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Selection of a Pure Cerebellar Granule Cell Culture by Kainate Treatment

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Chronic exposure of dissociated cerebellar cultures to 50 μM kainate results in a complete loss of [^{3}H]-GABA release which is a marker of GABAergic interneurons. No loss of granule cells was found and the glutamatergic nature of the granule cells appeared unaltered by the kainate treatment, since evoked release of [^{3}H]-D-aspartate was maintained after kainate exposure. Glial cells in such cultures are virtually eliminated by treatment with an antimitotic such as cytarabin. In consequence a pure culture of cerebellar granule cells virtually free of stellate, basket and glial cells may be obtained by a combined chronic treatment of the cultures with kainate and cytarabin.

KEY WORDS: Kainate; cerebellar granule cells; neuronal culture; exitotoxicity; [^{3}H]-GABA release; [^{3}H]-D-aspartate release.

INTRODUCTION

Dissociated cell cultures derived from early postnatal mouse cerebellum contain 80–90% small bipolar granule cells and a small proportion of GABA-ergic interneurons (1, 2, 3). It has been shown that the glial contamination of these neuronal cultures may be kept below 2% by treatment of the cultures with antimitotics such as cytarabin (2–4).

Such neuronal cultures have been widely used during the last decade primarily as a neurochemical model for studies of excitatory amino acid mediated neurotransmission (5-8). Due to the fact that the majority of cells in such cultures are granule cells the cultures are often referred to as granule cell cultures. However, from autoradiographic studies of [^{3}H]-GABA uptake (2, 9, 10), [^{3}H]-GABA release studies (11, 12) and studies of immunoreactivity for the GABA synthesizing enzyme glutamate decarboxylase (12, 13) it is evident that 5–10% of the neurons in the cultures are GABA-ergic interneurons of the basket and stellate types. Most neurochemical studies using these cerebellar cultures are performed under the assumption that the culture is a pure glutamatergic granule cell culture. However, the presence of a small but significant number of GABA-ergic neurons in the cultures may seriously complicate the interpretation of such studies.

In the cerebellar cortex in vivo (14, 15) and in vitro (16–18) it has been found that GABAergic neurons are selectively sensitive to kainate toxicity. Here we have studied the effect of chronic kainate exposure on cerebellar neuronal cultures with respect to viability and glutamatergic function.

EXPERIMENTAL PROCEDURE

Cultures obtained from dissociated cerebella of 7-day-old NMRI-mice were maintained for 8 days in vitro in the presence of cytarabin (5) and subsequently used for release experiments and viability testing.

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Some cultures were exposed to kainate (10–200 μM) from day 5 in vitro. Release of ^{3}H-GABA (80 μCi/mmol, Amersham) was studied in cerebellar cultures grown in Petri dishes (60 mm) using the model described by Drejer et al. (19) for cultured cortical neurons. Cells were preloaded for 30 min by addition of 50 μCi [^{3}H]-GABA to each culture. The GABA transaminase inhibitor γ-vinyl-GABA (100 μM) (kindly donated by Merrell Dow Research Institute) was added at the same time in order to prevent degradation of [^{3}H]-GABA (19). After the preincubation period cells were superfused with a HEPES buffered saline and stimulated every 4 min for 30 sec by changing the superfusion medium to a corresponding medium containing L-glutamate (range 5–200 μM). Superfusate was collected in a fraction collector and radioactivities in each fraction determined by liquid scintillation counting. Potassium evoked release of [^{3}H]-D-aspartate was studied as detailed by Drejer et al. (20) using a similar setup as described above.

Cell viability in kainate treated cultures was measured by incubating for 20 min with the tetrazole compound MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenylterazoleum, Sigma) (0.2 mg/ml) (21). The blue formazan formed from MTT in the mitochondria of the live cells was solubilized in isopropanol and measured spectrophotometrically at 570 nm.

RESULTS

Figure 1 shows the relative number of live cells in cerebellar cultures chronically exposed to increasing concentrations of kainate. In the presence of 10–50 μM kainate more than 90% of the neurons appeared to survive. Chronic exposure to significantly higher concentrations of kainate (≥ 200 μM) were found to cause significant cell death in the cultures.

