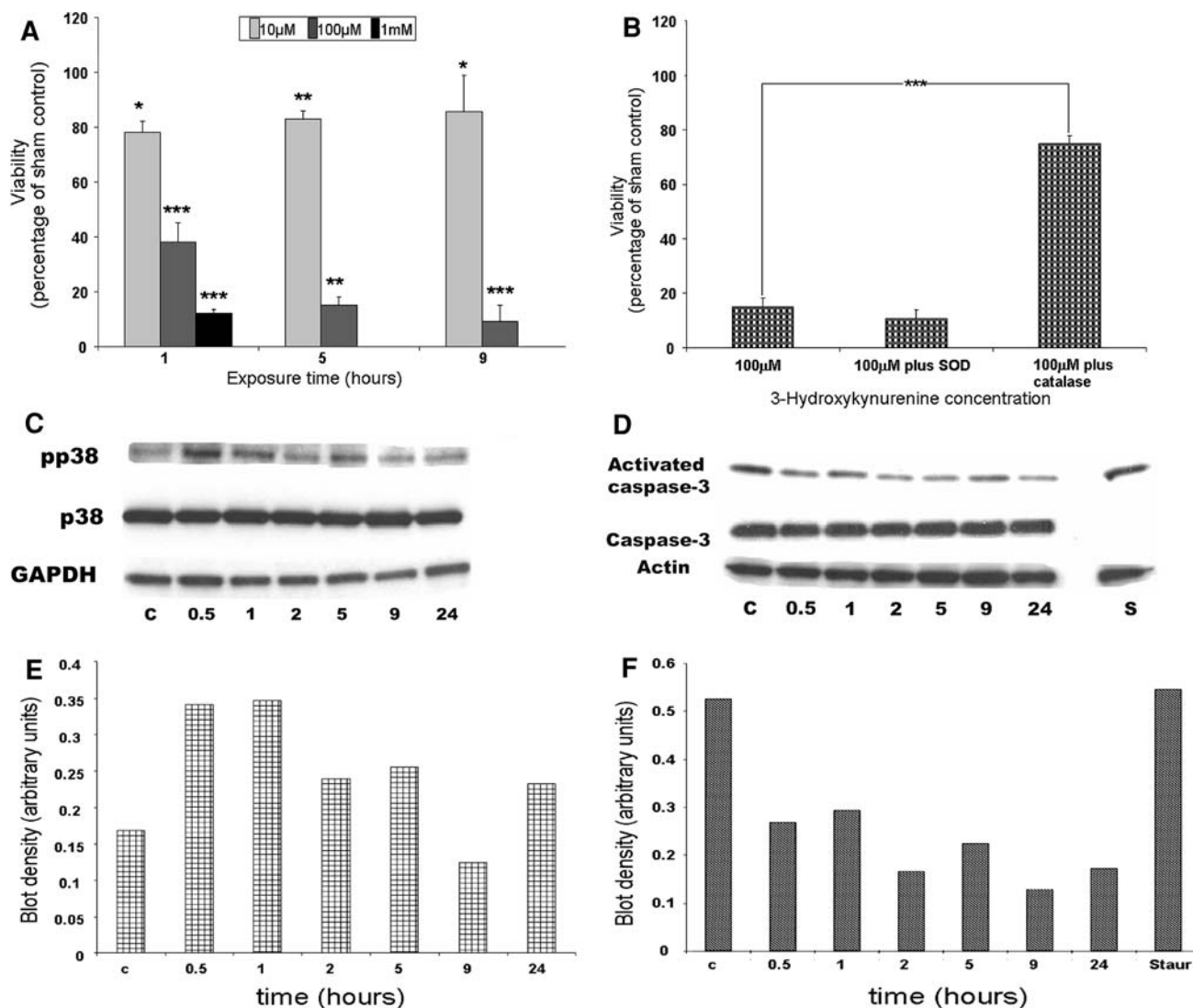
Fig. 1 Toxic effects of 3-hydroxykynurenine on CGNs. The bar chart **a** summarises the toxic effects on CGNs at 9 DIV of 3-HK at concentrations of 10 μM, 100 μM and 1 mM, based on the mean ± SEM for cell viability relative to untreated control cultures. $P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for $n = 5$ in each case. The bar chart in **b** illustrates the suppression of toxicity by catalase (200 U/ml) but not SOD (200 U/ml) when 3HK was present together with these enzymes for 5 h. ***$P < 0.001$ for $n = 5$. The Western blots at **c** show representative analyses for p38MAPK (p38) and phosphorylated p38 MAPK (pp38) of control cultures and cultures treated with 3HK for time periods of 0.5, 1, 2, 5, 9 or 24 h. GAPDH was used as a control/housekeeping marker. A chart of the density of the pp38 blot is shown as panel (**e**). The Western blots labelled **d** show representative analyses for activated and non-activated caspase-3 of control cultures and cultures treated with 3HK for periods of 0.5, 1, 2, 5, 9 or 24 h. S = 1 μM staurosporine applied for 6 h at 9 DIV as a positive control. Actin was used as a control/housekeeping marker. A chart of the density of the activated caspase-3 blot is shown as panel (**f**). (Staur = staurosporine 1 μM as positive control)

