IMPROVED RESPONSE OF ISOLATED LIVER CELLS TO GLUCAGON IN THE PRESENCE OF RAT SERUM

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SUMMARY: The stimulation by glucagon of gluconeogenesis from 10 mM lactate and urea formation in intact isolated rat liver cells is enhanced from about 33 % to 61 % and 92 %, respectively, by the addition of rat serum (30 % v/v) to the incubation medium. This effect is exerted by (a) dialysable, heat-labile serum component(s), different from known stimulators of gluconeogenesis, such as fatty acids, lysine, ammonium chloride or calcium, which do not improve the response of hepatocytes to glucagon. The factor(s) could provide a useful tool for the study of glucagon action(s) on liver metabolism.

INTRODUCTION

In the perfused rat liver glucagon increases gluconeogenesis from lactate by 75 - 100 % (1-5). In contrast, with isolated rat liver cells much smaller effects of the hormone or 3',5'-cAMP have been observed being in the range of 17-56 % (6-10). Therefore, experiments were started in our laboratory with the aim to improve the hormonal response of isolated hepatocytes. The present paper reports that the addition of serum to the incubation medium markedly increases the stimulation by glucagon of glucose and urea synthesis in isolated rat liver cells.

MATERIALS AND METHODS

Enzymes, coenzymes, fumarate, pyruvate, and the test kit for urea determination were purchased from Boehringer (Mannheim, Germany). Gelatin "non plus ultra" was obtained from Serva (Heidelberg, Germany) and bovine serum albumin from Behring Werke (Marburg, Germany). Glucagon was a gift from Hoechst (Frankfurt-Hoechst, Germany).

Parenchymal liver cells were prepared from 24 h - 36 h fasted male Sprague-Dawley rats (150 - 220 g body weight) according to Zahlten et al. (11). About 98 % of cells excluded 0.2 % trypane blue. Serum was obtained usually from 24 h fasted male Wistar

rats (200 - 250 g), in a few cases serum from fasted male Sprague-Dawley rats with equal results was used. To obtain "dialysate", serum was dialysed at 4°C against a 10-fold volume of distilled water overnight. Then the water volume was reduced to that of the starting serum by rotary evaporation, discarding any precipitate which might have formed. "Dialysed serum" was obtained by adding solid NaCl, 9 mg/ml, to the contents of the dialysis bag. Prior to use the pH of serum, dialysate, dialysed serum or ashed dialysate was adjusted to pH 7.4 with KOH (glass electrode). The incubation mixture consisted of 0.5 ml Ca^{2+} - free Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 1.5 % gelatin (KH-G), 0.1 ml 0.16 M l-lactate, 0.5 ml KH-G, or serum, or serum components, respectively, and 0.5 ml liver cell suspension in KH-G corresponding to about 50 mg fresh liver. Incubation at 37°C was performed, in stoppered plastic tubes (10 x 1.9 cm) with 95 % O_2 + 5 % CO_2 as the gas phase in a shaking waterbath. After 20 min of incubation 10 µg of glucagon in 5 µl 1.3 % $NaHCO_3$ or solvent only was added and the incubation continued for 45 min. Then the cells were removed from an aliquot of the incubation mixture by rapid centrifugation (about 20 sec at 10 000 xg) and the clear supernatant was analyzed for glucose (12) and urea. No difference in the pH value of the vials was detectable. The results given are corrected for glucose or urea present at the end of the 20 min preincubation period and hence represent the changes in product formation within the second incubation period of 45 min. Tabulated data are expressed in µmoles/g wet liver ± s.e.m. on the basis of 10^8 cells corresponding to 1 g wet liver (11). The number of independent experiments with different cell preparations is given in parentheses.

RESULTS AND DISCUSSION

Table 1 summarizes the effects of the addition of serum on gluconeogenesis from lactate by isolated hepatocytes. In the absence of glucagon the addition of rat serum caused an elevation of the rate of glucose production by 77 % (group A and C) and a similar effect was exerted by dialyzed serum (52 %, group C). Charcoal treatment of the dialyzed serum according to Chen (13) diminished the stimulation (not shown), indicating that it might be due to fatty acids bound to serum proteins. This view was supported by the fact that the addition of oleate to the charcoal treated serum restored the stimulation. Moreover, albumin-bound oleate (0.6 mM) elevated the rate of glucose production by 92 %, as illustrated in Table 2. Johnson et al. (14) and Cornell et al. (15) reported an increase in glucose form-

TABLE 1

STIMULATION BY SERUM OF THE EFFECT OF GLUCAGON ON GLUCONEOGENESIS FROM 10 mM LACTATE IN ISOLATED RAT LIVER CELLS.

