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Nimodipine Aggravates Systemic Kainic Acid Toxicity in Retinal Ganglion Cells of Intact Mice

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Abstract: The aim of the present study was to investigate the role of the L-type voltage dependent Ca^{2+} channel blocker, nimodipine, in kainate induced toxicity in retinal ganglion cells of mice. Kainate in 10mg/kg was administered intraperitoneally following the vehicle or nimodipine. Nimodipine, 45 minutes prior to kainate in 10, 15, 25, 50, 100 and 250 mg/kg doses was administered intraperitoneally. Surprisingly, nimodipine potentiated the ganglional cell death induced by kainate, with respect to the control and kainate+vehicle treated group. In accordance with our results, blockage of L-type calcium channels by nimodipine may worsen the prognosis and survival of retinal neurons in kainate receptor stimulated toxicity. Blocking of calcium channels may lead to the rebound activation of sodium channels or stimulate the release of intracellular calcium by activating ryanodine sensitive calcium channels.

Key Words: Kainate, retina, nimodipine, neurotoxicity, mice.

Introduction

The uncontrolled release of neurotransmitters has been suggested to play a major role in mediating ischemic damage in susceptible brain regions (1). Most studies have emphasized the detrimental role of excessive release of the excitatory amino acid, glutamate, in producing ischemic injury (2). The main glutamate receptor involved in the cytotoxic process is the receptor sensitive to N-methyl-D-aspartate (NMDA) which is permeable to Na^+ and Ca^{2+}(3). However, another ionotropic receptor, the α-amino-3 hydroxy-5-methyl-4-isoxasole propionate/ kainate (AMPA/KA) receptor, particularly distributed on retinal neurons, can also be involved in ischemic neuronal degeneration (4). In the retina, photoreceptors and bipolar neurons are immunoreactive for glutamate (5). In the retina, seven genes encoding functional subunits of AMPA/KA type glutamate receptors have been identified using in situ hybridization, suggesting the possible presence of Ca^{2+}-permeable AMPA/KA receptors in this tissue (6). Using the patch-clamp technique, the presence of calcium permeable non-NMDA Na^+/Ca^{2+} channels in retinal neurons was demonstrated (7). Na^+ influx can be involved in the toxic process by leading to osmotic stress (8) but also, via depolarization, by opening voltage operated calcium channels. Ca^{2+} influx can damage neurons by activating various enzymes such as xanthine oxidase and nitric oxide synthetase (9) that was shown to play an important role in excitatory amino acid-induced neuronal injury by generating oxygen derived free radicals. On the other hand, calcium channel blockers were reported to undergo selective neuronal degradation in cerebral cortical cultures of rats in a concentration dependent fashion (10) and this was attenuated by protein synthesis inhibitors. This result has been explained by the fact that a decrease in intraneuronal calcium levels may lead to synthesis of proteins mediating neuronal cell death.

Methods

Albino mice weighing 28-36 g, were used. Mice were housed at 23-25 °C and 60% humidity with a 12/12 hour light/dark (light on 06:00, off at 18:00) cycle. Mice were given rat chow and tap water ad libitum. Each experimental group consisted of eight mice. In accordance with a previous study (11), the most toxic systemic concentration of KA of 10 µg/kg was administered intraperitoneally following the vehicle or nimodipine. Nimodipine, 45 minutes prior to KA in 10, 15, 25, 50,

100 and 250 µg/kg doses was used intraperitoneally. Animals were allowed to survive for an additional 48 hours after the last injection. The mice were sacrificed by decapitation and the eyes were removed immediately and stored in 10% buffered formaldehyde for 72 hours, horizontal sections passing through the plate of optic nerve were taken, embedded in parafin, and cut in 4µm-thick sections. Following this procedure, the eyes were stained with hematoxylin-eosin. Slide labels were covered with tape to enable blind evaluation. Under light microscopic examination, ganglion cells were counted in the ganglion cell layer in 3 high-power (10x40 magnification) fields. For each preparation, three areas were examined. Average number of intact ganglion cells per preparation was calculated.

The scores were analyzed statistically with the Kolmogorov-Smirnov test with $p < 0.05$ required for significance.

Results

Mean±SEM scores of intact cells were 94.63±3.36 for the control and 82.33±3.12 for 10 mg/kg KA-administered group (Z:1.44 p:0.031). Surprisingly,

nimodipine induced cell death in systemic KA toxicity in ganglion cells of the retina with respect to the control and KA+vehicle treated group. Intact cell scores were 60.0±1.57 for 10 µg/kg, 58.28±1.85 for 15 µg/kg, 55.58±1.94 for 25 µg/kg, 55.39±3.21 for 50 µg/kg, 69.28±1.66 for 100 µg/kg and 50.58±1.44 for 250 mg/kg nimodipine plus 10 µg/kg KA injected groups. All the scores presented above were highly statistically significant with respect to the control ($p < 0.0001$ for all) and with respect to the 10 µg/kg KA+vehicle administered group ($p < 0.0005$ for 100 µg/kg nimodipine+KA, $p < 0.0001$ for others). The data are shown in figure 1. Microscopic photographs of intact and degenerated retinal ganglional cells are presented in figure 2 a-b-c.

