Titration of Rat Liver with Digitonin: a Well Defined Short Term Damage of Cellular Metabolism

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Z. Naturforsch. **36 c**, 880–883 (1981); received June 2, 1981

Digitonin, Liver Perfusion, Metabolic Compartmentation, Rat, Cell Damage

Carefully performed pulse titration of the isolated rat liver in the course of continuous erythrocyte free perfusion with small amounts of digitonin causes a short term permeability of liver cell plasma membranes with concomitant short lived release of intracellular low or high molecular compounds such as ATP or lactate dehydrogenase. Gluconeogenesis from lactate being completely inhibited during this period restores within about one minute up to a level that depends on the amount of perfused digitonin. The described experimental model is suggested to be useful for the measurement of cytoplasmic metabolites under physiological conditions. It moreover offers the possibility to import foreign substances into liver cells that normally do not penetrate liver cell plasma membranes.

Digitonin since long is used for hemolysis of erythrocytes in clinical routine [1]. Several publications are concerned with the basic mechanism of the digitonin-cholesterol interaction [2–5]. Zuurendonk and Tager [6] introduced this substance in the field of research on liver energy metabolism working with isolated liver cells. Since it becomes increasingly evident that isolated hepatocytes differ in a number of characteristics from liver cells in tissue entity, it seemed therefore desirable to adapt this method for the isolated perfused liver.

In this paper we present evidence that the short time opening of liver cells by digitonin is reversible and that during this period diffusion through the permeable plasma membranes takes place with subsequent loss of biosynthetic capacity of liver tissue at least for the investigated time period.

Materials and Methods

10 months old female Wistar rats with a mean weight of 240 g were used. The animals were fasted at least 24 h before operation. The isolation of the liver was performed according to Scholz [7] using Earle's [8] modified medium: 0.2% albumin, Cohn V fraction, Serva, no glucose, 2 mM lactate and 0.25 mM pyruvate. The bicarbonate buffer was replaced by 20 mM MOPS-buffer, pH 7.4, since oxygenation was accomplished by a modified membrane oxygenator [7]. Flowrate of at 37 °C tempered perfusion medium was kept constant at 36 ml/min. Were indicated, digitonin was injected as a single dose in a bubble collector before entering the liver by the portal vein. At the given flowrate, a mean concentration of digitonin for 15 sec of 0.250 mM can be calculated. Continuous digitonin perfusion was performed with 72 µM digitonin. Perfusate was collected in icecold tubes in time intervals as indicated and aliquots were precipitated with 1/10th of their volume of 6 N PCA. After neutralization metabolites were measured according standard procedures [9] and enzyme activities were determined according to standard procedures [9] from unprecipitated samples. Glucose was determined by the use of GOD-Perid kit from Boehringer, Mannheim. All biochemicals and chemicals used were of analytical grade.

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0341-0382/81/0900-0880 $ 01.00/0

Results and Discussion

Continuous perfusion of the isolated liver with 71 µM digitonin in Earle's modified medium leads to a complete and irreversible breakdown of gluconeogenesis within about 4–5 min (Fig. 1 a). Concomitantly released lactate dehydrogenase represents the total amount of LDH of the perfused liver (Fig. 1 b) amounting to about 2737 mU/g liver. The observation that neither glutamate nor β-hydroxybutyrate dehydrogenase activity was detectable in the perfusate indicates that only cytoplasmic compartment leaked out. Mitochondrial membranes are known to

