THE REGULATORY ROLE OF AMYLO-1,6-GLUCOSIDASE/OLIGO-1,4→1,4-GLUCANTRANSFERASE IN LIVER GLYCOGEN METABOLISM

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1. Introduction

The regulation of mammalian glycogen metabolism *in vivo* has hitherto been evaluated in terms of the well-established regulatory properties of glycogen phosphorylase (EC 2.4.1.1), UDPG:glycogen α-4-glucosyltransferase (EC 2.4.1.11, glycogen synthetase) and associated enzymes [1–3]. This approach cannot be regarded as totally comprehensive in that it fails to take into account the involvement in glycogen metabolism of branching and debranching enzymes specific for α-1,6-glucosidic linkages. The present investigation was directed towards evaluating the role of the debranching enzyme system, amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase (EC 3.2.1.33; EC 2.4.1.25) in the regulation of glycogen metabolism in mammalian liver. Evidence is presented to suggest that this system may assume a rate-limiting function when phosphorylase is subjected to hormonal control by adrenaline.

2. Experimental

Four dogs (15–27 kg), pre-fed on a high carbohydrate diet, were studied. The dogs were anaesthetized (sodium pentobarbitone, B.P.) and cannulae were inserted into the right brachial artery (blood pressure recording apparatus), the left saphenous vein (saline drip) and into the right femoral artery (blood sampling). The abdomen was opened to permit access to the liver. Multiple liver biopsies were taken in the course of the experiments (immediately following each dose of adrenaline) and were rapidly frozen in liquid nitrogen. Blood glucose [4], liver-glycogen content [5], the average unit-chain length and degree (%) of β-amylolysis of glycogen [5] and liver-glycogen phosphorylase activity [6] were determined by the documented methods. Amylo-1,6-glucosidase activity in liver homogenates was determined using the specific substrate 6-α-glucosyl α-Schardinger dextrin [7] (there is no specific method for the determination of transferase activity in crude homogenates). After the initial biopsy to determine base-line values, successive doses of adrenaline were administered via the left saphenous vein. Further biopsies were removed during the experiment. Minor bleeding resulting from liver biopsies was controlled with haemstat gauze (Ethicon Ltd., Edinburgh, UK), or with a ligature, or Spencer Wells forceps.

Abbreviation:

Amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase to glucosidase/transferase.

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3. Results and discussion

Glycogen phosphorylase is exo-acting and specific for α-1,4-glucosidic linkages. Accordingly, the α-1,6-linkages of glycogen impose a restriction on the extent to which the polysaccharide may be degraded by phosphorylase. The enzyme has unrestricted action on approximately 40% of the glycogen mole-

