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Effect of Lithium and Valproic Acid on Kainate-Induced Neurotoxicity in Cerebral Cortical Cell

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In the current study, the effects of valproic acid alone, valproic acid plus lithium, or lithium alone on kainate-induced neurotoxicity in cerebral cortical cell cultures of rat pups have been investigated.

Key Words: Kainate, Valproic acid, Lithium, Neuroprotection, Cortical neuron.

INTRODUCTION

Lithium has commonly used in the treatment of bipolar disorders since 1949[1]. Despite it has different chemical structure from lithium, valproic acid, also has quite high efficacy in mania. It seems more effective than lithium in rapid cycling and dysphoric mania[2]. It has showed that the valproic acid and the lithium protect neurons from apoptosis, induced by different apoptotic stimuli. The mechanisms underlying their mood-stabilizing actions and their antiapoptotic proporties remain to be elucidated. It was suggested that the drugs exert their actions by means of different mechanisms[3]. Recently, lithium was found neuroprotective against a variety of both; *in vitro* and *in vivo*[4]. Lithium has protective properties against toxic effects of glutamate and N-methyl-D-aspartate (NMDA) receptor activation, low potassium, nerve growth factor deprivation, radiation, β-amyloid, quinolinic acid, glycogen synthase kinase 3β (GSK-3β) overexpression coupled to staurosporine addition, calcium, 1-methyl-4-phenylpyridinium and ouabain-induced cell death[5,6]. The fact that it's mood stabilizing actions begin after a lag period, suggest the possibility of alterations at genomic level[7]. Lithium was also found to increase the expression of the antiapoptotic gene, B-cell lymphoma/leukaemia-2 (bcl-2), in rat cerebellar granular cells[8]. Additionally, both lithium and valproic acid regulate AP-1 mediated gene expression[9,10]. Unlike valproic acid, lithium provides neuroprotection by supressing the levels of caspase-3[11]. Finally, lithium was reported to inhibit glycogen synthase kinase 3β (GSK-3β) and reduces p53 levels which is a proapoptotic protein[12].

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Such as lithium, the possible neuroprotective mechanisms of valproic acid were reported as an increase in the levels of bcl-2, increase in glutamate release, decrease in inositol uptake and inhibition of GSK-3β[13,14]. In the state of epilepsy, several mechanisms of action of valproic acid has also been identified: Increase in GABAergic activity, blocking effects of NMDA receptors and ion channels[15]. Valproic acid protects mature cerebellar granule cells in cultures from blocking effects of glutamate-induced, NMDA receptor-mediated excitotoxicity[16].

Kainic acid used frequently in neurotoxicity researches. It is 30- to 100-fold more potent than glutamate as a neuronal excitant. Several mechanisms of action for kainic acid-induced neural cell death have identified, related to inhibiting the release of GABA, the influx of sodium and calcium[17]. It is hypothesized that the neuroprotective effects of both lithium and valproic acid may arise due to partly blocking activity of the kainate subtypes of glutamate receptors.

In this study, the effects of lithium and valproic acid on kainate receptor-mediated excitotoxicity in cultured cortical neurons of rats are evaluated and also discussed their possible underlying mechanisms.

EXPERIMENTAL

Cell culture: Primary cultures of parietal cerebral cortex cells were prepared from one day old newborn Sprague Dawley rats. Briefly, newborn rats were decapitated and parietal cortical area was dissected out. It was suspended in 5 mL of calcium-free Minimal Essential Medium (MEM, Sigma Co, St. Louis USA) containing 2 mL of trypsin-EDTA (0.25 % trypsin-0.02 % EDTA, Biol Ind, Haemek Israel) at 37 °C for 20 min. Trypsin digestion was ended by 10 mL HBSS containing deoxyribonuclease type 1 (120 units per mL, Sigma Co, St. Louis USA). After 3 min of centrifugation at 800 rpm, the pellet was resuspended in minimal essential medium. Cell suspensions (0.2 mL) were plated in 2.5 mL of medium that contained 10 % fetal calf serum (FCS, Biol Ind, Haemek Israel) and MEM without antibiotics in 25 cm^2, poly-D-lysine coated polypropylene tissue culture flasks. Following a 0.5 h period, media were changed for eliminating non-adhered cells. The culture dishes were kept at 37 °C in humidified 95 % air and 5 % CO_2. After 24-48 h, 10 µM cytosine arabinoside (cytosine 1-β-D-arabinofuranoside) was added to the culture medium to prevent the replication of non-neuronal cells. Culture media were changed twice a week, neurons were tested for neurotoxicity experiments after 8 d *in vitro*. Each experimental group was tested in 5 culture media (n = 5) and at least 20 microscopic areas were counted for each medium tested.

