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EFFECT OF ADRENALIN, HYDROCORTISONE, INSULIN, AND DIBUTYRYL-CYCLIC AMP ON GLYCOLYSIS AND GLYCOGENOLYSIS IN SURVIVING LIVER SPLICES FROM ALBINO RATS

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Adrenalin, hydrocortisone, and dibutyryl-cyclic AMP inhibited glycolysis and glycogenolysis in surviving liver slices from albino rats. An inhibitory effect also was found when glucose-6-phosphate (G6P), but not fructose-1,6-diphosphate, was used as the substrate for glycolysis. This indicates that activity of hexokinase and also, probably, of phosphorylase and phosphofructokinase, is inhibited under the influence of these hormones and dibutyryl-cyclic AMP. In a reconstituted cell-free system the hormone had no effect and dibutyryl-cyclic AMP inhibited hexokinase only. For the hormones to exert their effect interaction between them and the cell membrane was essential. Inhibition of glycogen and G6P breakdown to lactic acid in liver slices is not connected with the action of the hormones on the corresponding enzymes (phosphorylase and phosphofructokinase) directly through cyclic AMP and protein kinase. The results are evidence of an additional mechanism modifying the action of cyclic AMP on the activity of the above-mentioned enzymes. Insulin had no effect in all cases.
KEY WORDS: *adrenalin; hydrocortisone; insulin; cyclic AMP; glycolysis; glycogenolysis.*

Considerable progress has recently been made in the study of the hormonal regulation of metabolism. The mechanism of regulation of enzyme activity by the phosphorylation–dephosphorylation principle has been discovered [8]. The molecular mechanisms of action of adrenalin and glucagon on tissue phosphorylase activity have been elucidated [11, 13]. The important role of cyclic nucleotides as intermediate mediators of hormone action has been demonstrated. However, several fundamental problems still remain unexplained. This applies in particular to the mechanism of action of glucocorticoids and insulin on the state of glycolysis and glycogenolysis. It has been shown, for example, that hormones participate in the suppression and induction of hexokinase synthesis respectively in the liver [1, 14], whereas their role in the allosteric regulation of the key enzymes of anaerobic carbohydrate breakdown is not clear. It is not known whether penetration of glucocorticoids into the cell

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TABLE 1. Effect of Adrenalin, Hydrocortisone, Insulin, and Dibutyryl-Cyclic AMP on Velocity of Glycolysis and Glycogenolysis in the Liver (M ± m)

Substrate	Glycolysis in liver tissue	Glycolysis in slices				
		without additions	dibutyryl-cyclic AMP	adrenalin	hydrocortisone	insulin
Glucose (6)	9,52±0,92	9,58±1,1	6,9±0,7*	6,8±0,4*	7,7±1,4*	9,9±1,3
Glycogen (7)	13,0±1,2	12,3±1,3	7,5±1,1*	7,7±0,8*	9,2±1,6*	11,5±1,1
G6P (7)	22,1±1,4	16,8±1,1	12,7±1,3*	12,6±0,8*	13,5±1,4*	16,9±1,1
FDP (7)	40,8±3,6	42,3±4,0	41,9±2,6	42,1±3,2*	40,6±3,6	40,1±3,6

Legend. Here and in Tables 1-3. *)P < 0.01; significance calculated relative to control "without additions." Number of analyses shown in parentheses

is essential for the realization of "periodic" mechanisms of regulation of glycolysis and glycogenolysis. Do these hormones act directly on the key enzymes or indirectly through corresponding intracellular mediators? What compounds can play the role of these mediators in the action of glucocorticoids on metabolism? Are crossed effects found in the action of adrenalin, hydrocortisone, and insulin on the corresponding enzymes of glycolysis and glycogenolysis?

The investigation described below was undertaken in an attempt to shed light on some of these problems.

EXPERIMENTAL METHOD

Female Wistar rats weighing 150-200 g were used. The method of surviving tissue slices was used to study the molecular mechanisms of action of hormones. Compared with investigations *in vivo*, this method has certain advantages. Distortion of the results on account of changes in the character of interendocrine relations during prolonged administration of the hormone is eliminated. The effect of the numerous factors which attempt to restore homeostasis, when modified by exogenous hormones, is ruled out. Yet at the same time, this is a relatively complex system in which the special features of intercellular interaction and also the internal structure of the cells are preserved.

