Glucagon treatment of rats inhibits the accumulation of lysophospholipids by liver mitochondria during preparation and subsequent incubation

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1) Rat liver mitochondria isolated from rats exposed to $^{32}$P$_i$ for 24 h and treated with glucagon for 15 min before sacrifice contained less lysophosphatidylcholine and lysophosphatidylethanolamine than those from control animals. 2) Incubation of the mitochondria at 37°C for 15 min increased the lysophosphatidylcholine concentration of control mitochondria but not of those from glucagon-treated animals. 3) Lysophosphatidylethanolamine showed little change during in vitro incubation of mitochondria and this was not affected by glucagon treatment. 4) These results are discussed in relation to the effects of glucagon treatment on mitochondrial function in situ and in vitro.

Liver mitochondria isolated from rats treated with glucagon demonstrate changes in many aspects of their metabolism and an increased stability towards Ca$^{2+}$ loading (see Halestrap, 1981, and Halestrap et al., 1983, for reviews). We have described two in vitro treatments, hypo-osmotic treatment and aging of mitochondria, which can mimic or reverse the effects of glucagon respectively (Armston et al., 1982). This suggested that glucagon might exert its effect by either increasing the intramitochondrial volume or by inhibiting an endogenous mitochondrial phospholipase A$_2$ (Halestrap, 1981, 1982; Armston et al., 1982; Halestrap et al., 1983). Such an inhibition of phospholipase A$_2$ could account for the enhanced stability towards Ca$^{2+}$ loading of mitochondria from glucagon-treated animals (Armston et al., 1982). Siess et al. (1981) have also concluded that glucagon might inhibit endogenous phospholipase A$_2$ and suggested that the effects of glucagon on mitochondrial metabolism might be artefacts of mitochondrial preparation.

In isolated hepatocytes we have demonstrated a hormonally induced increase in intramitochondrial volume although no changes in lysophospholipid content were apparent (Quinlan et al., 1983). The increase in mitochondrial volume may account for those effects of glucagon on mitochondrial metabolism that have been demonstrated in intact hepatocytes. These include increased rates of citrulline synthesis, pyruvate metabolism, glutaminase activity, oxygen consumption, and
mitochondrial NADH/NAD$^+$ (see Halestrap et al., 1983, for review). In addition to these effects there are numerous other aspects of isolated mitochondrial function which glucagon treatment of animals has been reported to affect that are unlikely to be a consequence of an increase in volume (see Halestrap, 1981; Halestrap et al., 1983). Thus significant increases in mitochondrial volume are not always apparent in mitochondria isolated by conventional techniques even when hormone effects on other parameters are apparent (Armston et al., 1982; Hamman & Haynes, 1983; Whipps & Halestrap, 1984). In addition effects of glucagon treatment are apparent on the respiratory chain of both submitochondrial particles and broken mitochondria where intramitochondrial volume cannot be important (Titheradge et al., 1978; Halestrap, 1982). In the case of submitochondrial particles energization of the particles by ATP hydrolysis or respiration is also enhanced by glucagon treatment (Titheradge et al., 1978). These volume-independent effects of glucagon could be explained if glucagon inhibited phospholipase A$_2$ during preparation of the mitochondria as has been suggested by some (Halestrap, 1981, 1982; Siess et al., 1981; Armston et al., 1982; Quinlan et al., 1983). The experiments reported in this paper are designed to test this hypothesis. The data presented show that glucagon treatment causes an inhibition of lysosphospholipid formation by mitochondria during their isolation and subsequent incubation.

Materials and Methods

Materials

The sources of all chemicals, biochemicals, and radiochemicals were as described previously (Armston et al., 1982; Quinlan et al., 1983).

Female Wistar rats of about 100 g body weights and 4 weeks of age were used, and free access to food and water was allowed.

Method of preparation of liver mitochondria with $^{32}$P-labelled phospholipids

Rats were injected (intraperitoneally) with 2 mCi of $^{32}$P$_j$ (carrier-free and neutralized with NaHCO$_3$) 24 h before preparation of liver mitochondria. They were then anaesthetized by injection (intraperitoneal) of 0.1 ml of Sagatal, and when required glucagon (0.1 mg) was injected after 30 min as described previously (Halestrap, 1978). After a further 15 min, livers were removed and homogenized in 40 ml of ice-cold isolation buffer containing 300-mM sucrose, 10-mM Tris/HCl, and 2-mM EGTA before separation of mitochondria by differential centrifugation (Halestrap, 1978). The resultant mitochondrial pellet was further purified by resuspending in Percoll (17.5% v/v in isolation buffer) and centrifuging at 10 000 g for 15 min. Using this procedure more than 95% of the plasma-membrane and microsomal contamination was shown to be absent from the resulting mitochondrial pellet as judged by assay of suitable marker enzymes (Vargas, 1982). The mitochondria were washed free of Percoll by resuspension in isolation buffer and subsequent centrifugation at 10 000 g for 5 min.