BUTTERWORTH HEINEMANN

Low zinc status impairs calcium uptake by hippocampal synaptosomes stimulated by potassium but not by N-methyl-D-aspartate

Jimmy D. Browning and Boyd L. O'Dell

Department of Biochemistry, University of Missouri, Columbia, MO USA

Zinc deficiency results in neuropathology affecting both the peripheral and central nervous systems. A previous study showed that decreased calcium uptake by cortical synaptosomes was associated with the peripheral neuropathy in guinea pigs. Deficiency impaired the calcium uptake stimulated by high potassium and by additional glutamate. In this study, the effect of zinc status on potassium-stimulated and agonist (glutamate and N-methyl-D-aspartate [NMDA])–stimulated calcium uptake by both cortical and hippocampal synaptosomes was examined. Groups of guinea pigs were allowed to consume a low zinc (<1 mg/kg) diet ad libitum (−ZN) and an adequate zinc (100 mg/kg) diet either ad libitum (+AL) or restricted (+RF). Synaptosomes were prepared from cortex and hippocampus and calcium uptake measured using ^{45}Ca. Potassium-stimulated calcium uptake by cortical and hippocampal synaptosomes was significantly lower in synaptosomes from zinc deficient guinea pigs than in controls, 15% and 20%, respectively. Glutamate-stimulated calcium uptake by cortical synaptosomes from deficient animals was 32% less than that of controls; there was a similar trend in hippocampal synaptosomes. Zinc deficiency had no effect on the NMDA-stimulated uptake by synaptosomes from either source. Impairment of voltage-gated calcium channels appears to account for the decreased calcium uptake and may explain the neuropathology observed in zinc deficiency. (J. Nutr. Biochem. 6:588–594, 1995.)

Keywords: zinc status; guinea pig synaptosomes; calcium uptake; potassium depolarization; glutamate; N-methyl-D-aspartate (NMDA)

Introduction

Zinc plays key roles in both the central and peripheral nervous systems.[1] In the chicken[2] and the guinea pig,[3,4] zinc deficiency results in abnormal posture and locomotion, clinical signs that are associated with decreased sciatic motor nerve conduction velocity, and decreased muscle action potential in both species.[5,6] The clinical signs and depressed conduction velocity are similar to those of peripheral neuropathy and are readily reversed by zinc therapy. Zinc deficiency in rats results in impaired cognitive and learning ability.[7]

The role of calcium as a second messenger in the peripheral and central nervous systems (CNS) is well recognized. Depolarization of neuronal membranes by an action potential results in activation of presynaptic voltage-gated calcium channels (VGCC),[8] and the resulting transient increase in cytosolic calcium is coupled to neurotransmitter release at neuromuscular junctions[8] and at CNS synapses.[9] This presynaptic function of cytosolic calcium is sensitive to in vitro zinc concentration. Zinc blocks VGCC of dorsal root ganglion cells[10] and the VGCC at the mouse neuromuscular junction, resulting in decreased release of transmitter[11] and decreased magnitude of mini-end-plate potentials.[12]

Glutamate is the primary excitatory neurotransmitter in the CNS.[13] One type of postsynaptic glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, is associated

A contribution of the Missouri Agricultural Experiment Station. Supported in part by NRICGP/USDA grant 91-37200-62.
Address reprint requests to Dr. Boyd L. O'Dell at the Department of Biochemistry, 112 Animal Science Research Center, University of Missouri, Columbia, MO 65211, USA.
Received November 28, 1994; accepted January 6, 1995.

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(95)00125-J

with a calcium channel. Application of exogenous glutamate stimulates an increase in the cytosolic calcium concentration of neurons,[14,15] synaptosomes,[16] and microsacs.[17] In addition to the agonist receptor that binds glutamate and the more specific agonist, NMDA, the NMDA receptor/channel complex has modulatory binding sites for glycine, polyamines, dissociative anesthetics such as phencyclidine (PCP) and dizocilpine (MK-801), magnesium, and zinc.[18] Glycine potentiates the activating effect of glutamate[18] while zinc, added in vitro, blocks calcium uptake via the NMDA channel,[19] possibly by inhibiting glycine binding.[20]

Uptake of calcium via the NMDA channel is required for long-term potentiation, a phenomenon that serves as a model for memory.[21] Long-term potentiation has been observed most commonly in the hippocampus. The mossy fiber neurons of the intrahippocampal pathway are particularly rich in zinc,[22] and stimulation of hippocampal slices with electrical impulses[23] with elevated potassium or with excitatory amino acids[24] results in release of zinc from the tissue. Zinc coreleased with the neurotransmitter into the synapse may exert a regulatory effect on neurotransmission.[24] In this regard, it is noteworthy that dietary zinc deficiency has been shown to impair neurotransmission in the hippocampus.[25]