Designing experiment: Before 45 min of kainic acid (Sigma Co, St. Louis USA) addition, lithium in 10^{-4} M (distilled water for control) and valproic acid in 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M concentrations were applied to culture flasks. Lithium and kainic acid were tested in combined application in 10^{-3} M for each. Then, kainic acid (10^{-4} M) was added into the culture dishes. They were further incubated at 37 °C in humidified 95 % air and 5 % CO_2 for additional 16 h. Cultures were dyed with

1.5 mL 0.4 % trypan blue and between 5-15 min, neuronal cell death was assessed by dye exclusion test with an inverted light microscope by a scientist who was unaware of the content of the culture flasks.

Statistics: The mean ± SEM scores of the neurons were calculated. The scores were translated to the percentage of dead cell population. They were analyzed statistically by using ANOVA followed by a multiple comparison post-hoc test and then independent samples-t test with $p < 0.05$ was considered as indicative of significance.

RESULTS AND DISCUSSION

Valproic acid in only 10^{-3} M concentration, induced cortical cell death ($p < 0.001$), whereas other concentrations did not, as seen in Fig. 1. Kainic acid was able to induce cortical cell death at a 10^{-4} M concentration, as expected. Death cell score was found as 23.14 ± 1.8 %, while it was found 6.13 ± 0.7 % in the control group ($p < 0.0001$). Lithium was found ineffective in preventing (22.88 ± 3.3 %) cell death. Valproic acid was found to have protective property in only 10^{-4} M concentration (16.78 ± 1.7 %, t: 2.6, $p < 0.05$), while it was not protective in 10^{-3} (27.5 ± 2.5 %), 10^{-5} (20.8 ± 1.4 %) and 10^{-6} (21.67 ± 3.3 %) M concentrations. Combined applications of lithium and valproic acid in 10^{-3} M, for each, was found ineffective to prevent neurotoxicity of kainic acid (23.9 ± 2.8 %). However, when valproic acid in 10^{-4} M concentration was applied with lithium in 10^{-3} M, it was found effective statistically (14 ± 2.3 %, $p < 0.01$). For further details, please refer to Figs. 1 and 2.

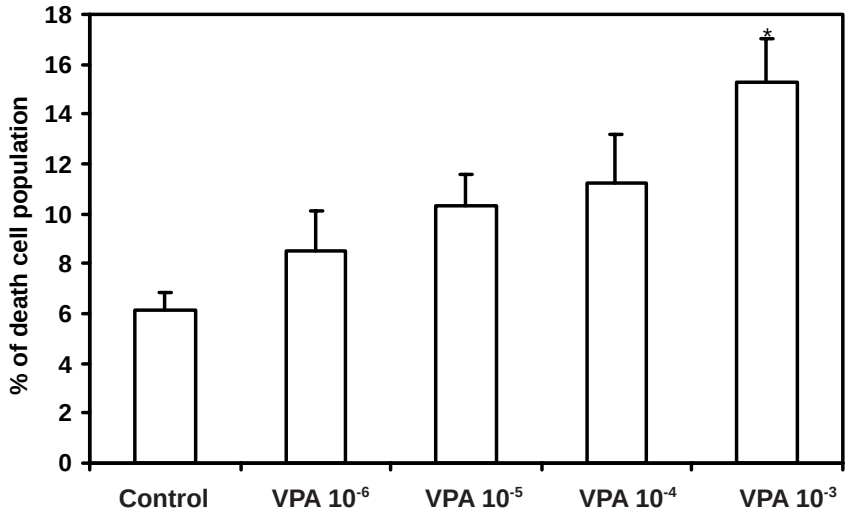

Fig. 1. Effects of valproic acid (VPA) on % of death cells in the population of cortical neuronal cultures. (*) $p < 0.001$ with respect to the control group

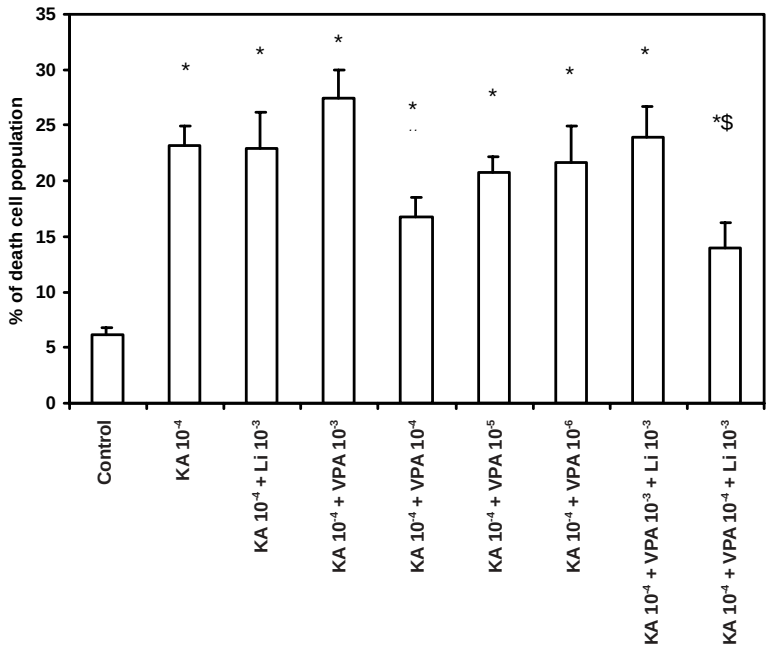

Fig. 2. Bar graph represents the effects of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M valproic acid alone or in combination with lithium at concentrations of 10^{-3} and 10^{-4} M or 10^{-3} M lithium alone in kainate-indued neurotoxicity in cerebral cortical cell cultures of rat pups. (*) $p < 0.0001$ with respect to control group. (#) $p < 0.05$ with respect to kainate group. ($) $p < 0.01$ with respect to kainate group

