EFFECT OF CADMIUM ON SODIUM-DEPENDENT L-GLUTAMATE TRANSPORT IN RENAL BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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One of the effects of cadmium on proximal tubular function is an aminoaciduria which results from inhibition of amino acid reabsorption. Since the rate-determining step in amino acid reabsorption is located at the brush border, it is likely that cadmium exerts its effect at this site. We have previously shown that Cd^{2+} inhibits the sodium-cotransport system of L-alanine in brush border membrane vesicles (BBMV) isolated from the kidney of the winter flounder (Bevan et al., Toxicol. Appl. Pharmacol. 101:461, 1989). This inhibition required prolonged exposure of the membranes to Cd^{2+} suggesting an interaction of Cd^{2+} ion with the cytoplasmic side of the membrane. Because under these conditions Cd^{2+} binds extensively to both sides of the membrane, direct evidence for the sidedness of cadmium action and characterization of the cadmium interaction with the sodium-alanine cotransport system was difficult. Therefore, we have examined the effect of cadmium on L-glutamate transport in renal BBMV from the winter flounder. It has been shown in BBMV from mammalian kidneys that sodium-dependent L-glutamate uptake is stimulated by the presence of K^+ at the inside of the membrane vesicle (Burckhardt et al., Biochim. Biophys. Acta 599: 191, 1980). This asymmetry should make it possible to determine the sidedness of Cd^{2+} action.

The time course of uptake of 50 μM L-[^{3}H]glutamate by flounder kidney BBMV preloaded with either 20 mM KCl or 20 mM choline is shown in figure 1A. Uptake in the presence of an inwardly-directed 100 mM NaCl gradient is significantly higher than in the presence of a 100 mM KCl gradient. There was, however, no significant difference in Na-dependent L-glutamate uptake in the absence or presence of a K^+ gradient ($K_i > K_o$). The effect of preincubation of membrane vesicles for 60 min with 100 μM $CdCl_2$ on L-glutamate uptake is shown in figure 1B. L-glutamate uptake in choline-preloaded vesicles in the presence of a sodium gradient was significantly reduced by Cd^{2+}; vesicles preloaded with K^+ showed only a slight reduction in L-glutamate uptake. No effect of Cd^{2+} was observed on L-glutamate uptake in the presence of an inwardly-directed 100 mM K^+ gradient.

The results demonstrate that the presence of K^+ at the inside of the vesicles blunts the effect of Cd^{2+} on L-glutamate transport. Further studies are necessary to determine more accurately whether this reaction takes place at the inner face of the carrier and how the Cd^{2+} effect can be incorporated into current models of L-glutamate transport (Heinz et al., Biochim. Biophys. Acta 937: 300, 1988).

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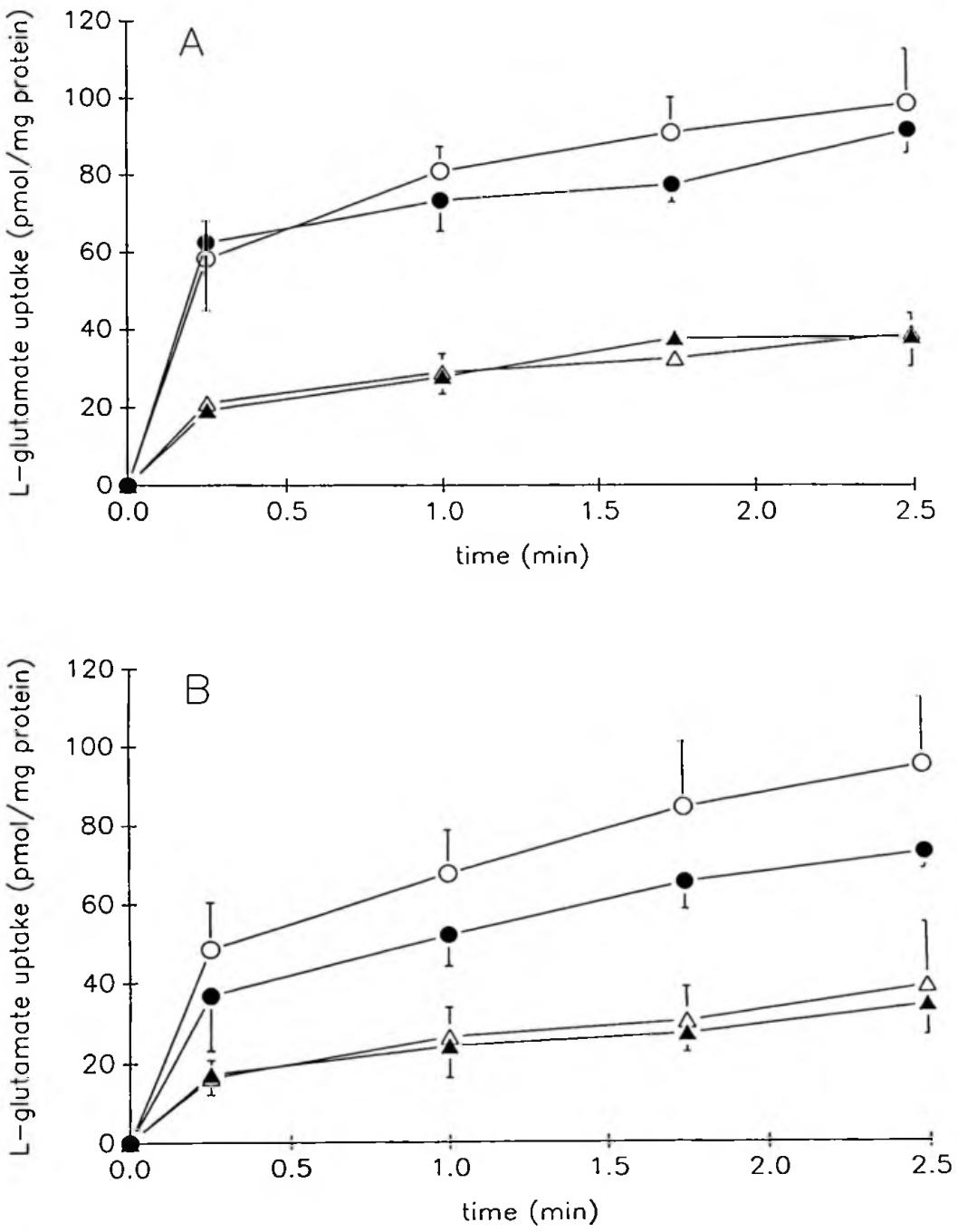

Figure 1. L-glutamate uptake into BBMV from flounder kidney in the absence (A) and presence (B) of Cd^{2+}. L-glutamate uptake was determined under four different conditions: (●) 100 mM NaCl ($Na_o > Na_i$), no K^+ present; (o) 100 mM NaCl ($Na_o > Na_i$), 20 mM K^+ inside vesicle; (▲) 100 mM KCl ($K_o > K_i$), no K^+ present inside vesicle; (△) 100 mM KCl ($K_o > K_i$), 20 mM K^+ inside vesicle. Vesicles were preloaded with either 20 mM KCl or 20 mM choline chloride for 60 min at 15°C in the absence or presence of 0.1 mM $CdCl_2$. L-glutamate uptake was determined in media containing 50 μM L-[^{3}H]glutamate and either 100 mM NaCl or 100 mM KCl. Values represent the mean ± S.D. from 3 experiments.