Discussion

Since the importance of the involvement of extracellular Ca^{+2} in the neurodegenerative process induced by excitatory amino acids in ischemia was unclear, to detect the neuroprotective effect of Ca^{+2} channel blockers, some important studies were carried out and some Ca^{+2} antagonists were reported to prevent the
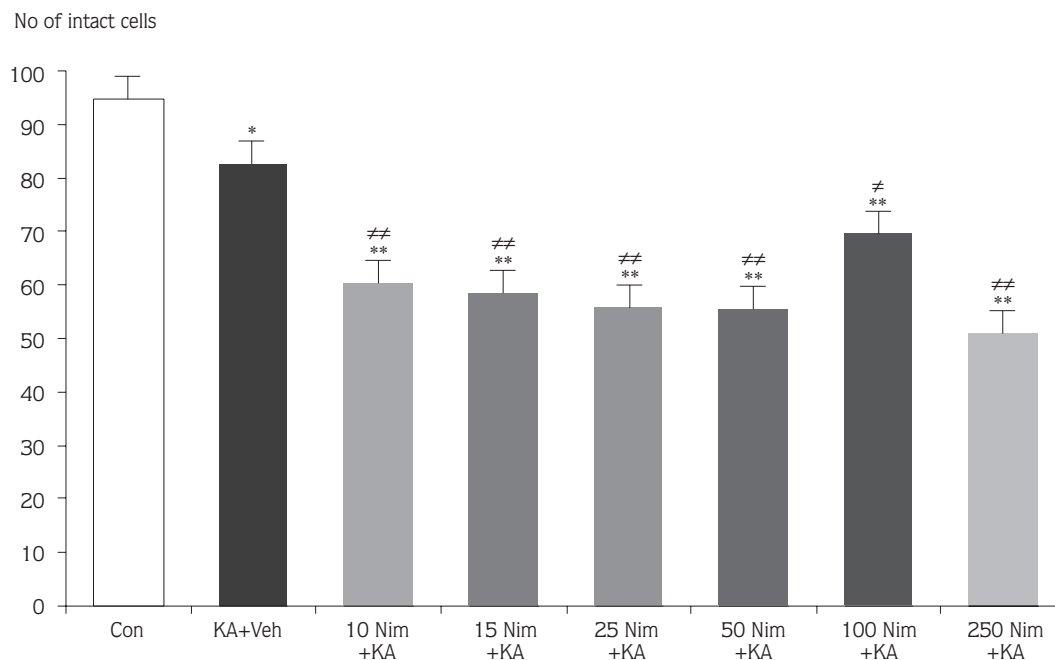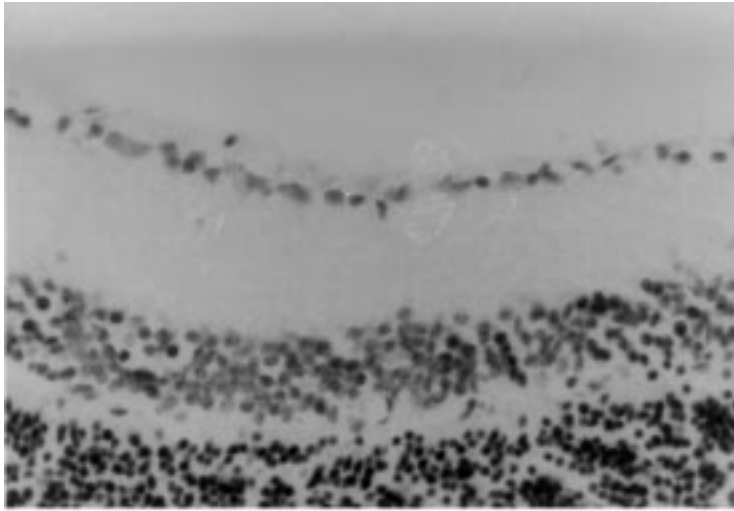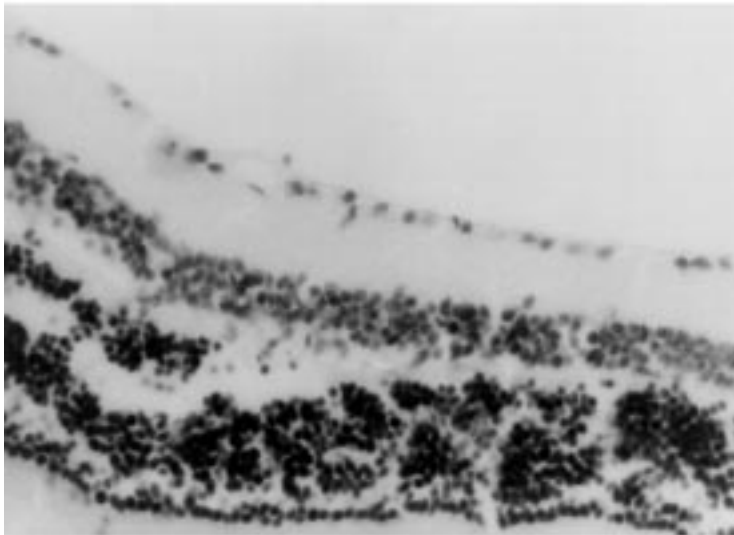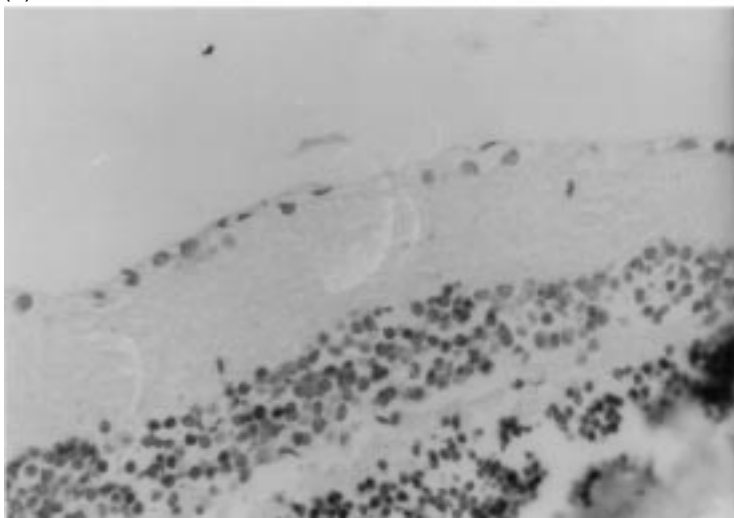