Previous results from this laboratory have shown that dietary zinc deficiency impairs calcium uptake by platelets[26] and by synaptosomes from guinea pig brain cortex.[27] In the latter case, calcium uptake induced by both potassium depolarization and by exogenous glutamate was decreased by zinc deficiency. Impaired calcium uptake may be the basis of both peripheral and CNS pathology in the guinea pig; a decrease in calcium uptake via VGCC at the neuromuscular junction could explain the observed peripheral neuropathy,[3,4] while a decrease in glutamate-stimulated uptake could cause the impairment of memory and cognition resulting from zinc deprivation.[27]

Because hippocampal function is important in long-term potentiation and memory and this function is initiated by way of the NMDA receptor/channel, it was deemed important to extend our prior observations[27] to the hippocampus. Thus, the purpose of this study was to determine the effect of zinc status on calcium uptake by cortical and hippocampal synaptosomes stimulated by high potassium, glutamate, and NMDA. The more specific agonist, NMDA, was used because the endogenous agonist, glutamate, binds to several other postsynaptic receptors in addition to the NMDA receptor.[13] The results confirmed the earlier observations made with cortical synaptosomes showing that zinc deficiency impaired the calcium uptake stimulated by potassium depolarization and by glutamate. Zinc deficiency had a similar effect on glutamate-stimulated calcium uptake by hippocampal synaptosomes, but the decrease was not statistically significant. The NMDA-stimulated uptake by neither cortical nor hippocampal synaptosomes was affected by dietary treatment.

Methods and materials

Animals and diets

Weanling guinea pigs (Hartley strain) of both sexes, weighing approximately 250 g, were obtained from the departmental colony and maintained as described previously.[27] The composition of the low zinc (0.9 ± 0.08 mg of zinc/kg) experimental diet has been described[3]; the control diet was the basal diet supplemented with $ZnCO_3$ to supply 100 mg of zinc/kg. Food was supplied fresh daily in moist form, and deionized water was supplied ad libitum from bottles or automatic waterers. Three guinea pigs of the same sex were assigned to each experimental group. One animal of each group was fed the low zinc diet ad libitum (−ZN), one the control diet ad libitum (+AL), and one the control diet restricted so as to maintain its body weight similar to that of the guinea pig on the −ZN regimen. Groups were fed the respective diets until the guinea pig fed the low zinc diet developed neurological signs (Score 3),[3] at about 5 weeks. The experimental protocol was approved by the University of Missouri, Columbia, Animal Care and Use Committee.

Tissue collection and synaptosome preparation

The procedures for collection and processing of tissues were the same as used previously.[27] The guinea pigs were euthanized by decapitation, trunk blood was collected, and serum was frozen for zinc analysis. Brain cortex and hippocampus were dissected on ice and used immediately for synaptosome preparation and calcium uptake assays.

Crude synaptosomes were prepared from cortex and hippocampus by sucrose gradient centrifugation as described.[27] Synaptosome pellets were suspended at a protein concentration of 5 mg/mL in cold basal assay solution (NaCl, 136; KCl, 5; $CaCl_2$, 0.085; EGTA, 0.025; glycine, 0.01; glucose, 10; Tris-HEPES, 20 mmol/L, pH 7.65). This solution is similar to the one used previously,[27] except that the calcium and EGTA concentrations were adjusted to increase the specific activity of calcium while maintaining the same calculated free calcium concentration (60 μmol/L). Synaptosomal membrane protein was determined by the method of Lowry,[28] using bovine serum albumin as the standard.

Synaptosome suspensions were kept on ice until they were used for ^{45}Ca uptake experiments. Before starting the uptake experiments, the synaptosome suspensions and other solutions were temperature equilibrated for 10 min in a water bath at 37°C.

^{45}Ca uptake assay procedure

Cortical and hippocampal synaptosome preparations from each of the three guinea pigs in an experimental group were assayed on the same day. To duplicate tubes were added 0.15 mL of synaptosome suspension and 0.20 mL of one of the ^{45}Ca assay solutions described below. Each assay solution contained $CaCl_2$ 0.085, EGTA 0.025, glycine 0.01, glucose 10; Tris-HEPES 20 mmol/L, pH 7.65, a total of 141 mmol/L NaCl plus KCl, and ^{45}Ca to supply 7.4 kBq (0.2 μCi) per tube. The solutions were supplemented with potassium (final concentration 5 or 45 mmol/L), glutamate, and NMDA as described for Experiments 1 and 2 below. Uptake was initiated by adding the appropriate ^{45}Ca solution to the synaptosome suspension. The contents were rapidly mixed and incubated at 30°C for 30 sec. Uptake was terminated by addition of 1.0 mL of ice-cold EGTA solution (NaCl, 136; KCl, 5; EGTA, 3; glucose, 10; Tris-HEPES 20 mmol/L; pH 7.0) and immediate chilling in an ice-water bath. Membranes were collected on glass fiber filters (Whatman GF/B), and ^{45}Ca radioactivity was determined as described earlier.[27] Results are presented as picomoles of calcium (calculated from specific activity of calcium added) divided by milligrams of synaptosomal protein.

