α-ADRENERGIC MOBILIZATION OF HEPATIC MITOCHONDRIAL CALCIUM

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1. Introduction

Recently it has been demonstrated that activation of α-adrenergic receptors by catecholamines in isolated rat liver parenchymal cells causes release of calcium from some intracellular store(s) [1–3]. It has been proposed that the resulting rise in cytosolic calcium is responsible for some of the metabolic changes observed [1–3]. With the identification of physiological α-adrenergic receptors in rat-liver plasma membranes [4,5], one may postulate activation of these receptors generates an intracellular second messenger which either stimulates the release or inhibits the uptake of calcium by an intracellular organelle [2].

Using methods specifically designed to prevent mobilization of mitochondrial calcium during isolation [6–8], we present data here showing that activation of α-adrenergic receptors in the perfused rat liver produces a large net loss of calcium from the mitochondria which correlates with the release of calcium from the liver into the perfusate. It is postulated that the calcium released from the mitochondria into the cytosol, allosterically activates phosphorylase *b* kinase [9], and is also transiently expelled from the cell by a calcium-extruding mechanism [10].

2. Materials and methods

Livers from 220–250 g male Sprague-Dawley rats were perfused with non-recirculating medium as in [2,11]. The calcium content of the medium was reduced from 2.4 mM to 50 μM in order to facilitate the measurement of calcium release from the liver. Sampling of the effluent medium was begun 2 min after insertion of the portal venous cannula. Samples were centrifuged (5000 × *g* for 10 min) to remove erythrocytes and analyzed for calcium and glucose content [2]. Mitochondria were isolated from separate liver lobes before hormone infusion and 6 min later. The liver lobe used to isolate the initial mitochondrial fraction was tied-off with a silk ligature immediately before sampling, to prevent leakage of perfusate. The liver samples were minced with scissors in 10 ml ice-cold homogenization buffer and washed once to remove excess perfusate. A 20% (w/v) homogenate was made using a Potter-Elvehjem glass–teflon homogenizer. The composition of the homogenization medium was 250 mM sucrose, 5.0 mM Hepes (*N*-2-hydroxy ethyl piperazine-*N'*-2-ethane sulfonic acid), 1.0 mM $LaCl_3$, 0.5 mM-EGTA (ethylene glycol bis (β-amino ethyl ether) *N,N'*-tetra-acetic acid) and ruthenium red (5 μg/ml) pH 7.5 [6–8]. The homogenate was diluted 2-fold and centrifuged at 900 × *g* for 5 min in a refrigerated RC-5 Sorvall centrifuge. The supernatant was then centrifuged at 10 000 × *g* for 5 min. The mitochondrial pellet obtained from this step was washed once with homogenization medium and recentrifuged (10 000 × *g* for 5 min). The mitochondria were then resuspended in 1.5 ml H_2O. Protein was determined by the biuret method with bovine serum albumin as standard [12] and calcium was determined by atomic absorption spectroscopy [2]. Source of materials are described elsewhere [1,2]. Ruthenium red was from British Drug Houses Ltd.

3. Results and discussion

The results in fig.1 (panel A) show the time course of calcium efflux from the perfused liver elicited by continuous infusion of phenylephrine (10^{-5} M final conc.), starting at 6 min. Despite the removal of ~20% of the liver at 4 min (for the initial mitochondrial sample), there was still evident a large and transient efflux of calcium [2]. When a supraphysiological concentration of glucagon (10^{-8} M) (fig.1, panel B) was infused into the liver, a much smaller efflux of calcium was observed, although the glucose output measured between 6 min and 12 min (0.112 mmol) was ~27% greater than that produced when 10^{-5} M phenylephrine was infused (0.082 mmol).

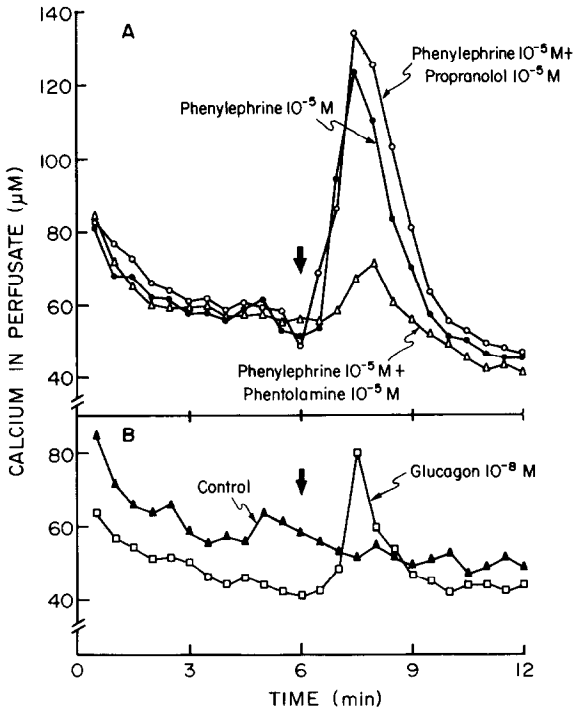

Fig.1. Effects of phenylephrine and glucagon on calcium release from the isolated perfused rat liver. Livers were perfused with non-recirculating medium as in section 2 and 3.5 ml aliquots of perfusate were collected every 30 s interval. Adrenergic blockers were added at zero time. Infusion of phenylephrine (10^{-5} M final conc.) and glucagon (10^{-8} M final conc.) into the inflowing medium was started after the 5.5 min sample was taken (indicated by arrow).