Liver slices 0.4-0.6 mm thick were incubated in an atmosphere of air for 1 h at 37°C in Krebs–Ringer–phosphate buffer with the addition of 2% albumin. The hormones were added in the following final concentration: insulin 0.7×10^{-6} M, hydrocortisone 0.5×10^{-6} M, adrenalin 5.0×10^{-4} M. The concentration of dibutyryl-cyclic AMP was 1×10^{-4} M. Adrenalin and dibutyryl-cyclic AMP were added 10 min before the end of incubation, and insulin and hydrocortisone were added immediately. After the end of incubation the slices were washed with cold physiological saline and homogenized. The 10% homogenate was centrifuged at 20,000g (15 min). The rate of glycolysis in the supernatant fraction was determined in a reconstituted system [4] of the following composition: Tris-HCl, pH 7.4 2 mM, K-phosphate buffer, pH 7.4 7 mM, $MgCl_2$ 5 mM, ATP 1 mM, nicotinamide 5 mM. To detect the limiting stages and to assess their state during the action of hormones and mediators the rate of anaerobic breakdown of carbohydrates was determined with various substrates: 10 mM glucose, 0.1% glycogen, and 3 mM glucose-6-phosphate (G6P) and fructose-1,6-diphosphate (FDP) and expressed in nanomoles lactate/min/mg protein. Lactate was determined by an enzymic method [9]. Two controls were set up: glycolysis in the intact liver and in slices after incubation without hormones or dibutyryl-cyclic AMP. The direct action of the hormones and dibutyryl-cyclic AMP also was studied on the enzymes of glycolysis in the reconstituted system. The supernatant fraction for this series was obtained from 10% liver homogenate after centrifugation (105,000g, 1 h).

EXPERIMENTAL RESULTS

Preincubation of the liver slices for 1 h was shown not to be reflected in the state of glycolysis and glycogenolysis (Table 1). Some decrease was observed in the rate of conversion of G6P to lactate, but this did not disturb the character of the interenzymic relationships formed: The velocity of glycolysis and glycogenolysis rose stepwise when substrates bypassing the key enzymes were used. The limiting enzyme of glycolysis in the liver is hexokinase, and that of glycogenolysis is evidently phosphorylase. It was shown previously that the activity of phosphoglucomutase and phosphohexoisomerase is considerably higher than the activity of the key enzymes [10, 12]. The next limiting enzyme of anaerobic conversion

TABLE 2. Effect of Adrenalin, Hydrocortisone, and Insulin on Velocity of Glycolysis and Glycogenolysis Directly in Reconstituted System (M ± m)

Substrate	Without addition (control)	Adrenalin ($1 \cdot 10^{-6}$ M)	Hydrocortisone ($0.5 \cdot 10^{-6}$ M)	Insulin ($0.7 \cdot 1 \cdot 10^{-6}$ M)
Glucose	6,6±0,6 (9)	6,7±0,9 (5)	6,0±0,9 (6)	6,5±0.6 (9)
Glycogen	11,5±0,5 (10)	11,5±0,6 (5)	13,1±0,9 (10)	11,6±0.6 (10)
G6P	16,1±0,7 (10)	15,0±1,2 (5)	18,8±0,5 (10)	16,4±0.6 (10)
FDP	36,0±1,3 (11)	36,6±2,8 (4)	38,9±1,0 (11)	36,2±1.7 (11)

TABLE 3. Effect of Dibutyryl-Cyclic AMP on Velocity of Glycolysis and Glycogenolysis in a Reconstituted System (M ± m)

Substrate	Without addition (control	Dibutylryl-cyclic AMP		
		10^{-5} M	10^{-4} M	10^{-3} M
Glucose	6,6±0,6 (9)	3,8±0,4* (6)	3,4±0,4* (8)	3,4±0,3* (6)
Glycogen	11,5±0,5 (10)	11,4±0,9 (7)	11,1±1,0 (7)	13,5±0,9 (6)
G6P	16,1±0,7 (10)	14,8±0,5 (7)	15,8±0,8 (8)	16,7±1,3 (5)
FDP	36,0±1,3 (11)	35,5±1,2 (8)	35,1±1,5 (9)	37,3±1,2 (6)

of carbohydrates in the liver is phosphofructokinase. For the process as a whole, especially when glycogenolysis is activated by glucagon or adrenalin, phosphofructokinase may play the role of the first limiting enzyme in muscle tissue, for example [2].