Experiment 1

The effect of a depolarizing level of potassium, and of exogenous glutamate, on calcium uptake by cortical and hippocampal synap-

Table 1 Zinc status of guinea pigs as indicated by weight gain and serum zinc concentration*

Dietary treatment	Weight gain (g/d)	Serum zinc (μmol/L)
−ZN†	−0.3 ± 0.4[a]	2.4 ± 0.2[a]
+RF	1.0 ± 0.3[b]	9.2 ± 0.4[b]
+AL	4.4 ± 0.5[c]	10.6 ± 0.6[c]

*Mean ± SEM, n = 17 for each dietary treatment. Values within columns with different letter superscripts [a,b,c] are significantly different, $P < 0.001$.
†Treatment abbreviations: −ZN refers to ad libitum consumption of the low zinc diet; +AL and +RF refer to ad libitum and restricted consumption, respectively, of the adequate zinc diet.

tosomes was determined. Calcium uptake for each synaptosome sample was measured using a 2 × 2 factorial design, with two concentrations of potassium (5 and 45 mmol/L) and two concentrations of glutamate (0 and 500 μmol/L). The experimental ^{45}Ca solutions were prepared by addition of glutamate to the basal solution and by substitution of KCl for NaCl; the total concentration of NaCl and KCl was held constant at 141 mmol/L. Duplicate tubes were assayed for each solution in the factorial design, for a total of 8 tubes per tissue (16 tubes per guinea pig).

Experiment 2

This experiment was similar to Experiment 1 above, except that the more specific NMDA receptor/channel agonist, NMDA, was used in lieu of glutamate.

Statistical methods

Calcium uptake data for the two experiments were analyzed separately as split-plot designs, using the General Linear Models (GLM) procedure (Statistical Analysis System, SAS Institute, Cary, NC). The factorial arrangement of potassium and glutamate (Experiment 1) or NMDA (Experiment 2) defined four subplots, with dietary treatment (DIET) as the whole plot for both experiments (REP = 8 for Experiment 1, REP = 9 for Experiment 2). Main plot effects for both experiments were tested using REP × DIET as the error term; the subplot effects were tested against the residual error. Combined potassium-stimulated uptake data and zinc status data for guinea pigs used in both experiments (n = 17) were analyzed by one-way ANOVA (GLM). Statistical differences between treatments were assessed using the least squares means component of the GLM procedure, with significance set at $P < 0.050$.

Results

Indices of the zinc status of the guinea pigs used in both experiments are shown in *Table 1*. Weight gain and serum zinc concentrations of animals fed the low zinc diet (−ZN) were significantly less than those of the two control groups.

Experiment 1

The calcium uptake data for this experiment are presented in *Table 2*. Both potassium depolarization and glutamate stimulation increased calcium uptake, and there was a significant overall effect of zinc status on calcium uptake in both hippocampus and cortex ($P < 0.05$), Statistically there was a highly significant interaction between dietary treatment and potassium concentration in both tissues, i.e., low zinc status impaired calcium uptake in response to potassium depolarization. Furthermore, there was a potassium-glutamate interaction in the case of cortical but not hippocampal synaptosomes, i.e., depolarization increased the response to glutamate by cortical but not by hippocampal synaptosomes.

Figure 1 shows the net calcium uptake that resulted from depolarization, i.e., the difference in calcium uptake by synaptosomes suspended in 45 and those in 5 mmol/L of potassium, without addition of glutamate or NMDA. The response of both cortical and hippocampal synaptosomes was affected significantly by dietary treatment; compared with ad libitum controls, those of low zinc status had lower calcium uptake, approximately 15% for cortical and 20% for hippocampal synaptosomes.

Figure 2 shows the net calcium uptake due to glutamate stimulation of cortical synaptosomes. Low zinc status decreased the glutamate-stimulated calcium uptake by cortical synaptosomes only when they were depolarized, i.e., diet had an effect at 45 but not at 5 mmol/L of potassium. This agrees with our earlier results.[27] Zinc status did not have a significant effect on the glutamate-stimulated calcium uptake by hippocampal synaptosomes at either potassium level, but there was a trend at 45 mmol/L of

Table 2 Effect of glutamate and potassium on calcium uptake by cortical and hippocampal synaptosomes from guinea pigs of low and adequate zinc status*