anthranilic acid, quinolinic acid, nicotinic acid or xanthurenic acid (Goldstein et al. 2000).

Iwahashi et al. (1988) have demonstrated that in cell-free buffer 3HAA produced hydroxyl radicals. This process was enhanced by SOD, a more alkaline pH, or the presence of EDTA, but was abolished by either desferrioxamine or catalase. The amount of hydroxyl radical produced in a Fenton reaction solution containing hydrogen peroxide and Fe^{3+} ions was increased by addition of 3HAA (3HK was less effective), and assays of Fe^{3+} reduction suggested that it was attributable to the presence of 3HAA under these conditions (Iwahashi et al. 1988). The same group showed that the generation of ROS was achieved more readily when *o*-, *m*- and *p*-aminophenol were added to solutions instead of 3HAA (Iwahashi et al. 1988). The presence of an aminophenol ring structure on the 5HAA molecule suggests that this may be the moiety in both 3HAA and 5HAA responsible for generating ROS. Our recent reports that inflammatory conditions, in which there is evidence for increased oxidative stress, are associated with lowered
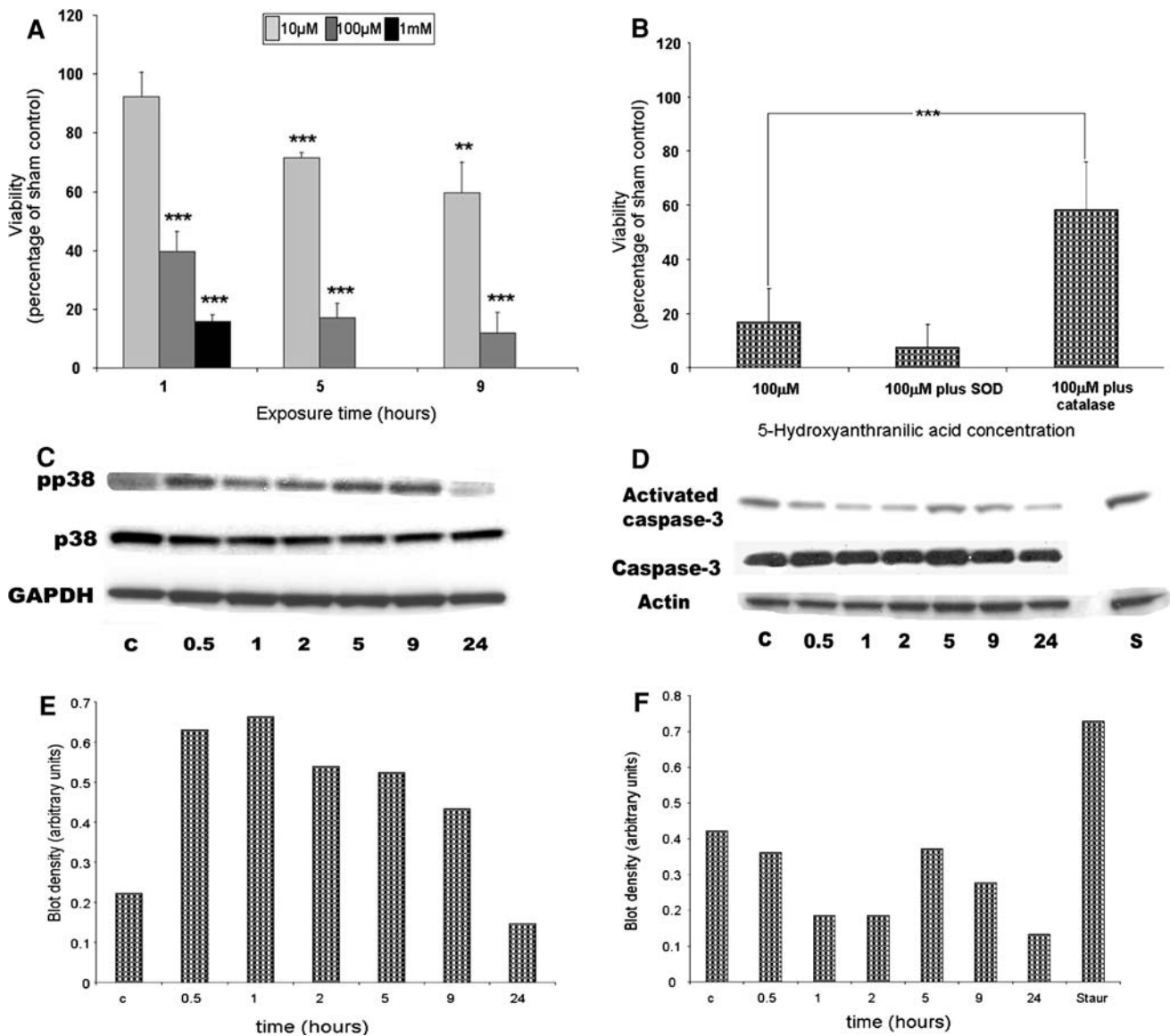
Fig. 2 Toxic effects of 5HAA on CGNs. The bar chart **a** summarises the toxic effects on CGNs at 9 DIV of 5HAA at concentrations of 10 μM, 100 μM and 1 mM, based on the mean ± SE mean for cell viability relative to untreated control cultures. $**P < 0.01$, $***P < 0.001$ for $n = 5$ in each case. The bar chart in **b** illustrates the suppression of toxicity by catalase (200 U/ml) but not SOD (200 U/ml) when 5HAA was present together with these enzymes for 5 h. $***P < 0.001$ for $n = 5$. The Western blots at **c** show representative analyses for p38MAPK (p38) and phosphorylated p38 MAPK (pp38) of control cultures and cultures treated with 5HAA

