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Comparison of the in vitro and in vivo neurotoxicity of three new sources of kainic acid

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Summary. Historically, all commercially available kainic acid has been derived from a single biological source using a consistent method of extraction and purification. That source became unavailable in 1995. Recently, three new commercial suppliers of kainic acid have made the product available, but the source of the material and the purification processes used differ. Our objective was to systematically compare the response produced by each of these new sources of kainic acid using three established neurobiological techniques: neuronal cell culture, hippocampal slice electrophysiology, and whole animal behavioural toxicity.

Results in all three systems indicated no overall differences between the three formulations, although studies in both cerebellar neuron cultures and whole animal toxicity testing in mice, revealed some significant differences that may imply subtle differences in receptor selectivity and/or potency.

We conclude that all three sources of kainic acid are viable alternatives to traditional kainate but they may not be identical. Until further information becomes available researchers may want to avoid using the three formulations interchangeably, and take note of the source of kainic acid when evaluating literature describing results from other laboratories.

Keywords: Glutamate – Excitatory amino acids – non-NMDA receptors – Excitotoxicity

Introduction

Kainic acid is a naturally occurring excitatory amino acid (EAA) that has been used extensively in neurobiological research for many years (for review see McGeer et al., 1987). Kainic acid is presumed to exert its actions via activation of kainate receptors, a subclass of non-NMDA receptors, that are distributed extensively throughout the mammalian CNS (Wisden and Seeburg, 1993). Kainate receptors, like all ionotropic glutamate receptors, are excitatory and contribute to excitotoxic cell damage and cell death when activated by high concentrations of kainic acid or other appropriate ligands (eg. domoic acid). Excitotoxicity produced by kainic acid can be studied in a variety of *in vitro* and *in vivo* experimental systems, including neuronal cell cultures, isolated tissue preparations, and whole animal models.

Prior to 2000, the only primary supplier of kainic acid was a Taiwanese company that extracted the toxin from a red seaweed (*Digenia simplex*) that grows in tropical and subtropical waters such as those of the South China sea. This seaweed, also known as "kaininso" in Japan, has been used for centuries as a deworming agent in both humans and animals. For largely unknown reasons, however, the company that was harvesting Digenia and extracting kainic acid to supply world demand, ceased operations in 1995. Over the next several years, supplies gradually dwindled and the shortage of kainic acid became a serious problem for the neuroscience community from 1998 to 2000.

Over the last two years, three new commercial sources of kainic acid have emerged. Bristol-based Tocris-Cookson began importing Digenia from the East China sea and extracting and purifying kainic acid from this natural source in 2000. It is believed that the company now grows Digenia at another location, but details of the extraction and purification process employed are proprietary. Around the same time,

Sigma-Aldrich (St. Louis, USA) began to advertise the availability of purely synthetic kainic acid. Previous attempts to synthesize kainic acid had proved unsuccessful, until a method was developed by a small Canadian company, Precision Biochemicals (Vancouver, BC). It was generally assumed that this company was the original supplier to Sigma-Aldrich although the distributor has chosen not to reveal its source(s) and it is not known whether Precision Biochemicals is currently supplying product to Sigma-Aldrich. It is also not known whether all of the product marketed by Sigma-Aldrich derives from a single, or from multiple, suppliers. Also in 2000, kainic acid became available from BioVectra dcl, a division of Diagnostic Chemicals Ltd of Charlottetown, PEI, Canada. Diagnostic Chemicals had been known previously to the neuroscience community as the main supplier of domoic acid, a closely-related EAA. The original source of the kainic acid marketed by BioVectra dcl is a Nova Scotia-based company, Ocean Produce Inc., who grow pure cultures of mutated algae that produce kainic acid. The extraction and purification of the compound is performed at BioVectra dcl in Charlottetown.

Because there is a considerable literature base on the actions of kainic acid derived from a single source which is no longer available, our intent was to provide comparative data on the actions of kainic acid derived from each of the three new sources and suppliers, using models that are commonly employed in EAA research.

Cerebellar granule neurons in culture represent a neuronal system that has proved very useful in the study of the biochemical events coupled to excitatory amino acid neurotransmission, and the conditions controlling excitotoxicity, as these neurons express both ionotropic and metabotropic glutamate receptors. These cultures have been used previously to investigate the neurotoxic effects of kainic acid (McCaslin and Smith, 1988; Kato et al., 1991; Simonian et al., 1996; Savidge et al., 1997; Leski et al., 1999) and domoic acid (Novelli et al., 1990; Novelli et al., 1992, Fernandez-Sánchez and Novelli, 1993; Fernández-Sánchez and Novelli, 1995; Fernández-Sánchez and Novelli, 1996; Berman and Murray, 1997). Isolated hippocampal slice preparations are commonly used to explore the electrophysiological properties of both endogenous and exogenous EAAs. The hippocampus is particularly rich in kainate receptors (Wisden and Seeburg, 1993) and is known to be exquisitely sensitive to both kainic acid and domoic acid induced neurotoxicity (Debonnel et al., 1989). Whole animal toxicity models have also proven extremely useful in investigations of both kainic acid and domoic acid toxicity (Tasker et al., 1991; Tasker and Strain, 1998; Doucette et al., 2000) and in experimental investigations of epilepsy and other seizure disorders (for review see Ben-Ari, 1985).