These results confirm those of our experiments with isolated hepatocytes [2], which indicated that glucagon is much less effective at mobilizing intracellular calcium than are α-agonists. Evidence in [1,2] has indicated that the cAMP-dependent mechanism by which glucagon activates phosphorylase is unrelated to its small action on cell calcium.

Figure 1 (panel A) also shows that the effect of phenylephrine was referable to its interaction with α-receptors since the effect was substantially blocked by the α-antagonist phentolamine (10^{-5} M) and unaffected by the β-blocker propranolol (10^{-5} M). The administration of the α- and β-blockers alone did not have any effect on calcium efflux [2].

Table 1 shows the calcium content of the mitochondria isolated from the livers perfused in fig.1. Phenylephrine produced a large (51%) decrease in mitochondrial calcium content. The inclusion of propranolol did not alter the effect of phenylephrine on mitochondrial calcium content, whereas it was substantially blocked by phentolamine. Thus the changes in mitochondrial calcium induced by the blockers correlated with the alterations in perfusate calcium seen in fig.1. Consistent with the small release of liver calcium produced by 10^{-8} M glucagon, there was little or no decrease in mitochondrial calcium content (table 1).

The average level of calcium in the control mitochondria isolated in the present investigation (1.9 nmol/mg protein) was lower than the range reported [8,13–16] in perfused or non-perfused liver

Table 1
Effects of phenylephrine and glucagon on mitochondrial calcium content

Agent(s)	Mitochondrial calcium at 12 min as % of value at 4 min	
Saline (control)	98 ± 5.9	(n = 3)
Phenylephrine 10^{-5} M	49 ± 7.9	(n = 5)
Phenylephrine 10^{-5} M + Propranolol 10^{-5} M	47, 47	
Phenylephrine 10^{-5} M + Phentolamine 10^{-5} M	82, 95	
Glucagon 10^{-8} M	95, 75	

Mitochondria were isolated from separate liver lobes sampled at 4 min and 12 min in the perfusion experiments shown in fig.1. The mean calcium content of the mitochondria isolated at 4 min was 1.9 ± 0.6 nmol/mg protein (n = 14). Other details are given in the legend to fig.1 and section 2

(4–15 nmol/mg protein). One of the reasons for this is that the livers were perfused with low calcium medium (50 μM), which was necessary in order to accurately measure the release of calcium. In support of this explanation, it was found that when the livers were perfused with medium containing normal levels of calcium (2.4 mM), the mitochondrial calcium content was approximately doubled. Another factor was the further reduction of calcium due to the inclusion of EGTA in the homogenization medium to prevent calcium re-uptake during mitochondrial isolation. Treatment of liver mitochondria with EGTA or EDTA has been shown to markedly decrease the calcium content [14,15].

The α-adrenergic-stimulated efflux of calcium from the mitochondria demonstrated in this communication would be expected to produce a relatively large, transient rise in the level of calcium in the cytosol before it is expelled from the cell. During this rise in cytosolic calcium, phosphorylase *b* kinase would be activated [9], eliciting the conversion of phosphorylase *b* into phosphorylase *a* and hence stimulation of glycogenolysis. The resulting glucose output was observed to follow the efflux of calcium from the liver by ~0.5 min. The maximal rate of calcium efflux was seen at 2 min after hormone infusion while that of glucose output occurred at 3 min.

The mechanism by which mitochondrial calcium is mobilized following binding of catecholamine to α-adrenergic receptors in the plasma membranes [4,5] is unknown. Since no specific α-adrenergic binding sites have been found in liver mitochondria [5], one could postulate the generation of a putative second messenger at the plasma membrane by a mechanism analogous to the β-receptor–adenylate cyclase system. The messenger could be a natural calcium ionophore, which would act to release calcium from the mitochondria [2]. Alternatively, it could be an inhibitor of mitochondrial calcium uptake.

It must be emphasized that the results presented here do not preclude the involvement of other calcium-containing organelles or membranes in the α-adrenergic response. The wet weight of each perfused liver is ~10 g and if it is assumed that there is ~50 mg mitochondrial protein/g wet wt liver [17], it can be calculated from the data in table 1 that the loss of calcium from the whole liver mitochondrial pool is ~0.5 μmol/10 g wet wt liver in the presence of phenylephrine. As the flow rate is constant (7 ml/min), and as the increase in calcium concentration of each fraction in the presence of phenylephrine is known (fig.1), it can be calculated that between 6 and 12 min, the total amount of calcium lost is ~0.8 μmol/10 g wet wt whole liver. Therefore, ~60% of the released calcium can be accounted for by the loss from the mitochondrial pool. This suggests the involvement of another pool. However, two factors may complicate these calculations.

(1) The assumption that 1 g liver contains 50 mg mitochondrial protein [17] may be incorrect, especially in the light of the recent findings that the rat liver mitochondrial population is heterogeneous [18] with respect to, e.g., cytochrome *c* oxidase activity. Light mitochondria contain ~50% less cytochrome *c* oxidase activity than heavy mitochondria [18] and since the present study does not examine which mitochondrial population is affected by phenylephrine, therefore, the 50 mg figure [17], which was based on cytochrome *c* oxidase measurements, may be an underestimate.

(2) The inclusion of 1 mM La^{3+} in the homogenization buffer may act to precipitate some non-mitochondrial protein [7], and this precipitated protein may be carried along with the isolated mitochondria, hence, the calcium content/mg protein may be higher than that reported here. Measurements of calcium changes in other cell organelles or membranes will be required to determine whether or not these structures are involved in the mobilization of intracellular calcium by α-agonists or other hormones.

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