No of intact cells

Figure 1.   Intact neuronal cell population mean scores per area in 10x40 magnification in kainate and nimodipine or vehicle administered mice. Con: Control, KA: Kainate (10 mg/kg intraperitoneally), Veh: Vehicle, Nim: Nimodipine (µM). *p<0.05 **p<0.0001 with respect to control, ≠p<0.0005, ≠≠p<0.0001 (n=7 for each group).

(a)

(b)

(c)

Figure 2. Photomicrographs show control group (a), slight retinal ischemic damage belongs to 100 nM Nim+KA group(b) and severe ischemic damage belongs to 250 nM Nim+KA group (c) . (10X40).

neuronal damage of ischemia in vivo (12). In general, in vitro experiments revealed only a slight effect of these drugs (13).

Our present paper demonstrates that as a Ca^{+2} channel blocker, nimodipine, had no neuroprotective effect and even worsened KA-induced ganglional cell death in mice retina. In the literature, a few studies described the neuroprotective effects of calcium channel blockers in retinal ischemia (14, 15). In primary culture of rat cerebellar granule cells, Pizzi and co-workers found that nifedipine used at 100 nM concentration, significantly counteracted the neuronal death induced by 15 minute application of 50 µM glutamate (16) Zhang and co-workers also showed that nifedipine had less effect on reducing the rise in intracellular Ca^{+2} level that causes neuronal death (17).

The efficacy of different Ca^{+2} entry blockers was evaluated in preventing the neurodegenerative effects induced by stimulation of distinct glutamate receptor subtypes so far and it was evaluated that different Ca^{+2} antagonists produced different pharmacological potentiation on blockage of voltage operated channels and receptor operated channels in the central system (17). These differences may be due to the different binding affinities and concentrations of these antagonists. It is quite clear that the calcium channel blockers do not show equal activity towards all calcium channels and some major differences in selectivity occur between tissues and also it is possible that selectivity of antagonism may occur between different vascular beds (18). In retinal cells, Ca^{+2} influx is induced through AMPA/KA receptor stimulation

(19), however intracellular Ca^{+2} is also subsequently mobilized through a Ca^{+2} induced Ca^{+2} release in KA induced retinal neurons (3). The using patch-clamp technique, the presence of non-NMDA Na^+/Ca^{+2} channels in retinal neurons was also demonstrated (7). On the other hand, the involvement of Na^+ influx in excitatory amino acid induced toxicity was reported (8).

Koh and Cotman reported that cerebral cortical cultures exposed for 2 days to different Ca^{+2} channel blockers undergo selective neuronal degeneration in a concentration dependent fashion and this degeneration could be attenuated by protein synthesis inhibitors. It was suggested in their studies that a decrease in intraneuronal calcium levels using Ca^{+2} channel blockers may trigger synthesis of proteins mediating neuronal cell death (10).

In accordance with our results, blockage of L-type calcium channels by nimodipine may worsen the prognosis and survival of retinal neurons in KA receptor stimulated toxicity. Since there is now evidence for a bidirectional Na^+ - Ca^{+2} exchange system that mediates movement of Ca^{+2} across the sarcolemma, blocking of calcium channels may lead to the rebound activation of sodium channels or stimulate the release of intracellular calcium from stores by activating ryanodine sensitive calcium channels (20).

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