for time periods of 0.5, 1, 2, 5, 9 or 24 h. GAPDH was used as a control/housekeeping marker. A chart of the density of the pp38 blot is shown as panel (**e**). The Western blots labelled **d** show representative analyses for activated and non-activated caspase-3 of control cultures and cultures treated with 5HAA for periods of 0.5, 1, 2, 5, 9 or 24 h. S = 1 μM staurosporine applied for 6 h at 9 DIV as a positive control. Actin was used as a control/housekeeping marker. A chart of the density of the activated caspase-3 blot is shown as panel (**f**). (Staur = staurosporine 1 μM as positive control)

levels of 3-HAA and raised levels of anthranilic acid (Stoy et al. 2005; Forrest et al. 2006; Mackay et al. 2006) would be consistent with the above findings if 3HAA is partly responsible for the oxidative stress, being reduced in the process to anthranilic acid.

The present results further suggest that 3HK and 5HAA-induced cell death is independent of caspase-3 activation

but that p38 MAPK phosphorylation was involved, and thus an alternative mechanism of cell death associated with p38 signalling. One possibility is the activation of the pro-apoptotic signalling molecule tumour necrosis factor alpha (TNF-α), since TNF-α production can be attenuated by blockade of p38 (Chaparro-Huerta et al. 2005). TNF-α activates caspase-8, which can in turn trigger either