Exp. group	Additions	µmoles glucose/g wet liver/45 min		change due to glucagon	% stimulation by glucagon
		No glucagon	glucagon (6µg/ml)		
A a)	Control	27.8±3.2(13) (100 %)	36.1±3.9(13) (p<0.001)	8.3±1.2	30
b)	Rat serum (30 % v/v)	49.2±6.9(13) (177 %)	79.1±9.6(13) (p<0.001)	29.8±2.9 (p<0.001)	61
B a)	Control	20.7±2.3(9)	27.5±3.3(9) (p<0.001)	6.8±1.2	33
b)	Dialysate from rat serum (30 % v/v)	20.8±2.1(9)	35.4±3.9(9) (p<0.001)	14.6±2.2 (p<0.01)	70
c)	Dialysate after ashing (30 % v/v)	19.4±2.8(4)	25.3±3.0(4) (p<0.001)	5.9±0.6 (n.s.)	30
C a)	Control	30.5±7.0(5) (100 %)	38.5±8.2(5) (p<0.01)	8.0±1.7	26
b)	Rat serum (30 % v/v)	54.0±13.4(5) (177 %)	87.0±19.4(5) (p<0.005)	33.3±6.3 (Pvsc)< 0.025)	61
c)	Dialysed rat serum (30 % v/v)	46.3±10.9(5) (152 %)	60.3±12.6(5) (p<0.0025)	14.0±1.9	30
D a)	Control	21.1±3.4(4)	27.8±5.9(4) (p<0.05)	6.7±2.7	32
b)	Human serum (30 % v/v)	30.9±3.5(4)	51.4±5.3(4) (p<0.005)	20.5±3.4 (p<0.025)	66

TABLE 2

STIMULATION BY OLEATE OF GLUCONEOGENESIS FROM 10 mM LACTATE IN ISOLATED HEPATOCYTES.

Oleate (mM)	µmoles glucose/g wet liver/45 min	% stimulation by oleate
0	18.6 ± 1.7 (4)	
	($p < 0.0025$)	
0.6	35.8 ± 2.5 (4)	92

Experimental details as described in Materials and Methods except that 0.2 ml albumin-bound oleate was added per tube. The incubation was stopped by the addition of 0.1 ml 70 % $HClO_4$.

ation from lactate by about 30 % and 70 % in the presence of 1 mM and 2 mM oleate, respectively. Thus, gluconeogenesis in isolated liver cells is enhanced by fatty acids to the same degree as in the perfused liver (for a review see (16)).

By addition of glucagon to the serum-free incubation medium glucose production was increased by about 30 % (Table 1 and 4). Almost the same value was reported by Zahlten et al. (10). However, as may be seen from Table 1, in the presence of serum a marked increase of the glucagon effect (61 % in group A, C, 66 % in group D) was apparent. This effect is not attributable to the serum fatty acids, as indicated by experiments where 0.6 mM oleate did not raise the glucagon response (28 % stimulation by glucagon in 4 experiments). Furthermore, dialysed serum (which retains its fatty acids) no longer showed an enhancement of the

TABLE 3

STIMULATION BY RAT SERUM OF THE EFFECT OF GLUCAGON ON UREA FORMATION IN ISOLATED RAT LIVER CELLS.

Exp. group	Additions	µmoles urea/g wet liver/45 min: No glucagon	µmoles urea/g wet liver/45 min: glucagon (6µg/ml)	change due to glucagon	% stimulation by glucagon
A	Control	5.23±0.75(5)	7.06±0.83(5) (p<0.0025)	1.83±0.26	35
	Rat serum (30 % v/v)	6.38±1.12(6)	12.57±2.01(6) (p<0.0025)	6.19±0.92 (p<0.005)	92
B	Control	5.8o±0.93(8)	7.41±1.11(8) (p<0.001)	1.61±0.25	28
	Dialysate from rat serum (30 % v/v)	6.86±0.91(9)	12.03±1.48(9) (p<0.001)	5.17±0.83 (p<0.005)	75

glucagon action (Table 1, group Cc). This, however, was recovered in the dialysing fluid ("dialysate") (group Bb) indicating that the stimulatory factor(s) of the serum might be of low molecular weight(s). No stimulation was obtained with heated dialysate (group Bc), which might suggest an organic nature of the factor(s).

Since the perfusion studies of Miller (17) the stimulatory effect of glucagon on urea formation has become a well-documented fact. As shown in Table 3 this effect could now be demonstrated also with isolated hepatocytes. As may be furthermore seen in Table 3, serum or "dialysate" amplified the effect of glucagon on urea formation to an extent similar to that on glucose formation.

TABLE 4

EFFECT OF GLUCAGON ON GLUCONEOGENESIS FROM 10 mM LACTATE IN ISOLATED HEPATOCYTES IN THE PRESENCE OR ABSENCE OF NH_4Cl.