We report herein, on the comparative actions of kainic acid derived from each of the three new sources and suppliers in each of these experimental systems.

Materials and methods

The kainic acid used in all comparisons was obtained directly from the respective suppliers, ie. synthetic kainate (Sigma-Aldrich Chemicals, St. Louis, USA), kainate derived from Digenia simplex (Tocris-Cookson, Bristol, UK) or kainate derived from mutated algae (OPIKA) (BioVectra dcl, Charlottetown, Canada).

A. Comparisons in neuronal cell cultures

Cell culture

Primary cultures of rat cerebellar neurons were prepared as previously described (Novelli et al., 1988). Briefly, cerebella from 8-day-old pups were dissected and the cells were dissociated and suspended in basal Eagle's medium with 25 mM KCl, 2 mM glutamine, 100 μg/ml gentamycin and 10% fetal calf serum. Cells were seeded in poly-L-Lysine coated (5 μg/ml) 35 mm dishes at 2.5×10^5 cells/cm^2 and incubated at 37°C in a 5% CO_2, 95% humidity, atmosphere. Cytosine arabinoside (10 μM) was added after 20–24 h of culture to inhibit the replication of non-neuronal cells. After 8 days *in vitro*, morphologically identified granule cells accounted for more than 95% of the neuronal population, the remaining 5% being essentially GABAergic neurons. Astrocytes did not exceed 3% of the overall number of cells in culture. Cerebellar neurons were kept alive for more than 40 days in culture by replenishing the growth medium with glucose every 4 days and compensating for lost amounts of water, due to evaporation.

Neurotoxicity assays

Neurons were used between 14–20 days in culture. Drugs were added to the growth medium for the indicated time, unless otherwise noted. Growth medium was then removed and cultures were incubated for 5 min. with 1 ml incubation buffer A containing (in mM): 154 NaCl, 5.6 KCl, 5.6 glucose, 8.6 HEPES, 1 $MgCl_2$, 2.3 $CaCl_2$, pH 7.4, to which the vital stain fluorescein diacetate (5 μg/ml) was added. The staining mixture was then aspirated, replaced with incubation buffer, and cultures were examined for neurotoxicity. Under fluorescent light, live neurons showed a bright green color in the cell body and neurites, while dead neurons did not retain any fluorescein diacetate, and their nuclei could be stained in red by 1 min. exposure to 50 μg/ml ethidium bromide. Photographs of three randomly selected culture fields were taken, and live and dead neurons were counted. Total number of neurons per dish was calculated considering the ratio between the area of the dish and the area of the picture (~3000).

Data presentation and analysis

For statistical analysis the one-way or the two-way analysis of variance (ANOVA) was used to identify overall treatment effects, followed by the unpaired two-tailed Student's t-test for selective comparison of individual data groups. Only significances relevant for the discussion of the data are indicated in each figure.

B. Comparisons in hippocampal slice preparations

Slice preparation and maintenance

Hippocampal slices were prepared from young adult male Sprague-Dawley rats (250 ± 25 g) according to standard procedures. All animals were treated in accordance with the guidelines of the University of Otago Animal Ethics Committee. Animals were obtained several days prior to use to reduce the confounding influence of stress from travel or handling, and were housed within the Pharmacology Department animal room at a constant temperature on a 12 hour light/dark cycle. Briefly, animals were anaesthetized with CO_2 and rapidly decapitated. Brains were dissected into ice cold oxygenated artificial cerebrospinal fluid (ACSF) and allowed to cool for approximately one minute. Brainstem and thalamus were gently retracted and the hippocampi dissected free from adjacent medial temporal lobes. Region CA3 was manually dissected away from each hippocampal formation in order to reduce hyperexcitability during electrical stimulation. Hippocampi were placed on a Stoelting-type drop-blade tissue chopper and 400 micron thick sections were cut transverse to the long axis of the hippocampus and immediately placed into a full submersion brain slice recording chamber where they were allowed to equilibrate for at least 1.5 hours prior to experimentation. In all experiments slices were constantly superfused with an artificial cerebrospinal fluid (ACSF) consisting of 124 mM NaCl, 3.2 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, and 10 mM glucose (21–23 C°, saturated with 95% O_2/5% CO_2), administered at a constant rate of 1ml/minute.

Extracellular field potential recording

Somal excitatory post-synaptic potentials (somal EPSP's) and orthodromic population spikes were evoked by positioning a twisted, bipolar 50 μm-diam teflon-coated tungsten wire stimulating electrode (A-M Systems, Carlsborg, Washington, USA) in the Schaffer collateral-commissural pathway (*stratum radiatum)* of hippocampal area CA1. Single, 100 μsec stimulus pulses were presented to the afferent fibres at 0.17 Hz using a Grass SD9 stimulator. The resulting evoked potentials were recorded through a 50 μm teflon-coated tungsten wire electrode positioned just above *stratum pyramidale* of area CA1. Waveforms were digitally acquired at 10 kHz, and were amplified and displayed on a Macintosh computer via a MacLab 4-S analog-digital converter and analyzed using "Chart" software (Analog Digital Instruments Ltd, Dunedin, New Zealand). Population spike amplitude (mV) was calculated as the difference between the most negative point of the sharp negative-going spike and the two positive points of inflection on either side. EPSP strength was assessed by measuring the slope of the somal EPSP along its rising face ($mVms^{-1}$). Raw data were normalised as a percentage change from the average baseline.