Figure 2a and 2b show photomicrographs of cerebellar neurons cultured in the absence (a) or presence (b) of 50 μM kainate. The granule cells which are dominating the cultures appear morphologically identical under the two culture conditions. No differences in Ca-dependent K^+-stimulated release of [^{3}H]-D-aspartate was seen between untreated (Figure 2e) and treated (Figure 2f) cultures.

However, in the kainate treated cultures it was not possible to demonstrate a significant glutamate evoked [^{3}H]-GABA release (Figure 2d) whereas in control cultures a pronounced glutamate evoked release of preloaded [^{3}H]-GABA was seen (Fig. 2c).

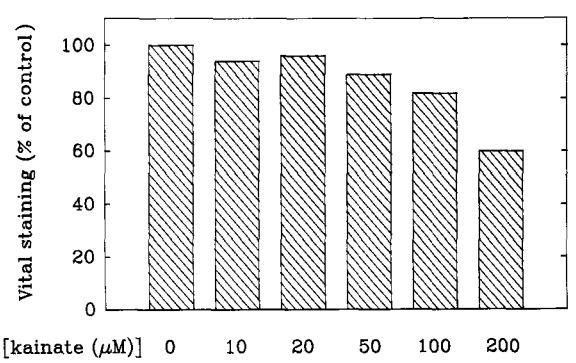

Fig. 1. Vital staining of cerebellar neurons cultured for 8 days in the absence or presence of kainate. The bars represents the mean vital stain from 3 individual Petri dishes expressed relative to the vital stain in control cultures (100%). Concentrations of kainate are given in the figure. The tetrazole compound MTT (see text) was used as vital stain (21).

DISCUSSION

The finding that the cerebellar neuronal cultures both morphologically and with respect to viability were more than 90% insensitive to 50 μM kainate, has been confirmed using the lactate dehydrogenase viability assay (A. Frandsen, unpublished). The selective survival of granule cells after kainate treatment has also been demonstrated both in cerebellar slices (18) in explant cultures (16) and in vivo (14, 15). That the neurons in these cultures are indeed granule cells has been confirmed by electron microscopy which showed cells with prominent nuclei, characteristic of granule cells (22).

The demonstrated specific degeneration of GABAergic functions, expressed as evoked [^{3}H]-GABA release, is in agreement with the previous finding by Messer and Maskin (17) that kainate treatment of cerebellar cultures resulted in a substantial loss of [^{3}H]-GABA uptake. Exposure of such cultures to kainate apparently also leads to a significant loss of the GABA synthesizing enzyme glutamate decarboxylase (17). Moreover, it should be emphasized that not only glutamate induced [^{3}H]-GABA release is eliminated by kainate treatment but also K^+-stimulated [^{3}H]-GABA release is completely abolished by kainate treatment (23). This is important since it might be argued that the kainate treatment could have desensitized glutamate receptors on the GABAergic neurons rather than actually destroying the cells.

The finding of the present study as well as that of Schousboe and Pasantes-Morales (23) that evoked [^{3}H]-D-aspartate release is insensitive to chronic kainate exposure indicate that the glutamatergic function of the granule cells is maintained.

In summary, it has been demonstrated that cerebellar granule cells in culture are insensitive to chronic exposure to low (≤ 50 μM) concentrations of kainate. However, the present as well as previous studies (16–18) have shown that 50 μM kainate is toxic to GABAergic interneurons (basket cells, stellate cells) in cerebellar cultures. This means that cerebellar neuronal cultures derived from early postnatal mice or rats may become

