Antagonism Between the Effects of Insulin and Glucagon on the Isolated Liver

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SUMMARY

Antagonism was demonstrated between the effects of insulin and glucagon, added simultaneously to the perfusing blood, on phosphorylase activity, glucose output and urea production of the isolated rat liver. However, the degree of antagonism varied greatly, according to the action under study, and there appeared to be a clear-cut hierarchy in the effects of each hormone. For glucagon, this was (stimulation of): (1) phosphorylase activity, (2) glycogenolysis and glucose release, (3) urea production. For insulin, it was (inhibition of): (1) urea production, (2) glycogenolysis, (3) phosphorylase activation. Thus, high concentrations of insulin completely failed to inhibit the phosphorylase activation induced by 1.4 mμg./ml. of glucagon, while modest concentrations of insulin totally suppressed the stimulation of urea production induced by sustained glucagon concentrations at least ten times greater, and well above the biologic range. Our findings argue against a single common primary site of action of these two hormones. DIABETES *18*:724-32, November, 1969.

Various effects of insulin on hepatic metabolism have been studied for many years but until recently there was considerable doubt whether insulin had any direct action at all on the liver.[1,2] At present, there is no hypothesis which satisfactorily explains all of the diverse effects of insulin.[1-4] The action of glucagon on the liver, however, has been characterized in considerable detail;[5,6] this hormone appears to play an essential role in stimulating hepatic production of glucose via glycogenolytic and gluconeogenic pathways.

Recent evidence that administration of small doses of glucagon to experimental animals, or man, results in an almost immediate stimulation of insulin secretion,[7,8] raises the question whether the effects of glucagon in vivo might not be significantly different from those defined by experiments with the isolated liver, because of the actions of endogenous insulin reaching that organ only moments later. Samols[7] has pointed out that glucagon, after secretion by pancreatic alpha cells, must be in proximity to beta cells in relatively high concentration and, hence, could stimulate insulin secretion even before reaching the general circulation.

The present study was undertaken to define the antagonism, if any, between the direct hepatic effects of insulin and glucagon, at portal vein concentrations of each hormone within or near the biologic range.

METHODS

Fed male Charles River CD rats weighing 250-400 gm. were used as liver donors. Blood donor animals were retired breeders of the same strain, fasted for eighteen to twenty-two hours to insure minimal blood insulin concentrations. Perfusion apparatus and technics have been described previously.[9] In brief, livers were perfused with diluted heparinized rat blood containing antibiotics (initial volume, 100 or 120 ml.) in a recirculating system. Blood entered the liver via the portal vein by free flow at a pressure of 13-15 cm. of blood. An opening in the reservoir flask permitted the direct collection of heptic outflow blood before it mixed with the pool in the reservoir. Blood entering the liver could be sampled by puncturing the rubber tubing of the inflow line immediately proximal to the portal vein cannula. Blood flow rate through the liver was determined directly by collecting hepatic outflow over an accurately timed interval. Minor hepatic lobes were ligated and removed at intervals to obtain specimens for phosphorylase and glycogen assay. Such specimens (except for two very small lobes near the hilum of the liver) are representative of the entire organ, and their removal does not affect the function of the remaining liver tissue. The weights of these specimens and of the liver tissue remaining at the end of an experiment were recorded; thus, liver weight at various times during perfusion could be calculated.

The principal measure of glycogenolysis used in this

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study was the hepatic glucose output rate. This is a very sensitive index, which can measure slight and transient glycogenolysis not detectable by serial glycogen determinations.[10] The glucose output rate was determined by measuring glucose concentrations in triplicate samples of heptic inflow and outflow blood by an AutoAnalyzer (Technicon Corporation). The mean hepatic outflow-inflow difference was multiplied by the blood flow rate through the liver, and the result divided by the liver weight at the time of sampling, to give hepatic glucose output in mg./min./gm. liver.