Experimental protocol

For each slice, prior to commencement of baseline recording, test stimuli were adjusted such that the level of evoked responding was at or near 50% of the maximal orthodromic population spike amplitude. Baseline potentials were continuously monitored for 30 min prior to the addition of any compound to ensure the overall health and stability of the preparation. Slices not exhibiting stable baseline potentials for at least 20 min prior to KA administration were discarded from the study. KA, dissolved in sterile deionized water was applied at a constant rate to the perfusion stream for up to 30 min, or until changes in CA1 responding indicated that maximal effects had been achieved. In no cases were any slices used following prior exposure to any of the toxins. For each compound, the concentration-dependence and time-course of specific effects were assessed off-line using automated analysis routines in Chart software, as decribed above. Three to five hippocampal slices were tested for each compound at each of the four doses examined (total $n = 45$ slices).

Data analysis

All data were expressed as mean % change from baseline levels of responding ± SEM. Neurophysiological effects were quantified in relation to the toxin concentration in the perfusate, and Student's t-test was performed to assess significance of changes after toxin application at various points along the dose-response curves. Statistical significance was determined at a confidence level of $p < 0.05$.

C. Comparisons in whole animal models

Experimental animals

Whole animal toxicity testing was conducted in groups (N = 4) of male CD-1 mice (20–24 g) obtained from Charles River Laboratories (St. Constant, Canada). Mice were group housed with food and water available ad libitum and maintained on a 12 hour light/dark cycle for a minimum of 7 days prior to experimentation. Animal housing and all experimental procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Behavioural toxicity testing

On the day of testing mice were habituated for 1 hour in a Plexiglas® test box (25 × 25 × 18 cm) that allowed for unobstructed observation by an experimenter who was blind to drug treatment. All drugs were dissolved in sterile physiological saline and administered via intraperitoneal injection in a volume of 10 ml/kg). Following drug injection mice were returned to the test box and behavioural changes occurring in 1 minute blocks were recorded every 5 minutes for up to 60 minutes according to a previously published 7 point rating scale (Tasker et al., 1991). Animals exhibiting hindlimb or whole body seizures for 2 consecutive blocks were humanely euthanized and assigned maximum scores for the remaining blocks. Each animal was used only once.

Data analysis

Cumulative toxicity scores and latency to the onset of motor seizures were analyzed by 2 way analysis of variance with post-hoc comparisons using Tukey's test where indicated. Statistical significance was determined at a confidence level of $p < 0.05$.

Results

A. Comparisons in neuronal cell cultures

Exposure of cultures to each of the three kainic acid (KA) preparations resulted in neuronal swelling and

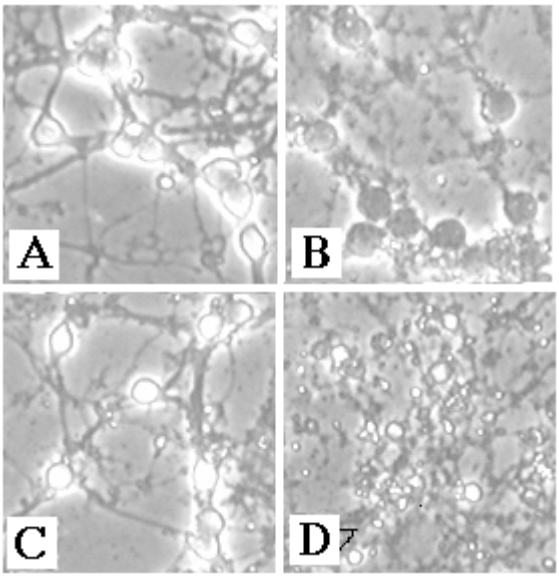

Fig. 1. Characterization of kainic acid neurotoxicity in cultured cerebellar neurons. **A** Untreated neuronal cultures; **B** Exposure to kainic acid for 30 min.; **C** Exposure to kainic acid for 30 min in the presence of 2 μM MK-801; **D** Exposure to kainic acid for 24 h. Similar data were obtained in all the experiments performed

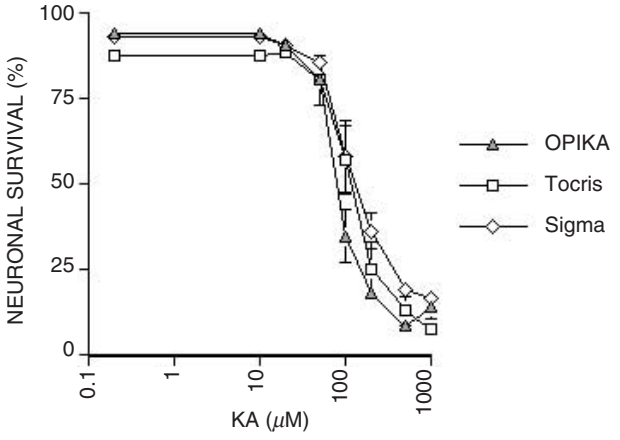

Fig. 2. Concentration-dependent toxicity by kainic acid from different suppliers. Kainic acid (*KA*) was added to the culture growth medium and toxicity was evaluated after 24 h. Data represent the mean ± SD (n = 4) from one experiment which has been repeated three times with similar results