	0 μmol/L of glutamate		500 μmol/L of glutamate	
Dietary treatment†	5 mmol/L of K	45 mmol/L of K	5 mmol/L of K	45 mmol/L of K
		(pmol of Ca/mg of protein)		
Cortex				
−ZN	607 ± 17	1965 ± 59[a]	663 ± 20	2113 ± 59[a]
+RF	650 ± 21	2132 ± 63[b]	725 ± 20	2349 ± 74[b]
+AL	683 ± 41	2242 ± 137[b]	772 ± 47	2438 ± 139[b]
Hippocampus				
−ZN	751 ± 19	2049 ± 59[a]	801 ± 19[a]	2152 ± 62[a]
+RF	752 ± 17	2238 ± 74[b]	826 ± 23[a]	2375 ± 72[b]
+AL	813 ± 32	2482 ± 125[c]	947 ± 47[b]	2611 ± 131[c]

*Values are mean ± SEM, n = 8 for each dietary treatment. Values within columns and within tissues with different letter superscripts [a,b,c] are significantly different, $P < 0.05$. Split Plot ANOVA, statistically significant contrasts: Cortex (pooled SEM = 41): potassium (K), $P < 0.001$; glutamate (G), $P < 0.001$; diet (D) × K, $P = 0.002$; K × G, $P = 0.018$. Hippocampus (pooled SEM = 42): K, $P < .001$; G, $P < 0.001$; D × K, $P < 0.001$.
†Treatment abbreviations: −ZN refers to ad libitum consumption of the low zinc diet; +AL and +RF refer to ad libitum and restricted consumption, respectively, of the adequate zinc diet.

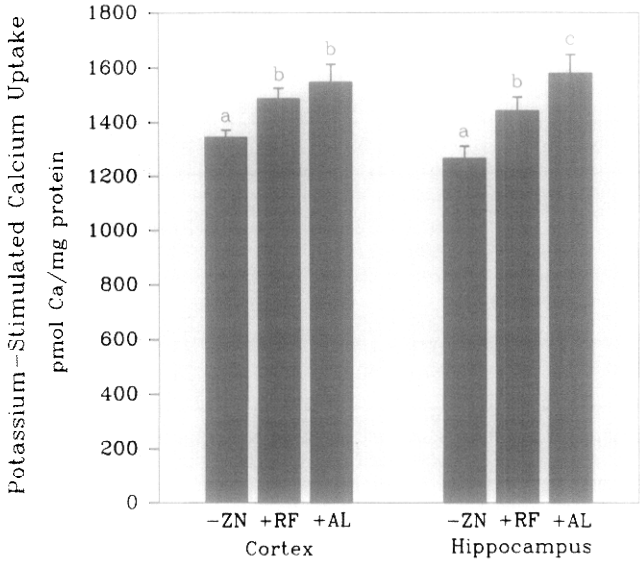

Figure 1 Calcium uptake of cortical and hippocampal synaptosomes from guinea pigs of low and adequate zinc status when stimulated by potassium depolarization. The values, in picomoles per milligram of protein, are means ± group SEM (bar extensions) of the differences between uptake at 5 and 45 mmol/L of potassium. The bars represent the means of 17 animals (Experiments 1 and 2 combined), pooled SEM = 41 and 46 for cortex and hippocampus, respectively. Within tissues, statistical significance ($P < 0.05$) is indicated by different letters.

potassium similar to that observed in cortical synaptosomes *(Figure 3)*. In hippocampal synaptosomes, glutamate stimulated calcium uptake of ad libitum control synaptosomes in the presence of 5 mmol/L of potassium to the same extent as it did in 45 mmol/L of potassium. Zinc deficiency impaired the response to glutamate at 5 mmol/L, but the effect appeared to be due to decreased food intake *(Figure 3)*.

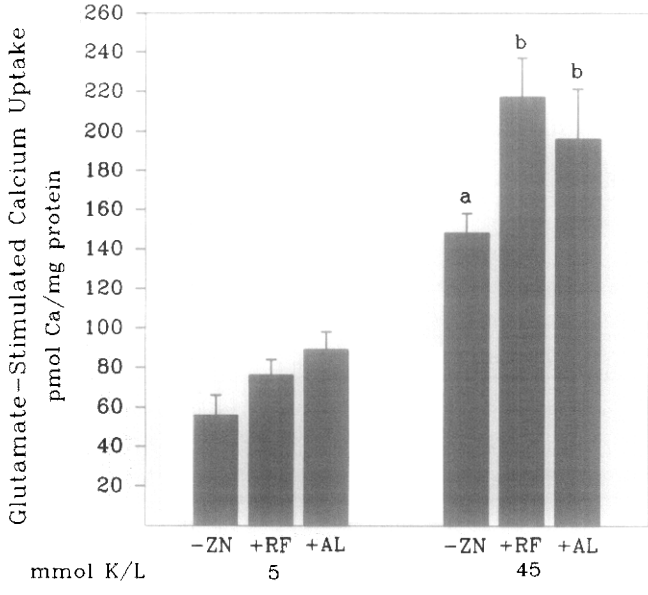

Figure 2 Calcium uptake by cortical synaptosomes from guinea pigs of low and adequate zinc status when stimulated with glutamate (500 μmol/L) in the presence of 5 and 45 mmol/L of potassium. $n = 8$ per group, pooled SEM = 14. Other designations are as in *Figure 1*.

