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INDUCTION OF GLUCOKINASE BY INSULIN UNDER THE PERMISSIVE ACTION OF DEXAMETHASONE IN PRIMARY RAT HEPATOCYTE CULTURES

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SUMMARY

Glucokinase, the organ specific key enzyme of glucose metabolism in liver, was studied in primary cultures of adult rat hepatocytes during the first two days after cell preparation. In the presence of dexamethasone low concentrations of insulin (10^{-9} mol/l) prevented the observed time dependent decrease of glucokinase activity while higher insulin concentrations (10^{-8} and 10^{-7} mol/l) led to a twofold increase of enzyme activity. The enhancement of glucokinase activity was completely blocked by either actinomycin D or cycloheximide. The degree of this insulin dependent induction was correlated with the concentration of added dexamethasone, which seemed to perform a permissive function. The induction of glucokinase activity could be prevented by addition of glucagon (2×10^{-7} mol/l).

INTRODUCTION

Glucokinase (E.C. 2.7.1.2) is of fundamental significance for the regulation of glucose metabolism in liver (1). This high K_m-glucose-ATP-phosphotransferase which is present in higher concentration only in hepatocytes (2,3) plays a key role in the glucose-homeostasis: it responds to the variation of the substrate level in an autoregulatory manner (4) and to changes of hormone levels and food intake by variation of its cellular level (5-10). In the intact animal glucokinase has been found to be induced by insulin (8-11); however, different and partly contradictory observations exist with regard to the influence of glucocorticoids on this enzyme level (10-13). This may be due to the complex system of the whole animal as well as to a multihormonal action on enzyme activity. Less complicated systems like incubated liver slices, isolated perfused liver or suspension of isolated hepatocytes are not suitable for induction experiments because of their short survival time (14, 15).

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On the other hand proliferating liver cell cultures and permanent cell lines apparently do not any longer contain the liver specific marker enzyme glucokinase (1). Only non-proliferating primary cultures of adult hepatocytes preserve most of their liver specific functions during the first days after cell preparation (16-18). In the present investigation the regulation of the glucokinase level by insulin, glucagon and glucocorticoids was studied in primary rat hepatocytes cultures. It was found that glucokinase was induced by insulin only under the permissive action of dexamethasone. Activity was increased twofold in hepatocytes of fed and fourfold in hepatocytes of fasted animals. The induction was prevented by addition of glucagon.

MATERIALS AND METHODS

Fetal calf serum, culture medium, enzymes and coenzymes were from Boehringer GmbH, D 6800 Mannheim, except collagenase type II which was supplied by Worthington, Freehold, N.J., USA. Bovine insulin, bovine glucagon and dexamethasone as well as penicillin, streptomycine sulfate, actinomycin D and cycloheximide were obtained from Sigma, D 8021 Taufkirchen. Bovine serum albumin fraction V was supplied by Roth, D 7500 Karlsruhe, 60 mm culture dishes by Greiner, D 7440 Nürtingen.

Rat liver parenchymal cells were isolated from fed or 48 h starved Wistar rats (150-180 g, Ivanovas, D 7967 Kissleg) by an in situ recirculating collagenase perfusion (19,20), cells were cultured in Medium 199 (Earle's salts) (21) containing 2 g/l serum albumin, 18 mmol/l $NaHCO_3$, 30 mmol/l N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 117 mg/l streptomycin sulfate, 60 mg/l penicillin and 2 µl/l each of oleic, linoleic and linolenic acid. The osmolarity was adjusted to 290 mosm/l. Hormones and inhibitors of protein synthesis were added as indicated for each experiment. 1.5-2.4 x 10^6 cells in 3 ml medium containing 5 % fetal calf serum and 10^{-9} mol/l insulin and 10^{-9} mol/l dexamethasone were inoculated into each culture dish, which had been coated with collagen gel (22). Incubation was carried out at 37°C in gas-tight containers gassed with a humidified atmosphere of 13 % O_2, 5 % CO_2 and 82 % N_2. After 4 h, when the cells had adhered to the collagen gel, medium was changed and fetal calf serum was then omitted. Collagen gels were made to float by loosening them from the bottom of the culture dishes. Induction of glucokinase was initiated by addition of hormones at this time or later as indicated. The next medium change was performed after 28 h.