darkening within 30 min. (Fig. 1B), and a significant reduction of neuronal survival 24 hours later (Fig. 1D). This pattern of toxicity was similar to that produced by exposure of cultures to toxic concentrations of exogenous glutamate (Novelli et al., 1988). Furthermore, the early signs of KA toxicity indicated by swelling and darkening of the neuronal cell bodies were completely abolished by the specific NMDA receptor antagonist MK-801 (2 μM) (Fig. 1C), indicating that this phase of KA toxicity was mediated through the release of excitatory amino acids acting at the NMDA receptor. The presence of MK-801 did not prevent the development of neurotoxicity after 24 h exposure to KA, which was similar to that produced by KA alone (data not shown).

Concentration-dependent neurotoxicity curves for each KA formulation revealed no statistically significant differences, with each product producing 50% and 100% neurotoxicity at approximately 100 μM and 500 μM respectively (Fig. 2). In order to further investigate possible differences in the toxic action of the three KA formulations, cultures were incubated in the absence of glucose for 30 min. followed by exposure for 60 min. to a concentration of KA that does not elicit excitotoxicity in glucose supplemented cultures (Novelli et al., 1988, 1990). Cultures were then returned to their own growth medium for 24 h to allow for the possible development of neurodegeneration. As shown in Table 1, 10 μM KA was able to produce early (30–60 minute) signs of neurotoxicity only in glucose-deprived cultures, and to produce a small but significant reduction in neuronal survival after 24 h. However, no differences in neuronal survival were observed when KA from the three commercial sources was compared.

To further investigate the actions of each KA formulation, a series of experiments were conducted in which cultures were exposed to both KA and a variety of EAA agonists and antagonists. It is well established that KA may act at non-NMDA receptors composed by different subunits (Chittajallu et al., 1999; Dingledine et al., 1999) although KA toxicity in cerebellar granule cells in culture is believed to be mediated only by AMPA and GluR5–6 receptors, as it can be effectively prevented by quisqualic acid (McCaslin and Smith, 1988).

No differences (data not shown) between the kainate formulations were observed when cultures were pre-treated with NS-102, a specific antagonist of GluR6 (Johansen et al., 1993; Tasker et al., 1996), GYKI 52466, a non-competitive AMPA/kainate antagonist, or AMPA (in order to desensitize the response through this receptor) (Lerma et al., 1993; Fernández-Sánchez and Novelli, 1996), although possible effects of AMPA at the heteromeric GluR6-KA2 receptor can not be excluded. Pre-treatment with

Table 1. Glucose deprivation facilitate kainate neurotoxicity

Drugs (μM)	Conditions			
	Acute low glucose		Normal glucose	
	Toxicity 30′	Neuronal survival 24 h	Toxicity 30′	Neuronal survival 24 h
None	−	82 ± 1.3	−	92 ± 3.3
KA-OPIKA 10	++	68 ± 1.5	−	94 ± 2.8
KA-Sigma 10	++	75 ± 3.6	−	93 ± 1.0
KA-Tocris 10	++	67 ± 1.0	−	88 ± 1.6

Cerebellar neurons in primary culture were exposed to 10 μM Kainic Acid (KA) from three different suppliers for 60 min in the incubation buffer A either with or without glucose. Cultures were observed under phase contrast microscopy either for the presence (+) or the absence (−) early signs of toxicity such as swelling and darkening of the cell body, after 30 min. Morphological changes were recorded with a TV camera. Then the incubation solution was replaced for the culture own incubation medium, and neuronal survival was evaluated after 24 h as indicated in the methods. Data are the mean ± SD (n = 4) from one experiment that has been repeated with similar results.

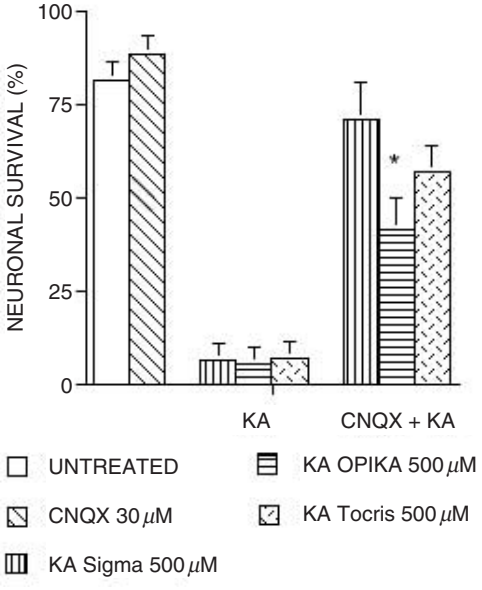

Fig. 3. Effect of CNQX on kainic acid toxicity. Neuronal survival after 24 h exposure to 500 μM KA from the indicated suppliers was determined in untreated neurons and in neurons pretreated for 1 min with 30 μM CNQX. All the drugs were added to the culture growth medium. Represented values are the mean ± SD (n = 4). *p < 0.01 vs. KA Sigma