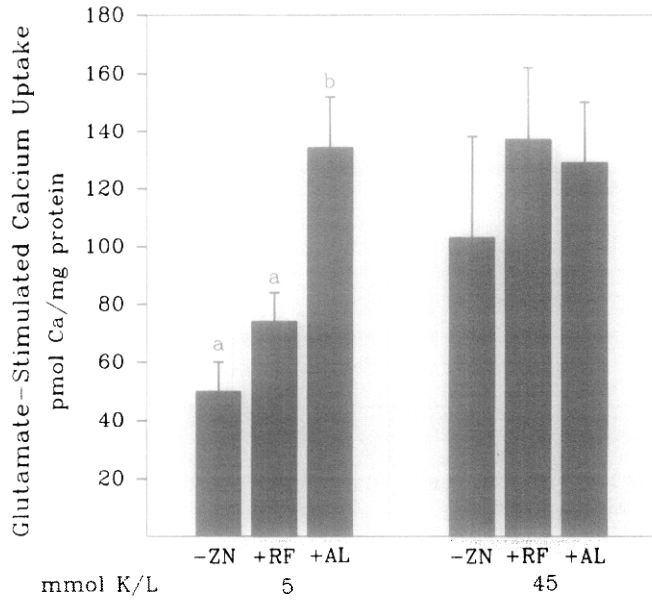

Figure 3 Calcium uptake by hippocampal synaptosomes from guinea pigs of low and adequate zinc status when stimulated with glutamate (500 μmol/L) in the presence of 5 and 45 mmol/L of potassium. $n = 8$ per group; pooled SEM = 21. Other designations are as in *Figure 1*.

Experiment 2

The calcium uptake data from this experiment are presented in *Table 3*. Both potassium depolarization and NMDA stimulated calcium uptake. As in Experiment 1, there was an interaction between dietary treatment and potassium concentration in both cortical and hippocampal synaptosomes. This interaction reflects the fact that low zinc status decreased the calcium uptake response to depolarization *(Figure 1)*. As in Experiment 1, when glutamate was the agonist, potassium depolarization significantly increased the response to NMDA in cortical but not in hippocampal synaptosomes.

Dietary treatment had no effect on NMDA-stimulated calcium uptake in either cortical or hippocampal synaptosomes regardless of potassium concentration *(Figures 4 and 5)*.

Discussion

Previous results[27] showed that zinc deficiency in the guinea pig impairs potassium-stimulated calcium uptake by cortical synaptosomes. Glutamate stimulation in the presence of high potassium resulted in additional calcium uptake, and this incremental uptake was also impaired by zinc deficiency. The results of this study confirm the earlier observations made with cortical synaptosomes and show that zinc deficiency also depresses potassium-stimulated calcium uptake by hippocampal synaptosomes. While glutamate-stimulated uptake by hippocampal synaptosomes was not significantly affected by zinc deficiency, the trend was the same as for cortical synaptosomes (25% reduction). NMDA-stimulated uptake was unaffected in both tissues. The glutamate results show that there is a difference between the cortical and hippocampal synaptosomes as regards the dietary effect on glutamate stimulation. The difference in response of the two tissues is demonstrated by the

Table 3 Effect of NMDA and potassium on calcium uptake by cortical and hippocampal synaptosomes from guinea pigs of low and adequate zinc status*

Dietary treatment†	0 μmol/L of NMDA		500 μmol/L of NMDA	
	5 mmol/L of K	45 mmol/L of K	5 mmol/L of K	45 mmol/L of K
	(pmol of Ca/mg of protein)			
Cortex				
−ZN	641 ± 19	1974 ± 32[a]	676 ± 22	2146 ± 45[a]
+RF	657 ± 26	2147 ± 83[b]	699 ± 27	2306 ± 96[b]
+AL	705 ± 32	2239 ± 113[b]	745 ± 35	2414 ± 126[b]
Hippocampus				
−ZN	774 ± 51	2011 ± 118[a]	809 ± 55	2153 ± 125[a]
+RF	781 ± 38	2184 ± 105[b]	831 ± 42	2319 ± 107[b]
+AL	818 ± 52	2315 ± 133[b]	874 ± 50	2456 ± 139[b]

*Values are mean ± SEM, $n = 8$ for each dietary treatment. Values within columns and within tissues with different letter superscripts [a,b,c] are significantly different, $P < 0.05$. Split plot ANOVA, statistically significant contrasts: Cortex (pooled SEM = 42): potassium (K), $P < 0.001$; NMDA (N), $P < 0.001$; diet (D) × K, $P = 0.004$; K × N, $P = 0.010$. Hippocampus (pooled SEM = 49): K, $P < .001$; N, $P = 0.002$; D × K, $P = 0.003$.

