Transient ischemic alteration of synaptosomal neutral amino acid uptake

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Summary. The effect of cerebral ischemia and postischemia on the synaptosomal uptake of some neutral amino acids was determined in mongolian gerbils at various periods of time. A transiently increased uptake of ^{3}H isoleucine, ^{14}C cycloleucine and ^{3}H phenylalanine in the synaptosomes was found after 3 min of bilateral common carotid occlusion which returned to normal at 30 min of reestablished cerebral blood circulation.

In our continuous effort of evaluating the effect of cerebral ischemia on the function of various cerebral compartments we investigated the amino acid uptake in synaptosomes obtained from brains of gerbils subjected to ischemia. This report will demonstrate a transient increase of neutral amino acids in the synaptosomes in cerebral ischemia and postischemia.

Materials and methods. Groups of 4–6 anesthetized animals (pentobarbital 20 mg/kg i.p.) were subjected to bilateral common carotid artery clipping for 1–30 min or to 3 min occlusion and 1–30 min clip release. Sham operated gerbils served as controls. The brains from the decapitated animals were removed quickly and homogenized immediately in 0.32 M sucrose at pH 7.0. The synaptosomes were prepared according to the method described by Whittaker and Barker[1]. Aliquots of the same sample suspended in 26 mM K phosphate-sucrose (147 mM) buffer at pH 7.4 were used in duplicate for the determination of ^{3}H isoleucine, ^{14}C cycloleucine and ^{3}H phenylalanine uptakes. The radioactive amino acids were obtained from New England Nuclear Corp. (Boston, Mass.) and their specific activities were as follows: ^{3}H isoleucine 80.6 or 103 mCi/mM, ^{14}C cycloleucine [aminocyclopentane-1-carboxylic acid 1-(carboxyl-^{14}C)] 29.9 mCi/mM and ^{3}H phenylalanine 21.6 Ci/mM. The assay procedures were the same as those used for the synaptosomal uptakes of ^{3}H 2-deoxy-D-glucose (^{3}H 2-DG) except for a shorter incubation period of 2 instead of 15 min. The nonspecific uptake of each amino acid was measured by addition of the unlabeled corresponding amino acids to the labeled incubation buffer. The rinsed and dried samples were mixed with Peterson and Green scintillation solution and the radioactivity was counted in a Beckman LS-250 liquid scintillation spectrometer[2, 3]. Protein was determined by Lowry's technique[4].

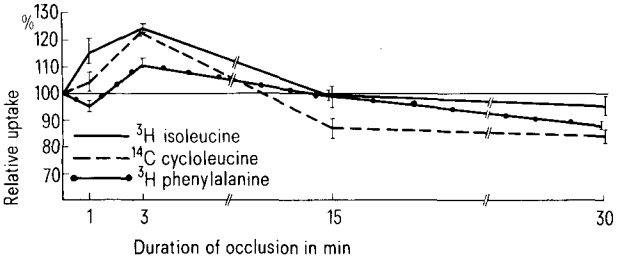

Fig. 1. Synaptosomal neutral amino acid uptake in ischemic gerbils. Each point represents mean values ±SEM. The final specific activity for ^{3}H isoleucine, ^{14}C cycloleucine and ^{3}H phenylalanine was 2.18×10^{7}, 2.1×10^{3} and 6.4×10^{7} cpm/min/μmole respectively.

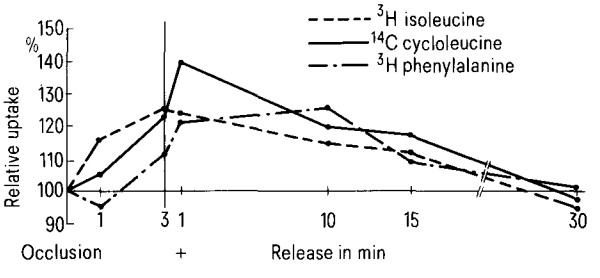

Fig. 2. The relative uptake of neutral amino acids in synaptosomes separated from brains of gerbils subjected to 3 min bilateral common carotid artery clipping and 1–30 min clip release. The yield of the total synaptosomal protein in all groups was similar to the controls. Each point is an average of 4–6 double determinations with variations of less than 5%.

Results and discussion. The specific synaptosomal uptake of the tested amino acids was found to be increased in cerebral ischemia of 3 min duration. This increased synaptosomal amino acids uptake was transient since no significant changes of either of these amino acids uptake were seen in the synaptosomes obtained from brains of gerbils subjected to 1, 15 and 30 min of cerebral blood flow deprivation as compared to controls (figure 1). However, a further increase in the uptake of these amino acids was seen in synaptosomes separated from brains of animals with 3 min of arterial clipping and 1 min clip release. Thereafter, the uptake progressively decreased and returned to normal at 30 min of reestablished circulation after 3 min of arterial occlusion (figure 2). These results suggest that the increased neutral amino acid uptake in synaptosomes was due to transport rather than metabolism since similar increase in the uptake of cycloleucine the nonmetabolizable analogue of isoleucine was seen as the one with the isoleucine. Similar changes were observed in the uptake of neutral amino acids in cerebral capillaries isolated from brains of gerbils subjected to bilateral common carotid artery occlusion and release (unpublished observations).

The mechanism for the transient increase of neutral amino acids into synaptosomes is unknown and needs to be clarified. However, the absence of reduced amino acid uptake in ischemia strongly suggests that the transport of these amino acids is most likely oxygen and energy independent. This concept is substantiated by the reported decrease of energy metabolites seen in the brains of gerbils under similar conditions[5]. In contrast, reduction of 2-deoxy-D-glucose was seen in synaptosomes and cerebral capillaries obtained from brains of ischemic gerbils[3, 6]. Likewise, studies on isolated dog brains demonstrated an unaltered leucine but decreased glucose transport in anoxia[7, 8].

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