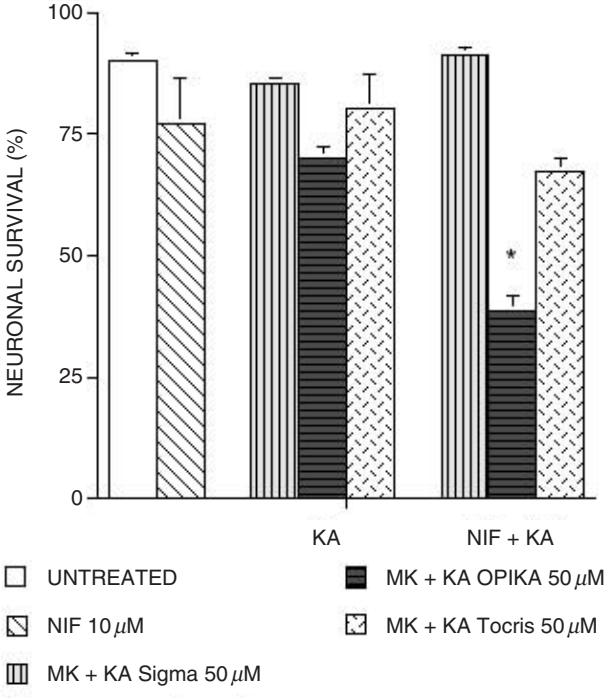

Fig. 4. Effect of nifedipine on kainic acid toxicity. Neuronal survival after 24 h exposure to 50 μM KA from the indicated suppliers, was determined in neurons pretreated for 2 min with MK-801, both in the absence or in the presence of 10 μM Nifedipine (*NIF*). All the drugs were added to the culture growth medium. NIF was added 1 min. before KA Represented values are the mean ± SD (n = 4). *p < 0.01 vs. KA alone

CNQX (a competitive AMPA receptor antagonist), however, provided significantly less protection against OPIKA exposure than the other two KA formulations (Fig. 3). Finally, we tried to discriminate among the activities of the three KAs by favoring KA toxicity through the block of L-type voltage sensitive calcium channels (VSCC) (Leski et al., 1999). As shown in Fig. 4, pretreatment of neurons with nifedipine (10 μM), selectively and significantly increased the toxicity of 50 μM OPIKA relative to the Tocris or Sigma products.