†Treatment abbreviations: −ZN refers to ad libitum consumption of the low zinc diet; +AL and +RF refer to ad libitum and restricted consumption, respectively, of the adequate zinc diet.

fact that there is a consistent potassium/agonist interaction in the cortex but not in the hippocampus. The hippocampus is particularly rich in zinc,[22] and therefore may be less susceptible than the cortex to the effects of dietary zinc deprivation.

Although the results of these studies clearly show that zinc deficiency impairs calcium uptake by synaptosomes prepared from both cortex and hippocampus, the specific calcium channel(s) affected is unknown. The impairment of potassium-stimulated calcium uptake in both cortical and hippocampal synaptosomes suggests that zinc deficiency has a direct effect on the function of voltage-gated calcium channels. However, it is possible that a proportion of the calcium uptake stimulated by potassium depolarization is the result of glutamate release and its subsequent activation of postsynaptic receptors. Synaptosome preparations contain a high concentration of glutamate, a proportion of which is released into the medium when they are depolarized by high potassium.[29] In cerebellar granule cells, 90% of the calcium uptake due to depolarization with 35 mmol/L of potassium is blocked by the NMDA channel blocker phencyclidine.[30] Recent results from this laboratory (Browning and O'Dell, unpublished results) show that 30% of the calcium uptake by rat synaptosomes depolarized by 40 mmol/L of potassium can be blocked by MK-801. Therefore, it is likely that the potassium-stimulated calcium uptake by the cortical and hippocampal synaptosomes as defined in this and the earlier study[27] was due to activation of both VGCC and the NMDA receptor/channel.

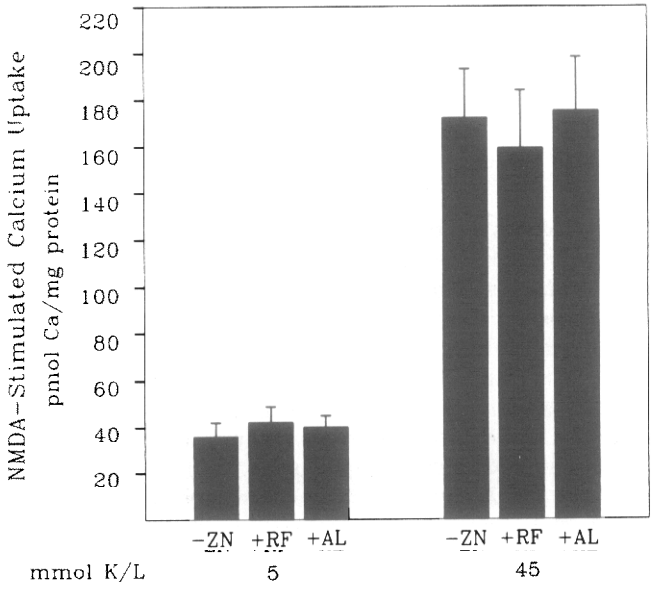

Figure 4 Calcium uptake by cortical synaptosomes from guinea pigs of low and adequate zinc status when stimulated with NMDA (500 μmol/L) in the presence of 5 and 45 mmol/L of potassium. $n = 9$ per group; pooled SEM = 16. Other designations are as in *Figure 1*.

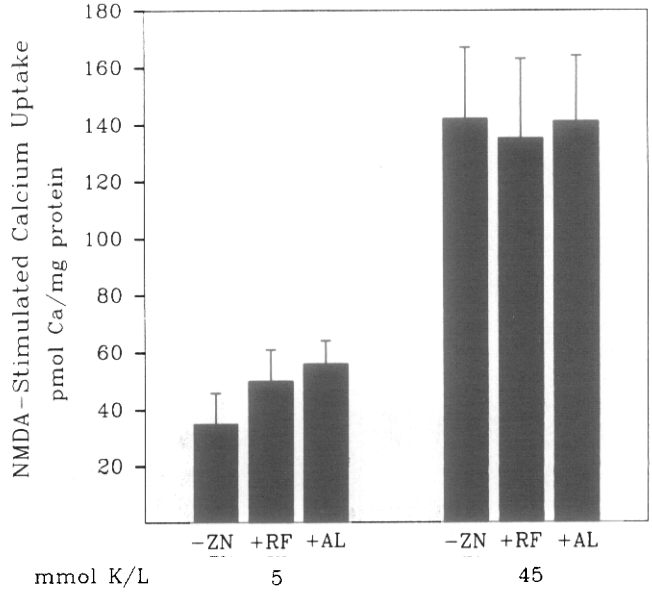

Figure 5 Calcium uptake by hippocampal synaptosomes from guinea pigs of low and adequate zinc status when stimulated with NMDA (500 μmol/L) in the presence of 5 and 45 mmol/L of potassium. $n = 9$ per group; pooled SEM = 19. Other designations are as in *Figure 1*.