Gels were processed for glucokinase assay as follows: they were at first washed in 0.9 % NaCl and then homogenized using an Ultra-Turrax (Janke und Kunkel KG, D 7813 Staufen) in 25 mmol/l glycylglycin, 35 mmol/l KCl, 6 mmol/l $MgSO_4$, 5 mmol/l ethylenediaminetetraacetic acid and 10 mmol/l 2-mercaptoethanol at pH 7.5. The glucokinase assay was modified according to Pilkis (23). For the measurement of hexokinase (E.C. 2.7.1.1) activity the glucose concentration (approx. 0.75 mmol/l) resulting from remaining culture medium was sufficient. Glucokinase plus hexokinase activity was determined after addition of glucose to a final concentration of 80 mmol/l. Glucokinase activity was obtained as the difference of

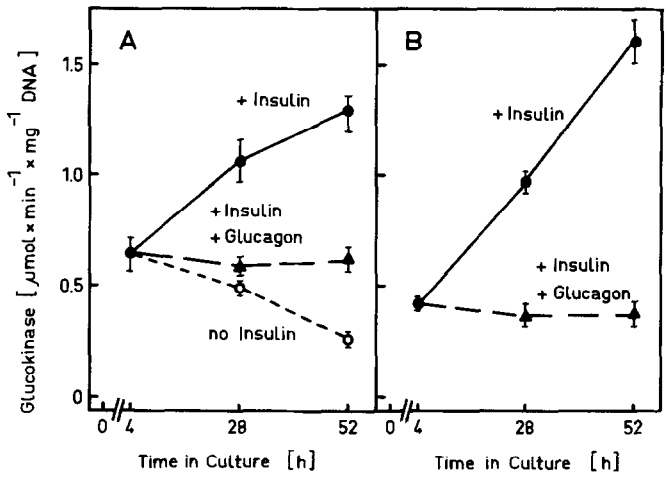

Fig. 1. Induction of glucokinase in primary cultured hepatocytes by insulin in the presence of dexamethasone. Liver parenchymal cells of fed (A) and 48 h starved (B) rats were cultured under standard conditions. 4 h and 28 h after plating medium was changed and 10^{-7} mol/l insulin with or without 2 x 10^{-7} mol/l glucagon were added as indicated. After the first medium change 10^{-7} mol/l dexamethasone was always present. Values are means ± SEM of 6 cultures from 2 representative experiments. The differences between the 4 h-value and the 28 h- or 52 h-values under dexamethasone or dexamethasone plus insulin were significant: $p < 0.01$.

total glucokinase plus hexokinase activity minus hexokinase activity. DNA was estimated as described (18).

RESULTS

In hepatocytes of fed rats cultured under standard conditions without hormones the glucokinase activity was decreased to less than 50 % of the initial value during the first two days. This behaviour was not altered by the addition of 10^{-7} mol/l dexamethasone (Fig. 1 A). In the presence of dexamethasone the addition of 10^{-7} mol/l insulin not only prevented the decrease but led to a significant increase of the enzyme activity to 165 % within 24 h and to 200 % within 48 h after the plating period during the first four hours (Fig. 1 A). This increase could be prevented by the addition of 2 x 10^{-7} mol/l glucagon Essentially no changes of the glucokinase activity were observed under these conditions.

In no case did the activity measured in presence of 0.75 mmol/l glucose exceed 8 % of the activity determined in the presence of 80 mmol/l glucose. Because glucokinase with a K_m between 10-20 mmol/l

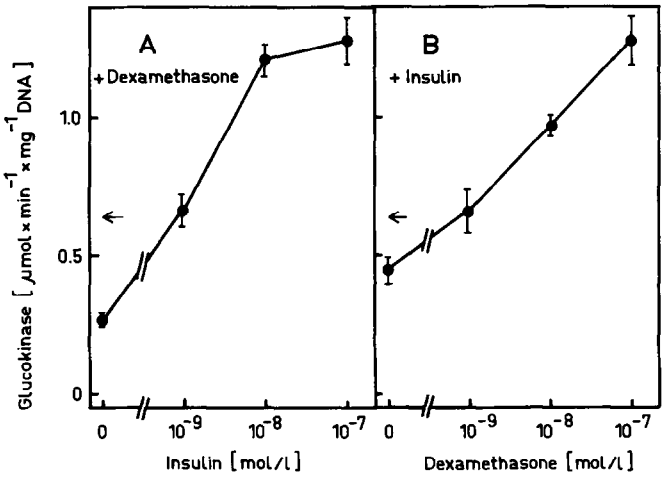

Fig. 2. Dependence of glucokinase activity on insulin in presence of 10^{-7} mol/l dexamethasone (A) and on dexamethasone in presence of 10^{-7} mol/l insulin (B) in primary hepatocyte culture after 48 h hormone treatment as indicated. 4 h and 28 h after plating medium was changed and hormones were added. Values are means ± SEM of 6 cultures from 2 representative experiments. Initial values were indicated by arrows. For comparison: physiological insulin level in the portal vein: ≦ 3×10^{-9} mol/l (26).