Kainic acid exerted a neurotoxic effect. However, low doses of kainate was reported to show trophic effects such as increases in survival and neurite outgrowth of cultured neurons derived from cerebellum, hippocampus and spinal cord[18]. Therefore, we used kainate in higher concentration (10^{-4}) to induce excitotoxicity in our model. The results of this study provide that valproic acid protects cerebral cortical neurons from kainate-induced excitotoxicity in culture. The neuroprotective effect was reported to occur at only 10^{-4} M concentration[16]. On the other hand, it has been documented that; valproic acid treatment after kainate-induced status epilepticus prevents many of kainate-induced neurologic sequelaes[19].

The selectivity of the protection was evaluated by using lithium, another mood stabilizer. Valproic acid and lithium share some possible mechanisms, such as an effect on glutamate release, decrease in inositol uptake, increase in the levels of bcl-2 and inhibition of GSK-3β[20]. In the present study, lithium alone and combined application with valproic acid were found ineffective to prevent neurotoxicity of kainate. This may show that the neuroprotective effect of valproic acid is selective and therefore the effect in the present study can not be explained by the mechanisms mentioned above.

Although little is known about the kainate receptors, as non-NMDA- receptor subtype, cloning studies have showed that kainate receptors consist of 5 different subunits including GluR5, GluR6, GluR7 (low-affinity kainate receptors), KA1 and KA2 (high-affinity kainate receptors). Kainate receptors modulate synaptic transmission by both pre- and postsynaptic mechanisms, generating large sodium influx through receptor-gated ion channels. Then, chloride and water molecules with sodium ion passively move into neurons and glial cells[21]. This osmotic overload may lead to neural cell damage. Furthermore, kainate produces focal swelling on the dendrites of hippocampal interneurons and primary cortical neuron cultures[16]. Valproic acid acts by blocking voltage-dependent sodium channels and increases calcium-dependent potassium conductance[22]. In the present case, valproic acid in 10^{-4} M concentration may block voltage-dependent sodium channels. In addition to sodium influx, it has been shown that the calcium influx also plays an important role in kainate-induced cell death[23]. In fact, the GluR2 receptor, an α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptor subtype, as well as kainate1 and kainate2 receptors, have very low calcium permeability and induced by kainate[17]. This intracellular calcium increase may be responsible for the stimulation of calcium-dependent enzymes including phospholipase A_2 and calpain II during kainate-induced neurotoxicity. It has also been reported that kainate-induced cell death may be due to some conditions, such as release of arachidonic acid from neural membrane phospholipids, accumulation of lipid peroxides, alterations in prostaglandin levels, decline in reduced glutathione (GSH) content and accumulation of 4-hydroxynonenal[17]. According to that, valproic acid may block one of the enzymatic cascade induced by calcium influx. In the present study, 10^{-4} M of valproic acid may prevent calcium influx by blocking external calcium channels. It was also reported that valproic acid blocked amyloid β-peptide neurotoxicity by attenuation of the elevation of intracellular free calcium levels elicited by amyloid β-peptide or glutamate[24]. Additionally, it has been recently demonstrated that valproic acid increases the expression of the chaperone GRP78 which is a protein that binds calcium in the endoplasmic reticulum and protects cells from the delerious effects of damaged proteins[25]. These possible mechanisms of valproic acid may also play an important role in valproic acid mediated neuroprotection in the present study. Kainate also stimulates AMPA receptors[17]. However, valproic acid was reported to modulate physiological responses mediated by AMPA receptors[26].

For lithium, it was shown that the drug has a neuroprotective effect against various insults as mentioned above[5]. In the present study, lithium alone was applied into the cultures in its therapeutic concentration, but it was not neuroprotective against kainate-induced cell death or in combined application with valproic acid either. However, acute treatment (45 min) may cause effectiveless of lithium. On the other hand, the neuroprotective effect of lithium in the literature, was reported to occur possibly *via* blockade of non-kainate receptors such as glutamate or NMDA. Nonaka *et al.*[27] also reported that chronic lithium treatment protected neurons against excitotoxicity by inhibiting NMDA receptor-mediated calcium influx.

Conclusion

According to the findings presented above, specifying the mechanism of action of valproic acid and further studies are necessary, especially with compounds that specifically block non-kainate receptors.

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