Recent results from this laboratory[31] show that zinc deficiency has a direct effect on the NMDA receptor/channel, since the building of MK-801 is decreased in cortical membranes from zinc-deficient guinea pigs. However, the results of the present study, which show no effect of zinc status on the response to NMDA, a specific postsynaptic agonist, suggest that the function of the NMDA receptor/channel complex is not affected. It is likely that impaired VGCC function is a primary defect in zinc deficiency.

The observation that glutamate-stimulated uptake by cortical synaptosomes is decreased by zinc deficiency while NMDA-stimulated uptake is not affected must relate to the difference in specificity of the two agonists. Unlike NMDA, glutamate binds to and activates several different postsynaptic receptors besides the NMDA receptor/channel, including the AMPA/kainate and metabotropic receptors.[13] The AMPA/kainate receptors are responsible for fast neurotransmission.[13] Binding of glutamate to the AMPA/kainate receptor gates an ion channel that is permeable to sodium and potassium, resulting in depolarization of the postsynaptic membrane. Since the depolarizing concentration (45 mmol/L) of potassium used in these experiments was submaximal,[27] the addition of saturating levels of glutamate provides an additional increment of depolarizing stimulus that would not be provided by NMDA. This increased depolarization could enhance calcium uptake by activating VGCC in the postsynaptic membrane.[32] The observed decrease in potassium-stimulated calcium uptake may due to reduced uptake via postsynaptic VGCC, resulting from either a direct effect on VGCC function or an impairment of AMPA/kainate channel function. Besides this indirect effect on calcium uptake, under some conditions AMPA/kainate channels are directly permeable to calcium.[33] Since the activity of the AMPA/kainate channel is enhanced by zinc added in vitro,[19,34] it may be affected by dietary zinc deficiency as well. Thus, part of the effect of zinc deficiency could be due to impaired calcium uptake by the AMPA/kainate channel.

Since VGCC are found in all types of excitable cells, this impairment of VGCC function may be relevant to other pathology observed in zinc deficiency. For example, platelets have voltage-gated calcium channels,[36] and platelets from zinc deficient rats, whether stimulated by ADP[26] or thrombin,[35] show impaired calcium uptake. Malfunction of pre- and postsynaptic voltage-gated calcium channels could explain the neuropathology observed in zinc-deficient animals. Peripheral neuropathy may result from impairment of VGCC at the neuromuscular junction,[3,5] and poor cognition and memory may arise from an indirect effect of impaired voltage-gated calcium channels in the postsynaptic membrane. Since change in cytosolic calcium concentration serves widely as a second messenger, impairment of plasma membrane calcium channels may be a general basis of zinc deficiency pathology.

References

1 O'Dell, B.L. (1993). Roles of zinc and copper in the nervous system. In *Essential and Toxic Trace Elements in Human Health and Disease: An Update* (A.S. Prasad, ed.), *Prog. Clin. Biol. Res.* Vol. 380, pp. 147–162, Wiley-Liss, New York, NY USA

2 O'Dell, B.L., Newberne, P.M., and Savage, J.E. (1958). Significance of dietary zinc for the growing chick. *J. Nutr.* **65,** 503–524

3 O'Dell, B.L., Becker, J.K., Emery, M.P., and Browning, J.D. (1989). Production and reversal of the neuromuscular pathology and related signs of zinc deficiency in guinea pigs. *J. Nutr.* **119,** 196–201

4 Quarterman, J. and Humphries, W.R. (1983). The production of zinc deficiency in the guinea pig. *J. Comp. Pathol.* **93,** 261–270

5 O'Dell, B.L., Conley-Harrison, J., Besch-Williford, C., Browning, J.D., and O'Brien, D. (1990a). Zinc status and peripheral nerve function in guinea pigs. *FASEB J.* **4,** 2919–2922

6 O'Dell, B.L., Conley-Harrison, J., Browning, J.D., Besch-Williford, C., Hempe, J.M., and Savage, J.E. (1990b). Zinc deficiency and peripheral neuropathy in chicks. *Proc. Soc. Exp. Biol. Med.* **194,** 1–4.

7 Sandstead, H.H., Fosmire, G.J., Mckenzie, J.M., and Halas, E.S. (1975). Zinc deficiency and brain development in the rat. *Fed. Proc.* **34,** 86–88

8 Atchison, W.D. (1987). Physiology of synaptic transmission. In *Electrophysiology in Neurotoxicology* (H.E. Lowndes, ed.), Vol. 1, p. 45–101, CRC Press, Boca Raton, FL USA

9 Miller, R.J. (1990). Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB J.* **4,** 3291–3299

10 Büsselberg, D., Michael, D., Evans, M.L., Carpenter, D.O., and Haas, H.L. (1992). Zinc (Zn^{2+}) blocks voltage gated calcium channels in cultured rat dorsal root ganglion cells. *Brain Res.* **593,** 77–81