Fig. 3 a Culture of control, untreated CGNs at 9 div in which extensive neurite outgrowth (*arrows*) is evident extending from the regular-shaped cell bodies. **b** Following exposure to 100 μM 3-HK for 5 h, neurites are lost and many of the cell bodies have a shrunken and irregular form (*). **c** Treatment for 5 h with 100 μM 5HAA also caused the appearance of damaged cell bodies (*) with a general loss of neurites. The damage produced by 100 μM 5HAA was ameliorated by the presence of catalase (**d**) throughout the 5 h treatment period, with CGNs retaining a phenotype similar to untreated controls in which neurites (*arrows*) continue to extend from the cell bodies. In contrast, 0.05 g/ml cycloheximide (**e**) or SOD (**f**) failed to prevent the morphological changes with many shrunken neurons evident (*). Bar = 50 μM throughout
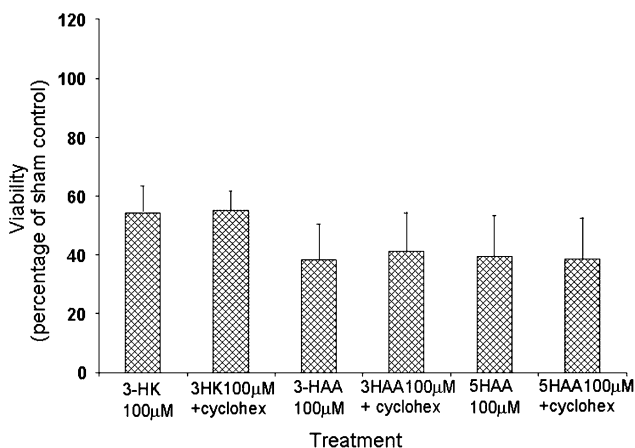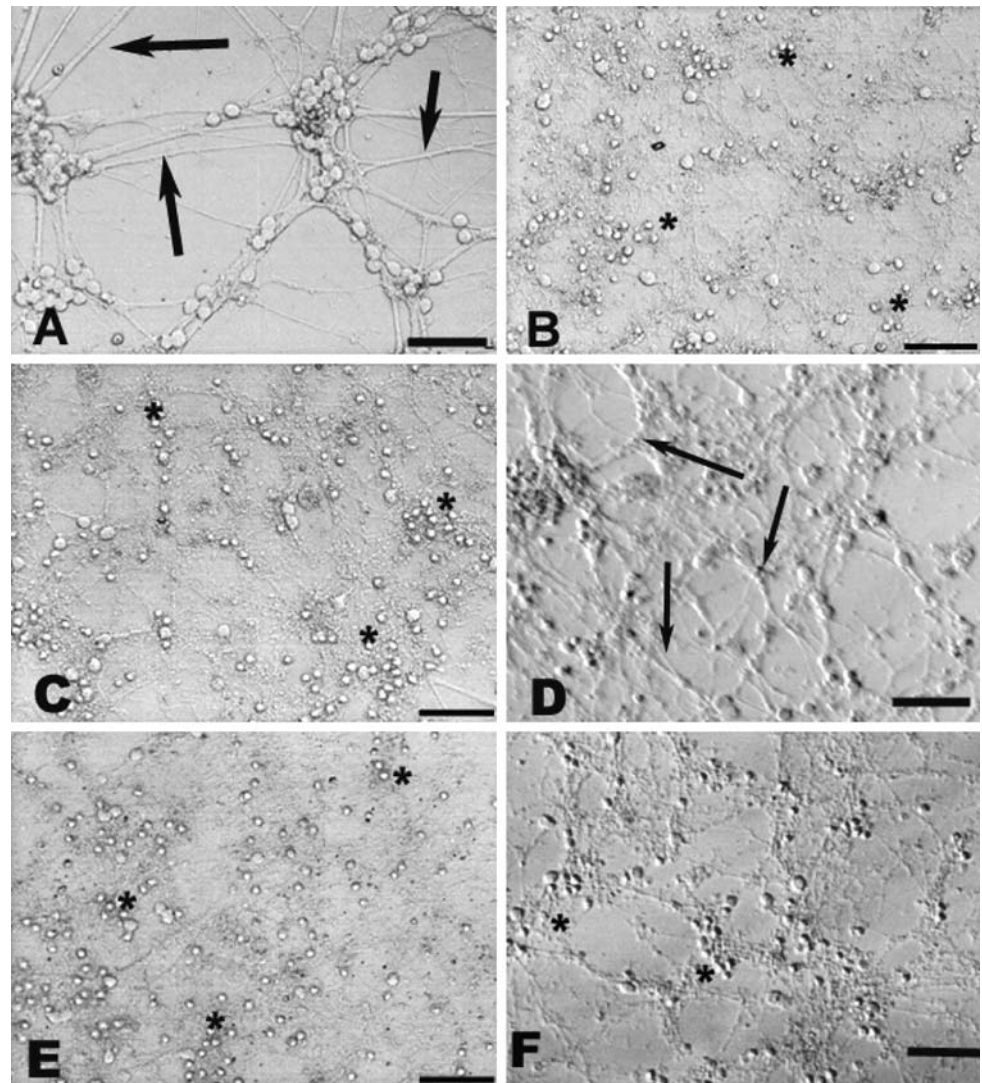