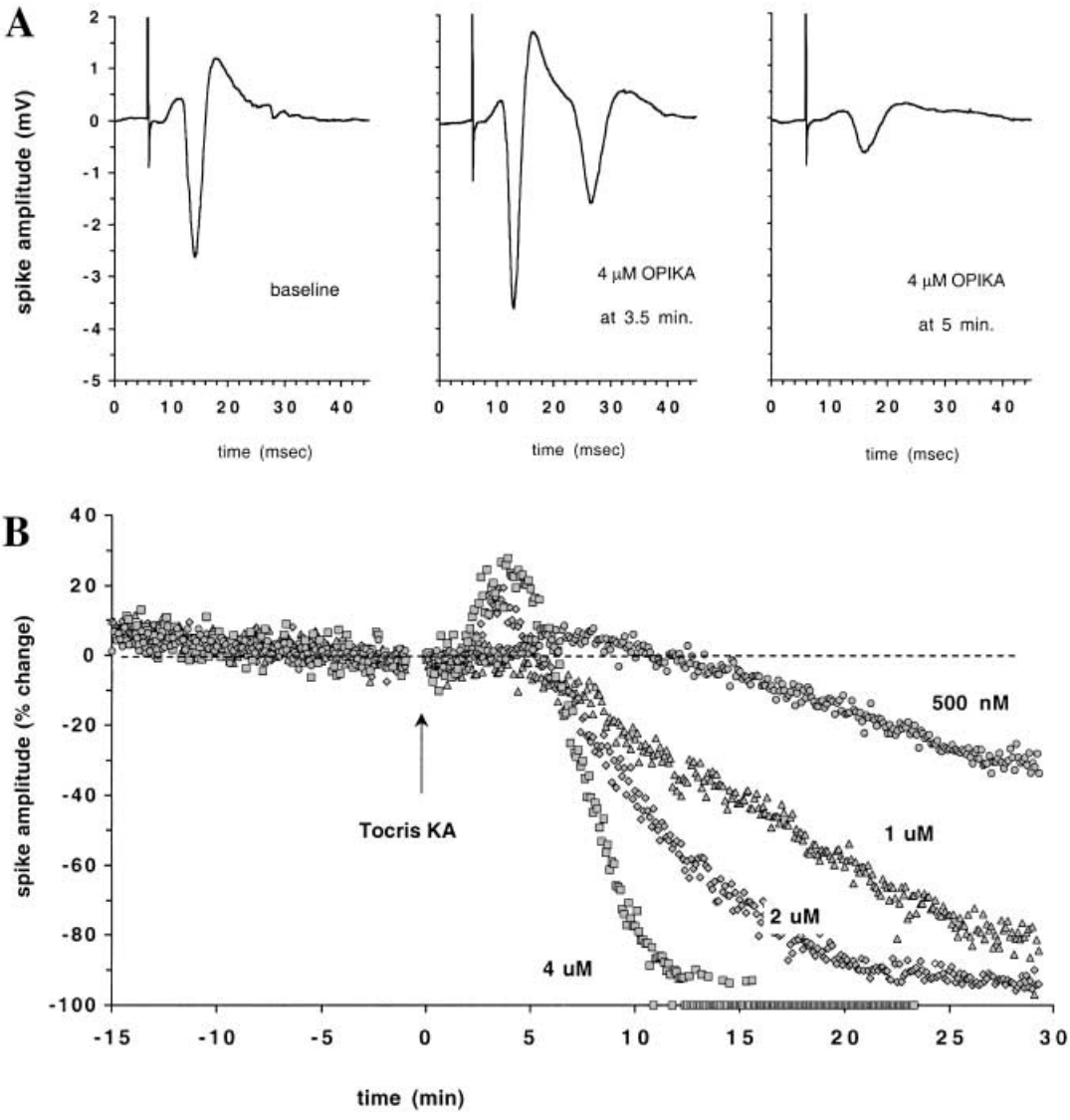

Fig. 5. Transient hyperexcitability followed by population spike suppression during administration of kainic acid to hippocampal region CA1. (**A**) Representative waveforms from a single hippocampal slice before (baseline) and after exposure to 4 μM OPIKA. Axes indicate waveform amplitude in millivolts (mV) vs duration in milliseconds (msec). Hyperexcitability, as evidenced by an increase in the absolute amplitude of the primary (ie., first) evoked population spike and the spontaneous appearance of second and third spikes, was routinely observed during KA administration. With prolonged administration profound spike suppression was observed at the highest doses. (**B**) Dot plots of % change in CA1population spike amplitude across time following Tocris KA administration at four doses shown

B. Comparisons in hippocampal slice preparations

Kainic acid obtained from all three suppliers (OPIKA, Tocris and Sigma) produced similar and essentially equivalent effects on hippocampal CA1 neuronal excitablity *in vitro.* Changes in CA1 responding were both dose- and time-dependent. For all three compounds, pronounced hyperexcitability was seen during the first few minutes of exposure as evidenced by significant increases in population spike amplitude and by the appearance of second and third spontaneous spikes, particularly at the higher doses (2–4 uM; Fig. 5A). With prolonged administration, population spikes underwent a dose-dependent suppression paralleled by a concomitant reduction in field EPSP strength over the 30 minutes of drug application (Fig. 5B). In addition, in a small subset of slices KA was washed out of the preparation, and in all cases, evoked responses were restored to baseline levels of responding, suggesting that the spike suppression seen during KA was not pathological (Fig. 6A). Taken together, these KA effects were qualitatively similar to those