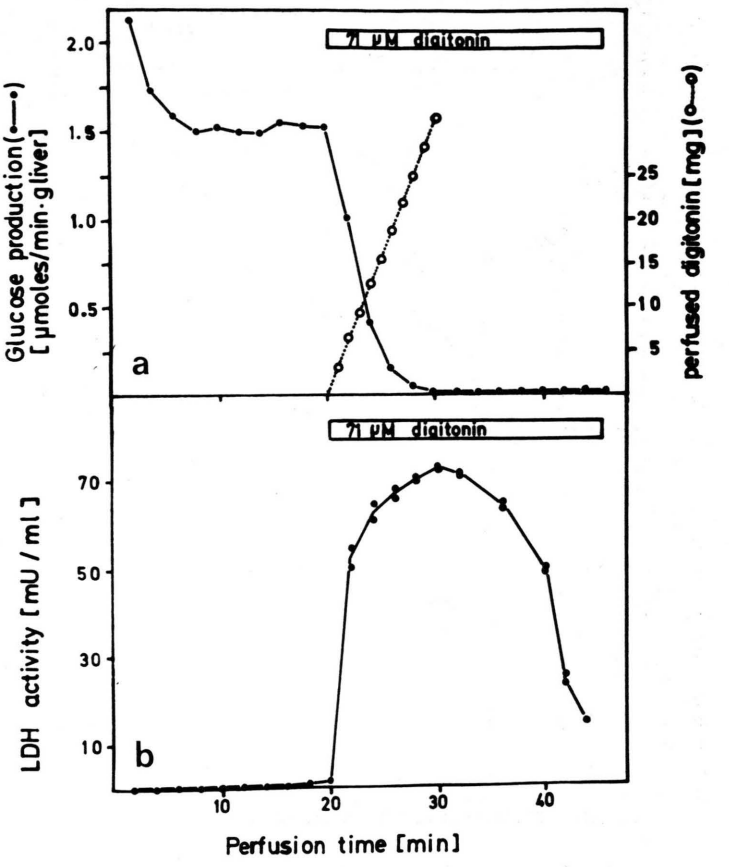

Fig. 1. Influence of continuous perfusion of digitonin containing Earles modified medium through isolated rat liver. Fig. 1 a: Glucose production (●—●) and total amount of perfused digitonin (○—○). Fig. 1 b: Release of lactate dehydrogenase (●—●). The data are taken from a typical experiment.

contain much lower cholesterol concentration [10, 11], thus being more resistent against digitonin treatment.

From the data of Fig. 1 a it can be calculated that an amount of about 25 µmol digitonin/g liver was sufficient to completely and irreversibly abolish gluconeogenesis. This value is in the same order of magnitude as that of free cholesterol of the plasma membranes of 1 g of liver tissue, estimated from the data of van Hoeven and Emmelot [10]. Fig. 1 a moreover shows the close correlation between the amount of perfused digitonin and glucose production.

In order to get a more sophisticated insight into this correlation, pulse titration was performed to reduce the activity of gluconeogenesis in a well defined and a reversible manner. The effect of repeated addition of 0.23 µmol digitonin/g liver on gluconeogenesis is depicted in Fig. 2 a. It clearly comes out that the liver cells are opened for a short time period as can be recognized by the leakage of ATP (Fig. 2 b) and of lactate dehydrogenase (Fig. 2 c). As was already observed in the first experimental device, correlation between glucose production and titrated digitonin again is found (see insert of Fig. 2 a). On the basis of further experiments with higher time resolution (data not shown), it can be estimated that liver cells are freely permeable for about 15–30 sec. Again, no glutamate dehydrogenase activity was detectable. The released ATP thus has to be considered to come mainly from cytoplasmic compartment; the more so, since once ATP has left the intracellular space, it is immediately taken away by the steady stream of perfuion medium. Thus, back diffusion is almost excluded.

Moreover, on the basis of the data from Fig. 1 b, it can be calculated that the amount of LDH released during the first digitonin pulse amounts to about 2.5% of total LDH (= 68 mU/g liver). Assuming about the same degree of relation for the ATP