Fig. 4 Effects of cycloheximide on toxicity. The bar chart shows the failure of 0.05 μg/ml cycloheximide to prevent the toxicity of 3-HK, 3-HAA and 5HAA when present for 5 h at 9 DIV. Columns show the mean ± SEM for $n = 4$ in each case

caspase-3 or the pro-apoptotic bcl-2 family protein (Bid), thus providing a mechanism for cell death independent of capase-3 activation. It has been further demonstrated that death due to ischaemia or oxygen-glucose deprivation in cerebral cortical neurones involves signalling through caspase-8 and BID (Plesnila et al. 2001).

The failure of cycloheximide to prevent toxicity of treatment with any of the compounds suggests that new protein synthesis was not required and, by implication, that activation of increased TNF-α production was not the major cause of cell death in CGNs. An alternative explanation would be that the p38 signalling leads to activation of another executioner caspase, such as caspase-7. A previous study using striatal neurones demonstrated chromatin condensation, consistent with the induction of apoptosis, was involved in cell death following 3HK exposure (Okuda et al. 1996, 1998).

It is possible that the mechanism activated could be affected by the specific type of ROS inducing cell death. In neonatal rat CGNs, the production of superoxide and molecular oxygen were associated with chromatin condensation and activation of caspases 3, 8 and 9, indicating apoptosis. In contrast, production of hydrogen peroxide caused no increase in any of these markers, while still producing comparable levels of cell death, suggesting a necrotic mechanism (Valencia and Morán 2004).

Phosphorylation of p38 MAPK has previously been reported in neurones exposed to glutamate or quinolinic acid (Ferrer et al. 2001; Kawasaki et al. 1997), linking this signalling molecule to neuronal cell death induced by excitotoxic or oxidative stress stimuli. The current evidence of phosphorylation of this molecule by 3HK and 5HAA further strengthens this association. The toxic effects of kynurenines may be relevant pathologically: 3HK in particular has been associated with Huntington's disease, since levels are increased in mouse models of Huntington's and in the striatum of post mortem Huntington's brain disease (Browne and Beal 2006; Guidetti et al. 2004, 2006) and Huntington's disease patients show abnormalities in kynurenine metabolism (Stoy et al. 2005). More recently, a study of pneumococcal meningitis in infant Wistar rats showed significant increases in 3HK levels in cortex and hippocampus 16–44 h post-infection, with no rise found in plasma samples (Bellac et al. 2006). There was a correlation between 3HK concentrations and increased apoptosis in the hippocampus, with 3HK levels of 190 nM or higher corresponding to higher levels of apoptosis. Additionally, alterations in the activity of enzymes along the kynurenine pathway were seen, with significant rise in the activities of kynurenine-3-hydroxylase (which converts kynurenine to 3HK) and kynureninase (converting 3HK to 3HAA) in both cortex and hippocampus (Bellac et al. 2006).

There is also strong evidence linking quinolinic acid to the AIDS dementia complex (Heyes et al. 1991; Medana et al. 2003) and cerebral malaria (Sanni et al. 1998; Clark et al. 2005). In Huntington's disease brain, quinolinic acid is present in higher concentrations than controls (Guidetti and Schwarcz 1999, Guidetti et al. 2004), and in animals generates a model in which the behavioural, neurochemical and electrophysiological consequences closely mimic those seen in the human disease (Popoli et al. 1994).

In summary, it is clear that the metabolism of tryptophan causes the formation of a number of potentially significant neurotoxins that are capable of inducing extensive oxidative damage. The previously demonstrated neurotoxic potential of 3HK and 3HAA is equalled by that of 5HAA, a compound previously little studied in neurones. The damage induced by 5HAA (and by 3HK) involves activation of the p38 death signalling pathway, but does not depend on caspase-3 activation to execute cell death.