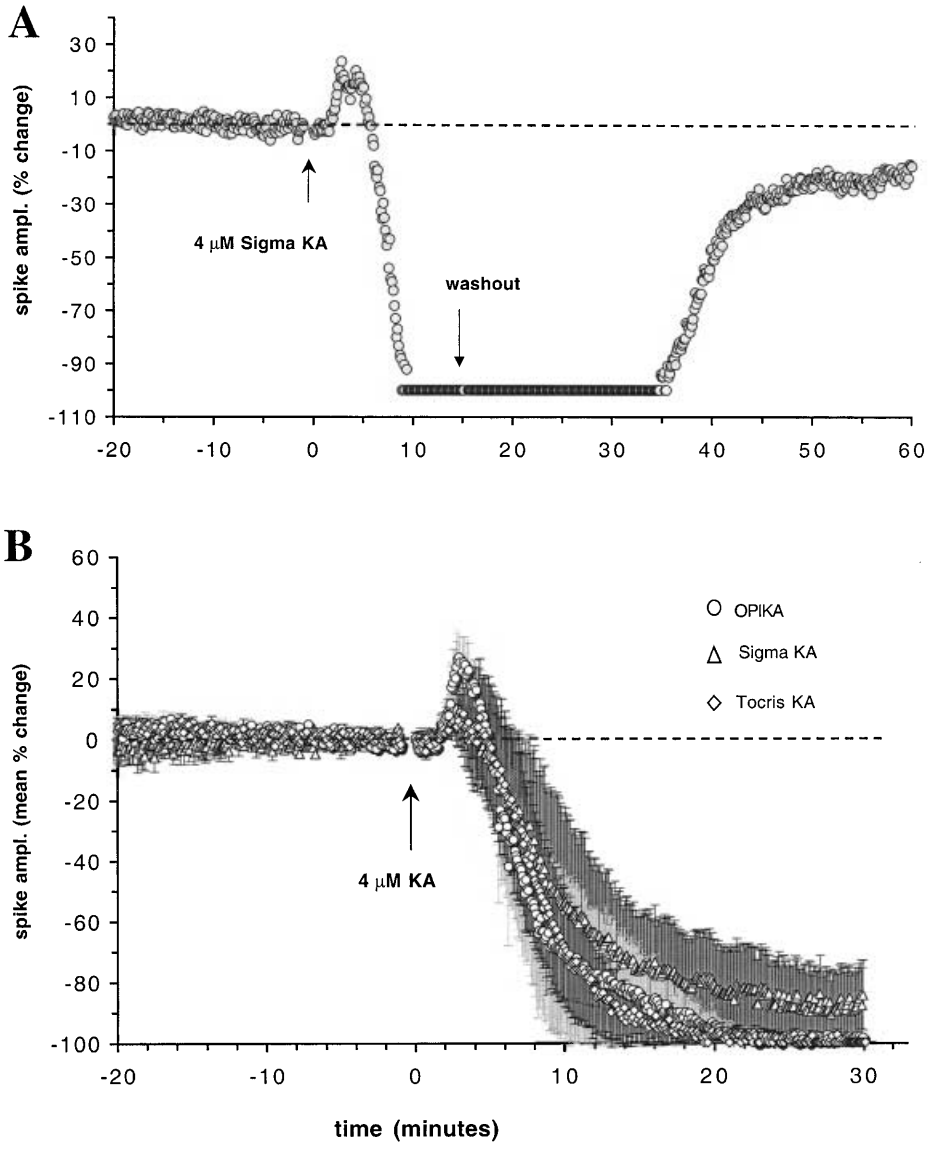

Fig. 6. Acute effects of high dose kainic acid on CA1 potentials. (**A**) KA effects are reversible with washout. Shown is a dot-plot of the time course of effects of 4 μM Sigma KA in a single hippocampal slice. After a lengthy washout, evoked responses recovered to near baseline levels. (**B**) Dot-plots of mean % change (±s.e.m.) in orthodromic evoked population spike amplitude across time following KA in hippocampal region CA1. Arrow indicates point of KA administration

effects observed for domoic acid under comparable *in vitro* conditions (Sari and Kerr, 2001).

The lowest dose of KA examined here (500 nM) generally produced little if any spike suppression with prolonged administration; at this dose, population spike amplitude slowly increased during wash-in and hyperexcitability (multiple spiking) often persisted throughout the drug application period. Higher doses (1–2 uM) typically produced a transient period of hyperexcitability followed by moderate to strong suppression of evoked responding over the next 20 to 30 minutes. At the highest dose (4 uM) KA produced a rapid and robust increase in primary population spike amplitude, strong driving of epileptiform activity, and a complete suppression of evoked responding within 15–20 minutes (Fig. 6B). As noted, all three compounds (Tocris KA, Sigma KA and OPIKA) produced virtually identical patterns of change in CA1, and no significant differences were detected between compounds in either induction of early hyperexcitability or suppression of neuronal activity over time.

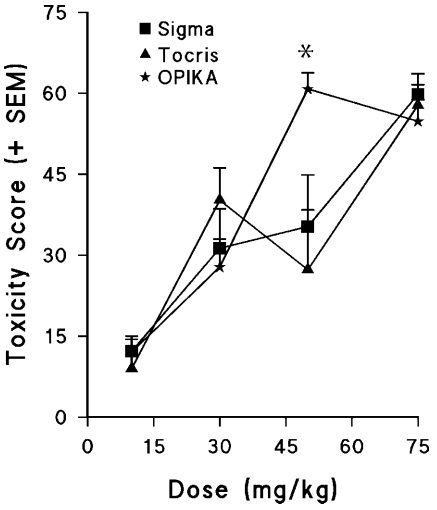

Fig. 7. Cumulative toxicity response curves for three formulations of kainic acid administered i.p. in mice (N = 4 per group). Maximum behavioural toxicity score is recorded every 5th minute over a 60 minute test session according to a previously published rating scale. * indicates $p < 0.05$

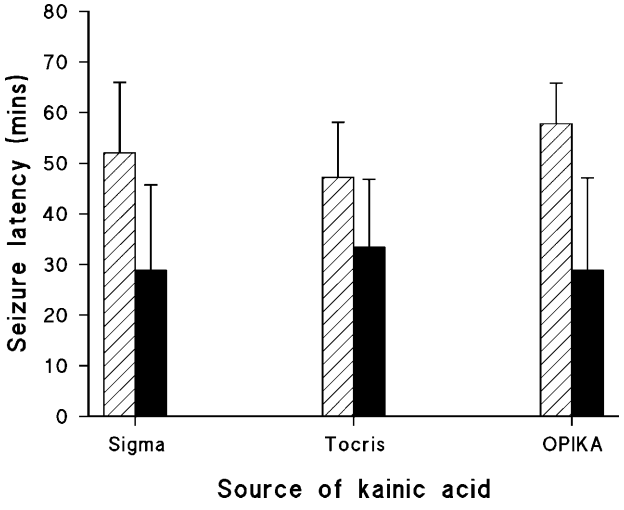

Fig. 8. Mean (+/−SEM) latency to the onset of hind-limb of whole body seizures in groups of mice (N = 4 per group) injected i.p with either 50 mg/kg (hatched bars) or 100 mg/kg (solid bars) kainic acid obtained from three different suppliers

Likewise, no differences between compounds were evident when assessing the slope of the CA1 somal EPSP *in vitro*. All three forms of KA produced steady, statistically significant dose-dependent reductions in EPSP strength during the 30 minutes of toxin administration (data not shown). However, pairwise comparisons between compounds on a dose-by-dose basis failed to reveal any differences in the magnitude of the effect on EPSPs.