Exp. group	NH_4Cl (mM)	µmoles glucose/g wet liver/45 min: No glucagon	µmoles glucose/g wet liver/45 min: glucagon (6µg/ml)	change due to glucagon	% stimulation by glucagon
A	0	24.7±2.4(6)	32.3±3.7(6) ($p<0.005$)	7.6±1.8	33
	0.61	26.8±3.9(6)	34.5±5.3(6) ($p<0.0025$)	7.7±1.4 (n.s.)	29
B	0	24.6±2.6(5)	30.4±4.3(5) ($p<0.05$)	5.8±2.0	24
	1.21	27.7±4.0(5)	37.5±5.1(5) ($p<0.025$)	9.8±2.8 (n.s.)	36

While these studies were in progress Zahlten et al. reported that the addition of 1 mM NH_4Cl to the incubation medium doubled the rate of gluconeogenesis from lactate and enhanced the stimulation by glucagon from 26 % to 61 % (10). However, in our experiments (Table 4) NH_4Cl displayed only a small (if any) increase of gluconeogenesis from lactate in the absence of glucagon, confirming the results of Cornell et al. (15). Furthermore, we were unable to detect a significant stimulation of the glucagon effect by NH_4Cl (Table 4). Thus our results obtained with serum or "dialysate" can hardly be related to an action of NH_4Cl.

TABLE 5

EFFECT OF GLUCAGON ON GLUCONEOGENESIS FROM 10 mM LACTATE IN ISOLATED HEPATOCYTES IN THE PRESENCE OR ABSENCE OF Ca^{2+}.

Exp. group	Ca^{2+} (mM)	µmoles glucose/g wet liver/45 min				% stimulation by glucagon
		no glucagon		glucagon (6µg/ml)		
			%		%	
A	0	14.2	100	19.2	100	35
	1.0	20.8	146	25.5	133	23
B	0	24.4	100	38.9	100	60
	1.0	32.9	135	46.9	120	43
C	0	30.0	100	36.4	100	21
	1.0	35.9	120	41.9	115	16
D	0	30.8	100	38.6	100	25
	2.5	43.9	142	60.0	155	37
E	0	24.4	100	38.9	100	59
	2.5	33.9	139	50.9	131	50

Besides fatty acids and NH_4Cl, lysine (15) and Ca^{2+} (9,10) have been reported to stimulate gluconeogenesis from lactate. As may be seen from Table 5, Ca^{2+} increased glucose production by 35 - 40 %, however, this was not accompanied by an enlargement of the glucagon effect. Lysine (0.6 mM) increased glucose formation by 65 % (mean of 3 experiments), in agreement with the results of Cornell et al. (15), but again the effect of glucagon was unchanged. Other serum constituents such as glucose, glycerol, ketone bodies, P_i or K^+ screened alone or in

combination with dialysed serum remained ineffectual as amplifiers of the glucagon effect. Thus it would appear, that serum contains a heat-labile, dialysable, as yet unidentified factor (or factors) that enable(s) isolated hepatocytes to respond to glucagon stimulation with respect to glucose and urea syntheses quantitatively almost in the same manner as observed in liver perfusion.

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REFERENCES

1. Schimassek, H. and Mitzkat, H.J. (1963) Biochem. Z. 337, 510 - 518.
2. Struck, E., Ashmore, J., and Wieland, O. (1965) Biochem. Z. 343, 107 - 110.
3. Ross, B.D., Hems, R., and Krebs, H.A. (1967) Biochem. J. 102, 942 - 951.
4. Exton, J.H. and Park, C.R. (1968) J. Biol. Chem. 243, 4189 - 4196.
5. Fröhlich, J. and Wieland, O. (1971) Eur.J.Biochem. 19, 557 - 562.
6. Veneziale, C.M. and Lohmar, P.H. (1973) J. Biol. Chem. 248, 7786 - 7791.
7. Cornell, N.W., Lund, P., Hems, R., and Krebs, H.A. (1973) Biochem. J. 134, 671 - 672.
8. Garrison, J.C. and Haynes, R.C., jr. (1973) J. Biol. Chem. 248, 5333 - 5343.
9. Tolbert, M.E.M. and Fain, J.N. (1974) J. Biol. Chem. 249, 1162 - 1166.
10. Zahlten, R.N., Kneer, N.M., Stratman, F.W., and Lardy, H.A. (1974) Arch. Biochem. Biophys. 161, 528 - 535.
11. Zahlten, R.N., Stratman, F.W., and Lardy, H.A. (1973) Proc. Nat. Acad. Sci. (USA) 70, 3213 - 3218.
12. Stein, M.W. (1962) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., ed.) pp. 117 - 123, Verlag Chemie, Weinheim/Bergstr.
13. Chen, R.F. (1967) J. Biol. Chem. 242, 173 - 181.
14. Johnson, M.E.M., Das, N.M., Butcher, F.R., and Fain, J.N. (1972) J. Biol. Chem. 247, 3229 - 3235.
15. Cornell, N.W., Lund, P., and Krebs, H.A. (1974) Biochem. J. 142, 327 - 337.
16. Wieland, O. (1968) Adv. Metab. Disorders 3, 1 - 47
17. Miller, L.L. (1961) Recent Progr. Hormone Res. 17, 539 -568.