References

Behan WMH, McDonald M, Darlington LG, Stone TW (1999) Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. Br J Pharmacol 128:1754–1760

Bellac CL, Coimbra RS, Christen S, Leib SL (2006) Pneumococcal meningitis causes accumulation of neurotoxic kynurenine metabolites in brain regions prone to injury. Neurobiol Dis 24:395–402

Browne SE, Beal MF (2006) Oxidative damage in Huntington's disease pathogenesis. Antioxid Redox Signal 8:2061–2073

Chaparro-Huerta V, Rivera-Cervantes MC, Flores-Soto ME, Gómez-Pinedo U, Beas-Zárate C (2005) Proinflammatory cytokines and apoptosis following glutamate-induced excitotoxicity mediated by p38 MAPK in the hippocampus of neonatal rats. J Neuroimmunol 165:53–62

Chiarugi A, Meli E, Moroni F (2001) Similarities and differences in the neuronal death processes activated by 3OH-kynurenine and quinolinic acid. J Neurochem 77:1310–1318

Clark CJ, Mackay GM, Smythe GA, Bustamante S, Stone TW, Phillips RS (2005) Prolonged survival of a murine model of cerebral malaria by kynurenine pathway inhibition. Infect Immun 73:5249–5251

Eastman CL, Guilarte TR (1989) Cyto-toxicity of 3-hydroxykynurenine in a neuronal hybrid cell line. Brain Res 495:225–231

Eastman CL, Guilarte TR (1990) The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine. Neurochem Res 15:1101–1107

Fatokun AA, Stone TW, Smith RA (2007) Cell death in rat cerebellar granule neurons induced by hydrogen peroxide in vitro: mechanisms and protection by adenosine receptor ligands. Brain Res 1132:193–202

Ferrer I, Blanco R, Carmona M (2001) Differential expression of active, phosphorylation-dependent MAP kinases, MAPK/ERK, SAPK/JNK and p38, and specific transcription factor substrates following quinolinic acid excitotoxicity in the rat. Mol Brain Res 94:48–58

Forrest CM, Mackay GM, Oxford L, Stoy N, Stone TW, Darlington LG (2006) Kynurenine pathway metabolism in patients with osteoporosis after two years of drug treatment. Clin Exp Pharmacol Physiol 33:1078–1087

Goldstein LE, Leopold MC, Huang X, Atwood CS, Saunders AJ, Hartshorn M, Lim JT, Faget KY, Muffat JA, Scarpa RC, Chylack LT Jr, Bowden EF, Tanzi RE, Bush AI (2000) 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen peroxide and promote α-crystallin cross-linking by metal ion reduction. Biochemistry 39:7266–7275

Guidetti P, Schwarcz R (1999) 3-Hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum. Eur J Neurosci 11:3857–3863

Guidetti P, Luthi-Carter RE, Augood SJ, Schwarcz R (2004) Neostriatal and cortical quinolinate are increased in early grade Huntington's disease. Neurobiol Dis 17:455–461

Guidetti P, Bates GP, Graham RK, Hayden MR, Leavitt BR, MacDonald ME, Slow EJ, Wheeler VC, Woodman B, Schwarcz R (2006) Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington's disease. Neurobiol Dis 23:190–197

Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, Sidtis JJ, Yergey JA, Mouradian MM, Sadler AE, Keilp J, Rubinow D, Markey SP (1991) Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. Ann Neurol 29:202–209

Iwahashi H, Ishii T, Sugata R, Kido R (1988) Superoxide dismutase enhances the formation of hydroxyl radicals in the reaction of 3-hydroxyanthranilic acid with molecular oxygen. Biochem J 251:893–899

Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J Biol Chem 272:18518–18521

Kostrzewa RM, Kostrzewa JP, Brus R (2000) Dopaminergic denervation enhances susceptibility to hydroxyl radicals in rat neostriatum. Amino Acids 191:183–199

Mackay GM, Forrest CM, Stoy N, Christofides J, Egerton M, Stone TW, Darlington LG (2006) Tryptophan metabolism and oxidative stress in patients with chronic brain injury. Eur J Neurol 13:30–42

Medana IM, Day NPJ, Salahifar-Sabet H, Stocker R, Smythe G, Bwanaisa L, Njobvu A, Kayira K, Tuner GDH, Taylor TE, Hunt NH (2003) Metabolites of the kynurenine pathway of tryptophan metabolism in the cerebrospinal fluid of Malawian children with malaria. J Infect Dis 188:844–849