C. Comparisons in whole animal models

Dose-response curves for cumulative behavioural toxicity produced by all three formulations of KA are presented in Fig. 7. Each form of KA produced responses typically associated with dose-dependent increases in KA concentration and there were no overall differences in DRCs, although OPIKA produced significantly greater toxicity than the other two formulations at the 50 mg/kg dose (Fig. 7).

Because KA is often used in whole animal models of epilepsy, we also examined the latency to the onset of motor seizures in mice. The results are presented in Fig. 8. Examination of these data reveals that there were no differences in seizure latency between the formulations at either of the two highest doses tested. None of the formulations tested produced motor seizures at either the 10 mg/kg or 30 mg/kg dose. While there were no significant differences in motor seizure latency, however, there did appear to be qualitative differences between the formulations; with mice receiving synthetic kainic acid tending to show intermittent low grade seizures (manifesting mainly as wet dog shakes and forelimb tremors) throughout the test period, whereas the two naturally-derived forms of kainic acid produced more of a traditional dose-dependency profile.

Discussion

Kainic acid has been used extensively in neurobiological research for many years. Because all previous supplies of kainic acid derived from a single biological source, results obtained in different laboratories could be compared with some degree of confidence. The sudden loss of this source in 1995 caused widespread concern throughout the neuroscience community. The recent emergence of three new commercial sources of kainic acid was welcome news, but as with any new supplier or source of an important research tool, it is necessary to systematically evaluate these new sources of kainic acid relative to both the historical literature and relative to each other.

Studies conducted in Dr. Novelli's laboratory using isolated cerebellar neurons in culture revealed characteristic patterns of excitotoxicity (Fig. 1) and no significant differences between the formulations with respect to dose-response (Fig. 2). However, in cell

cultures OPIKA was significantly less susceptible to antagonism with the AMPA antagonist CNQX (Fig. 3) and was more responsive to potentiation of toxicity by nifedipine (Fig. 4). Collectively these data could indicate that OPIKA is slightly more selective for kainate receptors and/or slightly more potent than the other two formulations, but neither of these interpretations is fully supported by the other results obtained in neuronal cultures, or indeed, by either of the other two experimental systems. These phenomena probably warrant further investigation in both cerebellar neurons and other types of neuronal cell culture.

In the hippocampal slice preparation KA is well known to produce a transient potentiation of CA1 population spikes, neuronal hyperexcitability as evidenced by multiple spiking, and prolonged spike suppression at higher doses (Robinson and Deadwyler, 1981; Collingridge et al., 1983; Westbrook and Lothman, 1983). The present findings are in agreement with recent results from Dr. Kerr's laboratory in which it was found that KA induces a transient hyperexcitability in isolated CA1 at doses up to 500 nM. The current study extended those findings by assessing KA at several higher concentrations (up to 4 uM) and has confirmed that KA induces patterns of change in area CA1 which are strikingly similar to those effects seen during the administration of domoic acid at much lower doses (Sari and Kerr, in press). The gradual reduction in field EPSP strength and population spike suppression seen during prolonged KA administration (Figs. 5 and 6) is most likely due to a depolarizing conduction block following prolonged AMPA or KA receptor activation (Lipski et al., 1988; Lees and Sandburg, 1991), and is consistent with the ability of AMPA to desensitize the response to KA-induced toxicity in neuronal cell cultures (see above). With respect to comparisons between the three formulations, however, in the hippocampal slice preparation all three compounds produced similar patterns of change in region CA1 and equivalent effects across a range of *in vitro* concentrations, suggesting that essential differences in commercial production do not affect the neuropharmacology or potency of KA.

In studies of whole animal toxicity in mice, similar results were obtained. Each of the three kainic acid formulations produced dose-dependent behavioural changes that are consistent with previous studies of both kainic and domoic acid (Tasker et al., 1991; Tasker and Strain, 1998). The dose-response curves for each compound following i.p. injection were very steep (Fig. 7), although it is possible that the DRC produced by OPIKA is slightly steeper than that produced by the other two formulations, because of the significantly increased toxicity of OPIKA at the 50 mg/kg dose (Fig. 7). More detailed dose-response curves are probably required to determine if this is true, but it is interesting to note that the finding is consistent with the results described previously in neuronal cell cultures (see above and Figs. 3 and 4). Similarly, the three KA products did not differ significantly in their ability to induce motor seizures in mice (Fig. 8), although there may be qualitative differences in the seizures produced by synthetic versus natural kainic acid. This speculation is supported in part by the finding that an experimenter-blind observer was able to accurately group all of the animals receiving synthetic kainate as being different from mice receiving either of the other two formulations. Quantitative analysis of this difference in whole animals, however, is problematic.

In summary, we have found that all three of the new sources of kainic acid produce comparable effects in each of the experimental systems studied. We did, however, find some subtle, but significant, differences between them. Until further information appears in the literature, therefore, it would seem prudent for individual laboratories to select a single supplier whose material is of consistent quality. Moreover, investigators comparing results between different laboratories should take careful note of the source of the kainic acid used.

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