Urea production during the hour after hormonal addition was used as a measure of protein/amino acid catabolism. Since the changes in total urea content of the system were relatively small, special care was taken to obtain these data in the most precise manner possible. Urea was determined in triplicate blood samples. Accurate estimation of blood volume changes during experiments and of urea losses from the system in the course of sampling had to be made. To accomplish this, several preliminary perfusions were performed, in which all materials used in collecting and processing liver and blood samples were tared and reweighed immediately after sampling to determine the weight (and hence, volume) of blood removed from the system in the course of an experiment. These data made possible a very close approximation of the actual blood volume in the perfusion system at any point in time and permitted calculation of the amount of urea removed in blood and liver samples.

Solutions of crystalline glucagon and/or glucagon-free insulin (Eli Lilly Co.) were added to the reservoir and were vigorously mixed with reservoir blood. Phosphorylase activity was determined by a method described previously,[11] using pieces of liver which had been frozen rapidly and stored in a dry ice refrigerator until assay. Glycogen was determined by the method of Good, Kramer and Somogyi[12] using the AutoAnalyzer for the determination of glucose after hydrolysis. Blood urea nitrogen was measured by the plasma method of Chaney and Marback.[13]

EXPERIMENTAL DESIGN

One hour was allowed for stabilization of the preparation, after initiation of liver perfusion. Then, serial blood and liver samples were obtained for determination of hepatic glucose output and phosphorylase activity, during a twenty-minute baseline period (i.e., at 60, 70 and 80 minutes of perfusion), and of urea concentration and liver glycogen immediately prior to hormonal additions. Additions of insulin and/or glucagon were then made into the blood reservoir. Additional samples of blood and liver were obtained during a one-hour experimental period. For each perfusion, mean baseline values for phosphorylase activity and hepatic glucose output were subtracted from values obtained after hormonal additions, to give the changes attributable to hormonal action. Urea production rates during the one-hour experimental period were calculated for each liver, and changes in liver glycogen recorded; the effects of hormonal addition on these parameters were determined by comparing data from the various groups of perfusions.

Hormonal additions were selected to reproduce (a) concentrations in the lower half of the biologic range for the portal vein, (b) relatively high biologic concentrations, and (c) concentrations at or above the upper limit of the biologic range. For insulin, these additions were 0.03, 0.10 and 0.30 units, giving calculated peak plasma concentrations of approximately 400, 1,400 and 4,000 μU./ml. In the perfusions at the lowest concentration, a second addition of insulin, 0.015 U., was made thirty minutes later, in order to compensate for hepatic inactivation of the hormone. A second addition was not made in the other perfusions, since it was believed that concentrations within the ranges desired would be maintained for one hour. To ensure that the insulin concentrations actually achieved were in the desired ranges, plasma samples were obtained during experimental periods in representative perfusions and subjected to immunoassay for insulin,* with the following results:

No insulin added—mean concentrations: 5, 6μU./ml.

Addition of 0.03 U., supplemented by 0.015 U.—mean concentrations: 140, 160 μU./ml.

Addition of 0.1 U.—mean concentrations: 850, 700 μU./ml.

Addition of 0.3 U. — mean concentrations: 3,000, 2,200 μU./ml.

The biologic range for portal vein glucagon concentrations has not been satisfactorily defined and, thus, glucagon additions were selected somewhat arbitrarily, on the basis of data from previous studies of glucagon action in this laboratory[10,11,14,15] and published figures for glucagon concentration in the pancreaticoduodenal vein, measured by radioimmunoassay.[16] The additions selected were 0.03 μg. (calculated peak plasma concentration, 0.4 to 0.5 mμg./ml.), an amount known

*Kindly performed by Dr. L. F. Frohman, Department of Medicine, State University of New York at Buffalo, Buffalo, N. Y.

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to cause distinct, but mild and relatively brief glycogenolysis in the isolated rat liver; 0.1 μg. (peak plasma concentration, 1.4 to 1.7 mμg./ml.), an amount known to give maximal phosphorylase activation and brisk glycogenolysis; 0.3 μg. (peak plasma concentration, 4 to 5 mμg./ml.), an amount known to give maximal glycogenolysis in the liver perfusion system; and 1.0 μg. (peak plasma concentration, 14 to 17 mμg./ml.), an amount which, from all available data, gives concentrations distinctly above the biologic range. Because glucagon is inactivated rapidly by the isolated liver,[14] these peak concentrations were maintained only for short periods, determined by the rates of blood circulation through the liver; one circulation time for the blood volume in the system was usually six to ten minutes, and the estimated glucagon half-life, about four minutes.