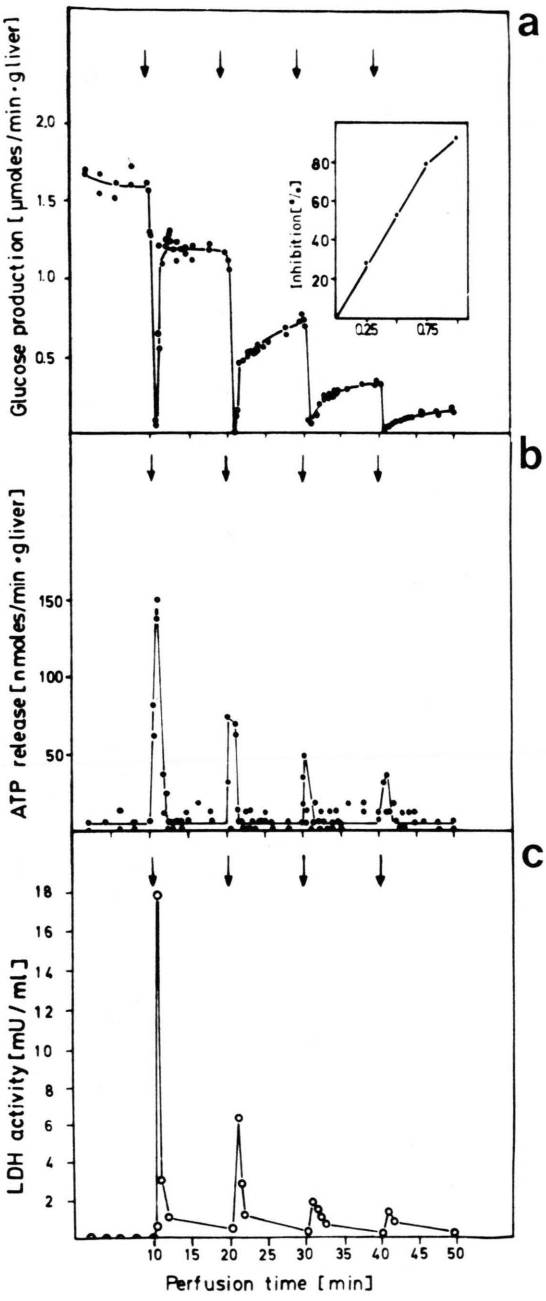

Fig. 2. Pulse titration of isolated rat liver with digitonin. The arrows indicate injections of 0.23 µmol of digitonin per gram of liver. Fig. 2a: Glucose production. Insert of Fig. 2a: correlation between inhibition of glucose production and the amount of perfused digitonin in µmol per gram of liver. (The data are taken from Fig. 2a.) Fig. 2b: ATP release and Fig. 2c: Release of lactate dehydrogenase activity. The data are taken from a typical experiment.

release, ATP content of cytoplasmic compartment comes to about 1.3 µmol/g liver. This value is in good agreement with the value for cytoplasmic ATP of 1.41 µmol/g liver tissue, which can be estimated from the data reported by Soboll *et al.* [12].

The question whether the observed digitonin effect might be interpreted 1) as a successive and irreversible damage of different cell populations [13] of 2) as a common damage of most liver cells together might be discussed as follows. Since concomitantly to the digitonin injection glucose production is completely inhibited followed by restauration depending on the total amount of perfused digitonin, second assumption has to be preferred. If really different cell populations would be damaged no short lived and complete inhibition of glucose production should be expected. The observation that repeatedly injected small amounts of digitonin produce from time to time decreasing effect, whereas cells are far from complete leakage, might be interpreted in favour of the existence of free and compartmentalized LDH [14] and free and bound ATP. In contrast to the effect of small amounts of digitonin, continuous perfusion of digitonin seems to liberate total compartimentalized LDH and bound ATP.

Although, Jungermann and Sasse [15] pointed the dynamic aspect of "metabolic zonation" meaning that glycolytic zone can be converted into a gluconeogenic zone, this can not be taken into consideration for an explanation of the described digitonin effect, since such changes are known to take place within several hours only.

The described method is suggested to represent a promising experimental device for *in vivo* cytoplasmic metabolic studies. Furthermore, it is under current investigations to be useful for the import on non-penetrating substances into liver cells under normal conditions.

Acknowledgements

We wish to thank to S. Hoppe for engaged technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Schwerpunkt „Biologie des Alterns").

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