11 Nishimura, M. (1987). Zinc competitively inhibits calcium-dependent release of transmitter at the mouse neuromuscular junction. *Pflügers Arch.* **410,** 623–626

12 Wang, Y.-X. and Quastel, D.M.J. (1990). Multiple actions of zinc on transmitter release at mouse end-plates. *Pflügers Arch.* **415,** 582–587

13 Monaghan, D.T., Bridges, R.J., and Cotman, C.W. (1989). The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–402

14 MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J., and Barker, J.L. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321,** 519–522

15 Villalba, M., Martinez-Serrano, A., Börner, C., Blanco, P., and Satrústegui, J. (1992). NMDA-induced increase in $[Ca^{2+}]_i$ and $^{45}Ca^{2+}$ uptake in acutely dissociated brain cells derived from adult rats. *Brain Res.* **570,** 347–353

16 Goddard, G.A. and Robinson, J.D. (1976). Uptake and release of calcium by rat brain synaptosomes. *Brain Res.* **110,** 331–350

17 Daniell, L.C. (1991). N-methyl-D-aspartate increases cytoplasmic free calcium in mouse hippocampus. *Neuropharmacology* **30,** 539–545

18 Reynolds, I.J. (1990). Modulation of NMDA receptor responsiveness by neurotransmitters, drugs and chemical modification. *Life Sci.* **47,** 1785–1792

19 Peters, S., Koh, J., and Choi, D.W. (1987). Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science* **236,** 589–593

20 Yeh, G.-C., Bonhaus, D.W., and McNamara, J.O. (1990). Evidence that zinc inhibits N-methyl-D-aspartate receptor-gated ion channel activation by non-competitive antagonism of glycine binding. *Mol. Pharmacol.* **38,** 14–19

21 Kennedy, M.B. (1989). Regulation of synaptic transmission in the central nervous system: long-term potentiation. *Cell* **59,** 777–787

22 Crawford, I.L. (1983). Zinc and the hippocampus. Histology, neurochemistry, pharmacology, and putative functional relevance. In *Neurobiology of the Trace Elements* (I.E. Dreosti and R.M. Smith, eds.), Vol. 1, p. 163–211, Humana Press, Clifton, NJ USA

23 Howell, G.A., Welch, M.G., and Frederickson, C.J. (1984). Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature* **308,** 736–738

24 Assaf, S.Y. and Chung, S.-H. (1984). Release of endogenous Zn^{2+} from brain tissue during activity. *Nature* **308,** 734–736

25 Hesse, G.W. (1979). Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. *Science* **205,** 1005–1007

26 O'Dell, B.L. and Emery, M. (1991). Compromised zinc status in

rats adversely affects calcium metabolism in platelets. *J. Nutr.* **121,** 1763–1768

27 Browning, J.D. and O'Dell, B.L. (1994a). Low zinc status in guinea pigs impairs calcium uptake by brain synaptosomes. *J. Nutr.* **124,** 436–443

28 Lowry, O.R., Rosebrough, N.J., Farr, A.L., and Randall, R.A. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

29 Nicholls, D.G. (1993). Ion channels and the regulation of neurotransmitter release. *Biochem. Soc. Trans.* **21,** 53–58

30 Didier, M., Héaulme, M., Gonalons, N., Soubrié, P., Bockaert, J., and Pin, J.-P. (1993). 35 mM K^+-stimulated $^{45}Ca^{2+}$ uptake in cerebellar granule cell cultures mainly results from NMDA receptor activation. *Eur. J. Pharmacol.* **244,** 57–65

31 Browning, J.D., and O'Dell, B.L. (1994b). Zinc deficiency decreases the concentration of N-methyl-D-aspartate receptor/channels in cortical synaptic membranes. *J. Nutr.* **125,** 2083–2089.

32 Jones, R.S.G. and Heinemann, U.H. (1987). Differential effects of calcium entry blockers on pre- and postsynaptic influx of calcium in the rat hippocampus in vitro. *Brain Res.* **416,** 257–266

33 Murphy, S.N. and Miller, R.J. (1989). Regulation of Ca^{++} influx into striatal neurons by kainic acid. *J. Pharmacol. Exp. Ther.* **249,** 184–193

34 Dreixler, J.C. and Leonard, J.P. (1994). Subunit-specific enhancement of glutamate receptor responses by zinc. *Mol. Brain Res.* **22,** 144–150

35 Xia, J. and O'Dell, B.L. (1994). Decreased calcium channel activity in thrombin-stimulated platelets of zinc deficient rats and relationship of protein kinase C to platelet function. *J. Nutr. Biochem.* In press

36 Palés, J., Palacios-Araus, L., López, A., and Gual, A. (1991). Effects of dihydropyridines and inorganic calcium blockers on aggregation and on intracellular free calcium in platelets. *Biochim. Biophys. Acta* **1064,** 169–174