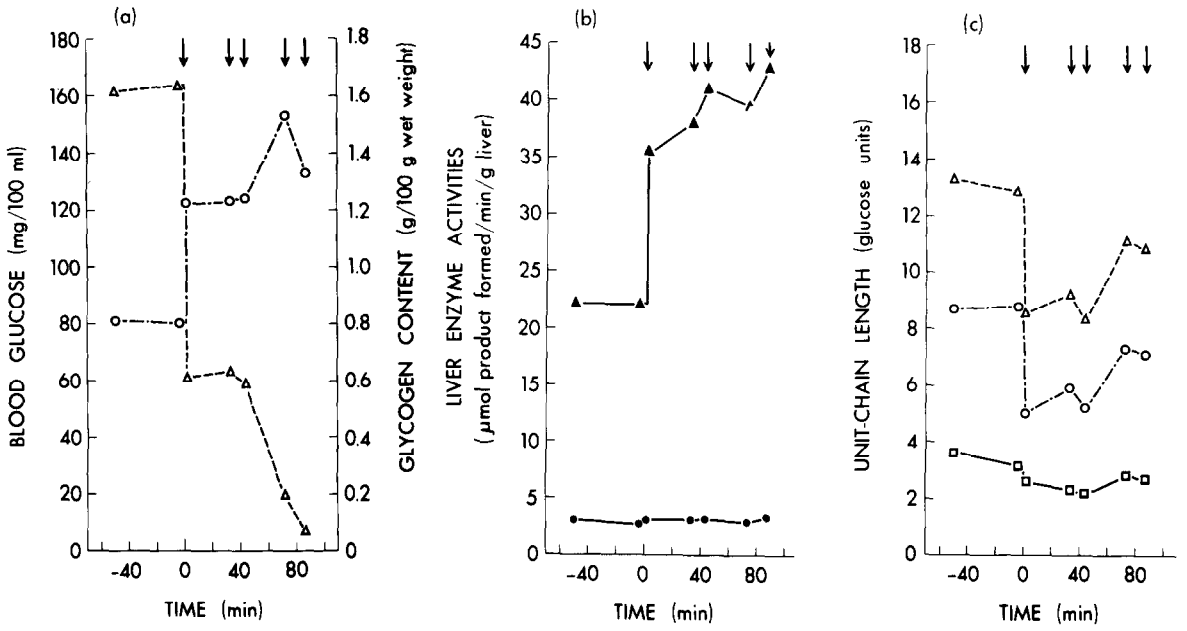

Fig. 1. The effect of successive doses of adrenaline (250 μg) on carbohydrate metabolism in the dog. (a) Effect on blood glucose (○) and liver-glycogen (△). (b) Effect on liver-glycogen phosphorylase activity (▲) and amylo-1,6-glucosidase activity (●). (c) Effect on average unit-chain length (△), external unit-chain length (○) and internal unit-chain length (□) of liver glycogen. The arrows refer to the times when adrenaline was administered.

cule. Total degradation of glycogen (to glucose-1-P and glucose) requires the combined actions of glycogen phosphorylase and glucosidase/transferase. The rate of *total* glycogenolysis is thereby subject to regulation in three possible ways: (a) The rate of debranching is regulated in concert with the regulation of phosphorylase activity. Thus, the rate of glycogenolysis is regulated by dual enzyme control. (b) The rate of debranching is in excess of the maximal rate of phosphorylase action. Thus, glycogen phosphorylase is the rate-limiting enzyme. (c) The rate of debranching is less than the maximal rate of phosphorylase action. Thus, in the primary phase of glycogenolysis (equivalent to approximately 40% mobilization), phosphorylase is the rate-limiting enzyme. If degradation proceeds beyond this point, glucosidase/transferase assumes the rate-limiting function.

These alternatives may be distinguished experimentally. Adrenaline stimulates glycogenolysis via a cyclic AMP-mediated activation of glycogen phosphorylase. Under these conditions the regulatory function of glucosidase/transferase may be assessed by (1) determining the relative activities of phosphorylase and glucosidase/transferase (the theoretical ratio of activities to permit parity in rates in glycogenolysis is dependent on average unit-chain length and is in the order of 9–14:1) and (2) by examining glycogen structure. The unit-chain length of glycogen is a direct index of the relative rates of α-1,4- and α-1,6-linkage turnover.

Adrenaline administration to sedated dogs resulted in immediate and excessive liver-glycogen mobilization with associated hyperglycaemia (the results for a typical experiment are shown in fig. 1a, b, and c). The stimulation of glycogenolysis was a direct consequence of hormonal activation of glycogen phosphorylase. Amylo-1,6-glucosidase activity was unaffected by hormone administration. The second and third doses of adrenaline produced no apparent stimulation of glycogenolysis. Subsequent doses, however, resulted in further mobilization of the polysaccharide to a point when the glycogen reserves were almost totally exhausted (residual liver glycogen content 0.07%).

The invariance in the glucosidase activity (and pre-

sumably transferase activity also) shows that this enzyme is not subject to control by adrenaline and is not regulated in a concerted fashion with glycogen phosphorylase activity. The apparent activity of the enzyme is approximately one order of magnitude lower than maximal phosphorylase activity, and is within the range required to provide for parity in the rates of phosphorolysis and debranching.

The glycogen unit-chain length decreased significantly following the initial adrenaline infusion. Determination of the degree of β-amylolysis coupled with knowledge of average unit-chain length permits calculation of the average external and internal chain lengths [5]. The internal unit-chain length was constant (2–4 glucosyl units). All structural changes resulting from stimulated glycogenolysis were in external unit-chain length, which decreased to six glucosyl units (the external unit-chain length of a phosphorylase-limit dextrin of glycogen is four glucosyl units). The unit-chain length increased in the terminal phase of glycogenolysis.

The results permit the conclusion that (1) glucosidase/transferase activity is not subject to regulation by adrenaline, (2) amylo-1,6-glucosidase activity is sufficiently high to guarantee that glycogen phosphorylase is the rate-limiting enzyme in glycogenolysis, at least when the enzyme is in its non-activated state, i.e. in the resting animal [1, 8], and (3) glucosidase/transferase may assume a rate-limiting function when phosphorylase is subject to hormonal activation. This regulation is viewed as a form of negative control and may well have a conservational function.

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