(15,24) should exhibit at 0.75 mmol/l glucose a substrate saturation of at least 3 %, hexokinase might be responsible only for less than 5% of the total hexokinase plus glucokinase activity.

In hepatocytes of 48 h starved rats cultured under standard conditions significantly lower glucokinase levels were observed. 10^{-7} mol/l insulin in the presence of 10^{-7} mol/l dexamethasone led to a significant increase of glucokinase activity to 230 % within 24 h and to 390 % within 48 h after the plating period (Fig. 1 B). The insulin dependent increase of glucokinase activity was prevented by glucagon also under these conditions.

In the presence of 10^{-7} mol/l dexamethasone glucokinase in cultures of fed hepatocytes was decreased without insulin to 45 %, it was maintained by 10^{-9} mol/l insulin and increased to 189 % by 10^{-8} mol/l insulin and only slightly more to 200 % by 10^{-7} mol/l insulin within the first two days (Fig. 2 A). A similar dependence of the glucokinase level on the glucocorticoid hormone was observed. In the presence of 10^{-7} mol/l insulin the enzyme activity was decreased without dexamethasone to 71 %, it was maintained by 10^{-9} mol/l dexamethasone and it was increased to 152 % and to 200 % by 10^{-8} mol/l and 10^{-7} mol/l dexamethasone, respectively (Fig. 2 B). The

Table 1. Inhibition of hormone dependent glucokinase induction by actinomycin D and cycloheximide

Inhibitor	Time after addition 0 h	4 h	12 h
	Glucokinase [μmol x min^{-1} x mg^{-1} DNA]		
no	0.59 ± 0.06	0.67 ± 0.03	0.90 ± 0.10 *
actinomycin D	-	0.54 ± 0.04	0.48 ± 0.07
cycloheximide	-	0.60 ± 0.06	0.37 ± 0.02 **

Values are means ± SEM of three determinations with each of three parallel cultures. Rat hepatocytes were cultured for 28 h under standard conditions in the presence of 10^{-9} mol/l insulin and 10^{-9} mol/l dexamethasone. With the medium change 10^{-7} mol/l insulin and 10^{-7} mol/l dexamethasone ± 0.4 mg/l actinomycin D or 10 mg/l cycloheximide were then added. Significant differences compared with the initial value are indicated by asterisks: * = $p < 0.025$, * = $p < 0.001$.

increase of glucokinase activity was prevented by either actinomycin D or cycloheximide which specifically blocked protein synthesis on the transcriptional or translational level (Table 1). During periods up to 12 h these inhibitors did not produce any dramatic changes of the cell morphology and it therefore could be concluded that the enhancement of glucokinase activity was due to enzyme induction. Due to the relatively slow glucokinase induction (6,15) it would have been desirable to study the induction and its inhibition also over a longer period of one or two days. Yet this was not possible, because the survival of cultured hepatocytes was impaired mainly by a longer lasting inhibition of protein synthesis.

DISCUSSION

In this communication it was shown for the first time that glucokinase could be induced in primary cultures of hepatocytes from adult rats by insulin under the permissive action of dexamethasone (Fig. 1 and 2). The induction which was observed in cultures during the first 48 h was prevented by the addition of glucagon. Previously glucokinase could not be maintained or induced in adult non proliferating (25) and proliferating hepatocyte cul-

tures (1). This advantage of the present culture system over other primary hepatocyte cultures may be due first to different cultivation conditions e.g. the presence of fetal serum only during the plating period or the use of floating collagen gels and second to the presence of hormones, such as insulin and dexamethasone.Similar to in vivo investigations using the whole animal (8-11) insulin concentrations (10^{-8} mol/l (Fig. 2)) somewhat higher than the physiological portal level were required for the induction of glucokinase also in primary liver cell cultures. This relatively high concentrations may be required because of the degradation of more than 90 % of added insulin within 24 h (27) and because of the fact that insulin receptors may be partially degraded by collagenase during the preparation of single hepatocytes.