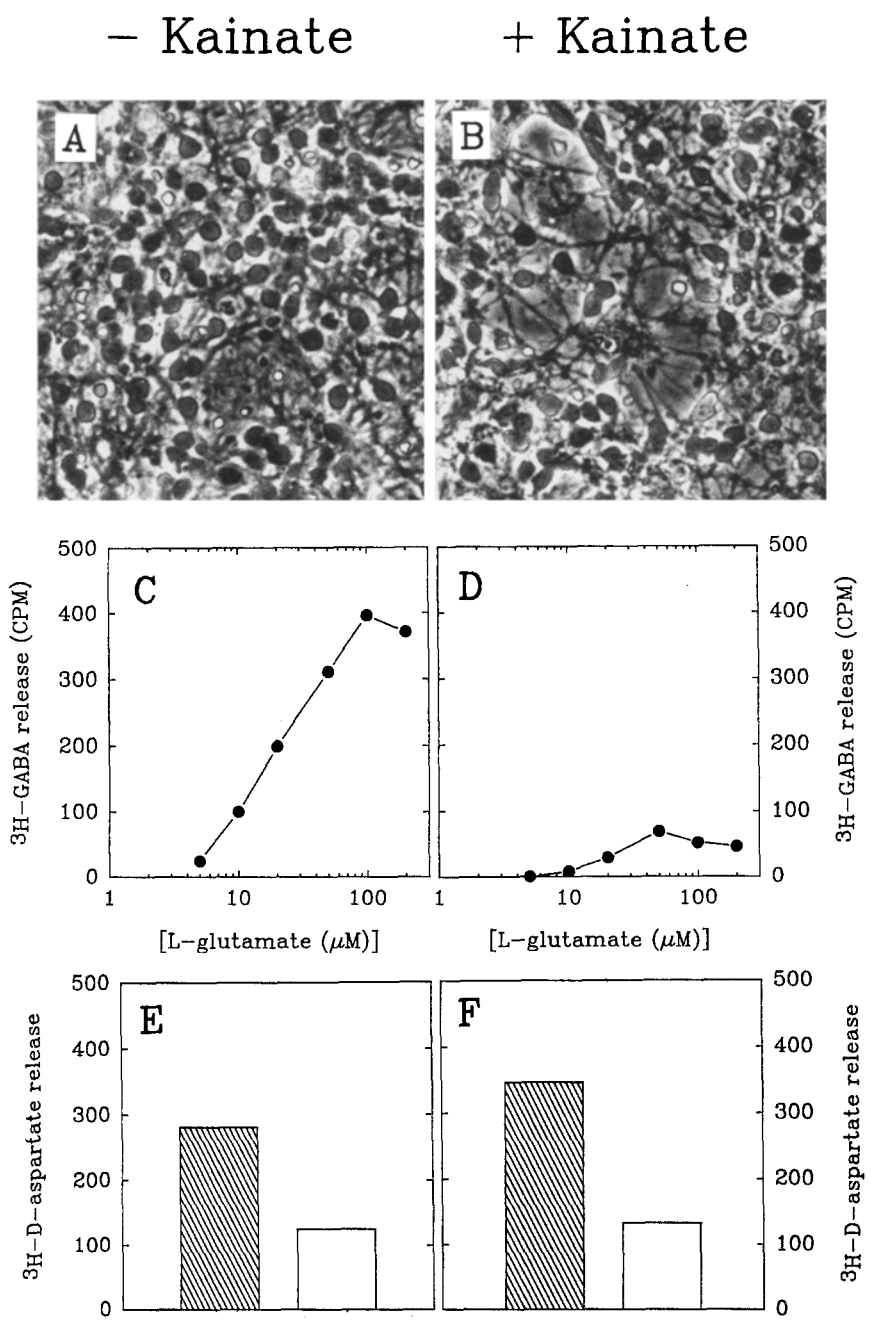

Fig. 2. (a.) Photomicrograph (200 ×) of cerebellar neuronal cultures maintained for 8 days in vitro in the absence of kainate. (b.) Parallel cultures supplemented with 50 μM kainate from day 5 in vitro. (c.) Glutamate induced [^{3}H]-GABA release from cerebellar neurons cultured in the absence of kainate and (d.) in the presence of 50 μM kainate. Results expressed in CPM are the means (corrected for basal release) of experiments performed in duplicates. Experiments were repeated with qualitatively similar results. (e.) Potassium (55 mM) induced release of [^{3}H]-D-aspartate from cerebellar neurons cultured in the absence of kainate and (f.) presence of 50 μM kainate. Release experiments were performed with Ca^{2+}-containing media (1 mM Ca^{2+}, hatched bars) and with Ca-free media (added 1 mM $CoCl_2$, open bars). Results expressed as % of basal release are the means of experiments performed in duplicates. Experiments were repeated with qualitatively similar results.

pure granule cell cultures by the inclusion of 50 μM kainate in the culture media. Since cerebellar granule cells are believed to be glutamatergic, pure granule cell cultures obtained by a combined kainate and cytarabin treatment may be a useful model in the neurochemical evaluation of glutamate mediated neurotransmission. This

procedure has recently been used to show that Ca^{2+} independent, K^+-stimulated release of taurine from cerebellar cultures originates specifically from granule cells (23).

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