Preincubation of the liver slices with adrenalin, hydrocortisone, and dibutyryl-cyclic AMP considerably reduced the velocity of breakdown of glycogen, glucose, and G6P to lactic acid. The rate of breakdown of FDP was unchanged. Insulin had no effect under these conditions. The results are evidence that adrenalin and hydrocortisone play an important role in the "routine" mechanisms of regulation of the anaerobic conversion of carbohydrates in the liver. A crossed effect was observed in the action of the two hormones, connected with inhibition of the initial enzymes of glycolysis and glycogenolysis, namely phosphorylase, hexokinase, and phosphofructokinase. The role of general intracellular mediator in this system is probably played by cyclic AMP. In experiments *in vivo* the writers showed previously that the activity of the initial enzymes of glycolysis and glycogenolysis is lowered under the influence of extraordinary external enviromental factors [5, 6]. According to existing views cyclic AMP increases phosphorylase activity. It is through this mechanism that adrenalin exerts its stimulating action on this enzyme [11, 13], an action connected with activation of the corresponding protein kinase. Inhibition of the initial stages of glycolysis and glycogenolysis under the influence of adrenalin, hydrocortisone, and dibutyryl-cyclic AMP in these experiments suggests the existence of additional mechanisms and, perhaps, of a second mediator modifying the effect of cyclic AMP.

Adrenalin, hydrocortisone, and insulin had no effect on the enzymes of glycolysis or glycogenolysis in the reconstituted system (Table 2). Comparison of the data in Tables 1 and 2 supports the view that reception of the hormones on the hepatocyte cell membrane is essential for realization of the metabolic effect of adrenalin and hydrocortisone. The presence of α- and β-adrenoreceptors is not now disputed. Probably the existence of cell receptors for glucocorticoids must also be accepted.

Dibutyryl-cyclic AMP had an inhibitory action in the reconstituted system only on glycolysis, over a wide range of concentrations (Table 3). The rate of conversion of G6P and FDP was unchanged under these conditions. The inhibitory action of dibutyryl-cyclic AMP on hexokinase is probably exerted through an allosteric mechanism [7]. In the presence of a high concentration of dibutyryl-cyclic AMP (1×10^{-3} M) a distinct tendency was observed toward activation of glycogenolysis. It can tentatively be suggested that insufficient activation of the process was due to the fact that the phosphorylase and its kinase were

present in the tissue in the inactive form as a complex with protein. During preparation of the supernatant fraction the complex with protein may have been broken down, with conversion of the phosphorylase into the active form [3]. In this case the addition of cyclic AMP could no longer increase the activity of the enzyme. It is interesting to note that under these conditions the inhibitory action of cyclic AMP on phosphorylase and phosphofructokinase likewise could not be reproduced. This is further evidence that in intact cells, as is observed during preincubation of the slices with dibutyryl-cyclic AMP, the action of the mediator is modulated by some form of additional mechanism. This role may perhaps be played by a second mediator, the nature of which is not yet clear. Insulin had no action in any of these cases on the enzymes of glycolysis and glycogenolysis. This hormone probably has no allosteric effect toward enzymes of glycolysis and glycogenolysis. However, it may perhaps participate in the allosteric regulation of anaerobic conversion of carbohydrates in the liver. This effect is mediated through phosphodiesterase, whose activity is increased by the action of insulin. As a result of destruction of cyclic AMP by phosphodiesterase the metabolic effect of catecholamines and glucocorticoids is blocked. Insulin can thus perform the role of a counterhormone relative to the action of glucocorticoids and also, to some extent, of catecholamines.

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