The insulin dependent induction of glucokinase in primary hepatocyte cultures required the simultaneous presence of dexamethasone which by itself was not even able to maintain the glucokinase level. Therefore dexamethasone seemed to have a permissive effect on insulin action similar to that demonstrated on the action of other hormones such as glucagon and catecholamines which operate through cyclic AMP (28). It is possible that the observed glucagon-dependent inhibition of the induction shown earlier in the whole animal (15), was also effected by antagonistic effects on the cyclic AMP level. Thus, the ratio of insulin and glucagon seems to be more important for the regulation of the glucokinase level than the absolute concentration of either of these hormones (29). Therefore the heterogeneous distribution of that enzyme over the liver acinus with low periportal and high perivenous activity (30) could be effected by an increase of the insulin/glucagon ratio during blood passage from the periportal to the perivenous area; an alteration of the hormone ratio could be effected by different degradation rates of both hormones in liver.

The advantage of the present system over studies with whole animals is based on the presence of differentiated parenchymal tissue independent of other complicating parameters. Earlier in vivo studies with the whole animal suffered from such complex interactions (1), which may explain the different and partly contradictory observations regarding the influence of glucocorticoids on the insulin dependent induction of glucokinase (11-14).

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REFERENCES

1) Weinhouse, S. (1976) Curr. Top. Cell. Regul. 11, 1-50
2) Sapag-Hagar, M., Marco, R. and Sols, A. (1969) FEBS Lett. 3, 68-71
3) Crisp, D.M. and Pogson, C.J. (1969) Biochem. J. 126, 1009-1023
4) Scrutton, M.C. and Utter, M.F. (1968) Ann. Rev. Biochem. 37, 249-302
5) DiPietro, D.L. and Weinhouse, S. (1960) J. Biol. Chem. 235, 2542-2545
6) Sharma, C., Manjeshwar, R. and Weinhouse, S. (1963) J. Biol. Chem. 238, 3840-3845
7) Niemeyer, H., Perez, N. and Codoceo, R. (1967) J. Biol. Chem. 242, 860-864
8) Adelman, R.C. and Freeman, C. (1972) Endocrinology 90, 1551-1560
9) Salas, M., Viñuela, E. and Sols, A. (1963) J. Biol. Chem. 238, 3535-3538
10) Pilkis, S.J. (1970) Biochim. Biophys. Acta 215, 461-476
11) Ilyin, V.S. (1964) Adv. Enzyme Reg. 2, 150-172
12) Sharma, C., Manjeshwar, R. and Weinhouse, S. (1964) Adv. Enzyme Reg. 2, 189-200
13) Walker, D.G. and Rao, S. (1964) Biochem. J. 90, 360-368
14) Ruderman, N.B., Lauris, V. and Herrera, M.G. (1967) Am. J. Physiol. 212, 1169-1173
15) Sols, A., Sillero, A. and Salas, J. (1965) J. Cell. Comp. Physiol. 66, 23-28
16) Bonney, R.J., Becker, J.E., Walker, P.R. and Potter, V.R. (1974) In Vitro 9, 399-413
17) Savage, C.R. Jr. and Bonney, R.J. (1978) Exp. Cell. Res. 114, 307-315
18) Oliver, J.T., Edwards, A.M. and Pitot, H.C. (1978) Eur. J. Biochem. 87, 221-227
19) Berry, M.N. and Friend, D.S. (1969) J. Cell. Biol. 43, 506-520
20) Katz, N. and Jungermann, K. (1976) Hoppe Seyler's Z. Physiol. Chem. 359, 359-375
21) Morgan, J.F., Morton, H.J. and Parker, R.C. (1950) Proc. Soc. Expl. Biol. Med. 73, 1-8
22) Michalopoulos, G. and Pitot, H.C. (1975) Exp. Cell. Res. 94, 70-78
23) Pilkis, S.J. (1975) Methods Enzymol. 42, 31-39
24) Parry, M.J. and Walker, D.G. (1966) Biochem. J. 99, 266-274
25) Walker, P.R. (1977) J. Cell. Physiol. 91, 169-180
26) Berger, W., Göschke, H. and Ohnhaus, E. (1975) in Journées de Diabétologie, Hotel-Dieu, Flammarion Médicine-Sciences pp.240-258
27) Bernaert, D., Wanson, J.-C., Drochmans, P. and Popowski, A. (1977) J. Cell. Biol. 74, 878-900
28) Jost, J.P. and Rickenberg, M.V. (1971) Ann. Rev. Biochem. 40, 741-774
29) Foa, P.P. (1977) in Glucagon: Its Role in Physiology and Clinical Medicine (Foa, P.P., Bajaj, J.S. and Foa, N.L., eds.) pp. XX-XLI, Springer, New York
30) Katz, N., Teutsch, H.F., Sasse, D. and Jungermann, K. (1977) FEBS Lett. 76, 226-230