Acta Neurobiol. Exp. 1996, 56: 703

Acidosis inhibits calcium accumulation in intrasynaptosomal mitochondria

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INTRODUCTION AND METHODS. Brain ischaemia is accompained by massive increase in intracellular calcium, its final level being dependent on functioning calcium stores. Since pathogenesis of ischaemia also involves acidosis, we tested the pH dependence of calcium transport into introsynaptosomal mitochondria. Synaptosomes were isolated from rat brain according to (2). Mitochondrial potential was tested by steady-state content of [^{3}H]-tetraphenylphosphonium bromide ([^{3}H]-TPP, 70 nCi). Incubation medium contained (in mM): NaCl-77, KCl-60, glucose-60, $MgCl_2$-1.3, NaH_2PO_4-1.2, $CaCl_2$-1.0, Tris-maleate-10, pH 6.0-7.4 (3). To measure $^{45}Ca^{2+}$ uptake into intrasynaptosomal mitochondria synaptosomes were permeabilized with 2.5 mg/ml digitonin and incubated for 30 s at 37°C with 1 μCi $^{45}Ca^{2+}$ in medium: KCl-137, glucose -10, $MgCl_2$-1.3, NaH_2PO_4-1.2, $CaCl_2$-0.025, Tris-maleate-10, pH 6.0-7.4) followed by rapid vacuum filtration through the filters GF/F followed and scintillation counting.

RESULTS AND DISCUSSION. In the presence of 60 mM K^+ a decrease of pH leads to loss of [^{3}H]TPP^+ from intact synaptosomes (Fig. 1), indicating mitochondrial depolarization. This effect was reversible up to pH 6.0 with distinct irreversibility beginning at pH<5.5. After permeabilization of synaptosomes a specific blocker of mitochondrial calcium transport ruthenium red (10 μM) at pH 7.4 inhibited the total uptake of $^{45}Ca^{2+}$ by 50% as compared with 20% value obtained on intact synaptosomes. Ruthenium red-inhibitable calcium transport in digitonin-treated synaptosomes exhibits the same sensitivity to low pH as [^{3}H]TPP^+ accumulation showing a link between acidosis-induced depolarization and a blockage of calcium uniporter function in mitochondria. We observed a similar pH dependence for calcium transport inhibitable by combination of CCCP (10 μM) and oligomycin (4 μg/ml) thereby supporting a mitochondrial nature of transport. Our results are in accordance with those described for mitochondria isolated from ferret heart (1). Thus, there is a decrease in accumulating activity of calcium in a mitochondrial store under acidosis. However, it is difficult to ascertain the exact extent of this impairment *in vivo* since simultaneous inhibition of calcium efflux *via* the Na^+/Ca^{2+} exchange takes place at low pH (1).

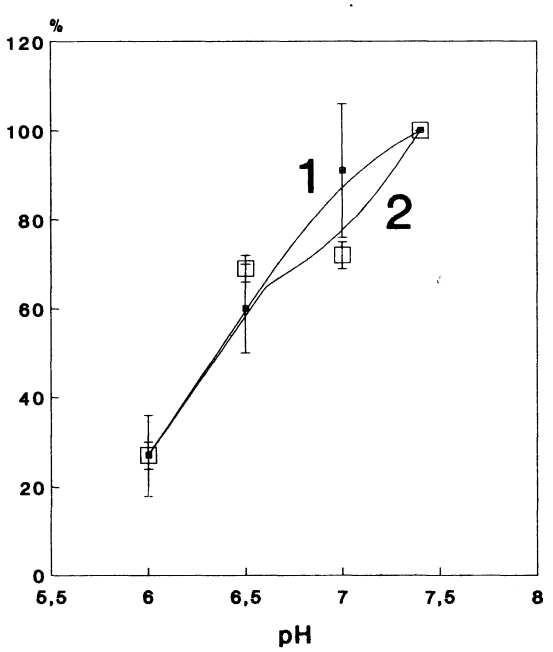

Fig. 1. Effect of acidosis on ruthenium red-sensitive $^{45}Ca^{2+}$ uptake (1) and steady state content of [^{3}H]TPP^+ (2) in intrasynaptosomal mitochondria. The results are expressed as percentage to control values measured at pH 7.4. Data are means ± SE of 3 experiments performed in quadruplicates.

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Accepted 24 April 1996