Morita AU, Saito K, Takemura M, Maekawa N, Fujigaki S, Fujii H, Wada H, Takeuchi S, Noma A, Seishima M (2001) 3-Hydroxy-anthranilic acid, an L-tryptophan metabolite, induces apoptosis in monocyte-derived cells stimulated by interferon-gamma. Ann Clin Biochem 38:242–251

Moroni F (1998) Tryptophan metabolism and brain function: focus on kynurenine and other indole metabolites. Eur J Pharmacol 375(special issue): 87–100

Okuda S, Nishiyama N, Saito H, Katsuki H (1996) Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. Proc Natl Acad Sci USA 93:12553–12558

Okuda S, Nishiyama N, Saito H, Katsuki H (1998) 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J Neurochem 70:299–307

Opitz CA, Wick W, Steinman L, Platten M (2007) Tryptophan degradation in autoimmune diseases. Cell Mol Life Sci 64:19–20

Perkins MN, Stone TW (1983) Pharmacology and regional variations of quinolinic acid-evoked excitations in the rat central nervous system. J Pharm Exp Ther 226:551–557

Plesnila N, Zinkel S, Le DA, Amin-Hanjani S, Wu Y, Qiu J, Chiarugi A, Thomas SS, Kohane DS, Korsmeyer SJ, Moskowitz MA (2001) BID mediates neuronal cell death after oxygen/glucose deprvation and focal cerebral ischaemia. Proc Nat Acad Sci USA 98:15318–15323

Popoli P, Pezzola A, Domenici MR, Sagratella S, Diana G, Caporali MG, Bronzetti E, Vega J, Decarolis AS (1994) Behavioral and electrophysiological correlates of the quinolinic acid rat model of Huntington's disease in rats. Brain Res Bull 35:329–335

Rotman B, Papermaster BW (1996) Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. Proc Natl Acad Sci USA 55:134–141

Sanni LA, Thomas SR, Tattam BN, Moore DE, Chaudhri G, Stocker R, Hunt NH (1998) Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and non-cerebral malaria. Am J Pathol 152:611–619

Santamaria A, Galvan-Arzate S, Lisy V, Ali SF, Duhart HM, Osorio-Rico L, Rios C, Stasny F (2001) Quinolinic acid induces oxidative stress in rat brain synaptosomes. Neuroreport 12:871–874

Schwarcz R, Pellicciari R (2002) Manipulation of brain kynurenines: glial targets, neuronal effects and clinical opportunities. J Pharmacol Exp Ther 303:1–10

Schwarcz R, Whetsell WO Jr, Mangano RM (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science 219:316–318

Smith AJ, Stone TW, Smith RA (2007) Neurotoxicity of tryptophan metabolites. Biochem Soc Trans 35:1287–1289

St'astny F, Lisy V, Mares V, Lisa R, Balcar VJ, Santamaria A (2004) Quinolinic acid induces NMDA receptor-mediated lipid peroxidation in rat brain microvessels. Redox Rep 9:229–233

Stone TW (2001) Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. Prog Neurobiol 64:185–218

Stone TW, Darlington LG (2002) Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov 1:609–620

Stoy N, Mackay GM, Forrest CM, Christofides J, Egerton M, Stone TW, Darlington LG (2005) Tryptophan metabolism and oxidative stress in patients with Huntington's disease. J Neurochem 93:611–623

Trenkner E (1998) Cerebellar cells in culture. In: Banker G, Goslin K (eds) Culturing nerve cells, 2nd edn, chap 12. MIT Press, Cambridge

Valencia A, Morán J (2004) Reactive oxygen species induce different cell death mechanisms in cultured neurons. Free Rad Biol Med 36:1112–1125

Wei H, Leeds P, Chen RW, Wei W, Leng Y, Bredesen DE, Chuang DM (2000) Neuronal apoptosis induced by pharmacological concentrations of 3-hydroxykynurenine: characterisation and protection by dantrolene and bcl-2 overexpression. J Neurochem 75:81–90

Wu HQ, Guidetti P, Goodman JH, Varasi M, Ceresoli-Borroni G, Speciale C, Scharfman HE, Schwarcz R (2000) Kynurenergic manipulations influence excitatory synaptic function and excitotoxic vulnerability in the rat hippocampus in vivo. Neuroscience 97:243–251