Phosphorylase activation by glucagon is very rapid, and the level of enzyme activity reached after a single addition of this hormone to the blood reservoir remains stable for at least twenty minutes.[10,11] Under the conditions of those experiments, reproducible figures for hepatic glucose output, near the maximal values reached after various additions of glucagon, are obtained by averaging the rates observed ten and twenty minutes after addition of the hormone to the blood reservoir. Thus, evaluation of the effects of hormonal additions on phosphorylase activity and hepatic glucose output was based on the data obtained at ninety and 100 minutes of perfusion (i.e., 9 to 10 and 19 to 20 minutes after addition of glucagon). Urea production rates could not be determined accurately over so short a time period. However, previous studies in this laboratory[15] showed that the stimulation of urea production induced by glucagon persists for at least one hour after addition of this hormone. Thus, despite the fact that glucagon concentrations were probably very low during the final thirty minutes of the experimental period, it seemed reasonable to evaluate effects on urea production over this time period. In a supplementary group of perfusions, performed specifically to check data for urea production, glucagon concentrations were maintained at high levels throughout the one-hour experimental period (see below).

RESULTS

In six control perfusions, in which Ringer's solution was added to the blood reservoir at the end of the baseline period, satisfactory stability of the preparation was demonstrated (figure 1). The blood glucose concentration, which had risen initially to levels above 200 mg./100 ml. as the result of glycogenolysis prior

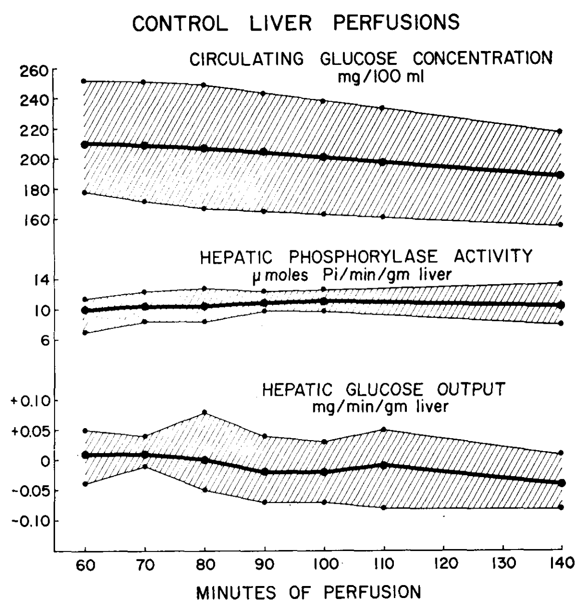

FIG. 1. Blood glucose concentration, hepatic phosphorylase activity and hepatic glucose output in six control perfusions. The highest, lowest and mean values at each time interval are plotted.

to and immediately after starting perfusion, remained stable or declined slowly after the sixty-minute equilibration period. Hepatic glucose output rates of individual livers at various times between sixty and 140 minutes ranged from –0.08 to + 0.08 mg./min./gm. liver, and averaged –0.01 mg./min./gm. Phosphorylase activity ranged from 8.1 to 14.3 μmoles Pi/min./gm. and remained stable in individual livers. Liver glycogen ranged from 11 to 53 mg./gm. liver and did not change significantly during the one-hour experimental period. Urea production ranged from 0.14 to 0.34 mg. urea N/gm. liver during the one-hour experimental period; approximately 60 per cent of this was accounted for by urea withdrawn during blood sampling, confirming the importance of meticulous control of experimental procedures in attempts to measure amino acid catabolism in this system.

Hormonal effects on phosphorylase activity

Increases in phosphorylase activity after addition of glucagon were seen in all groups of perfusions and, as in our previous experience,[11] maximal increases (7.3 ± 0.7 μmoles Pi/min./gm.) were observed after addition of 0.1 μg. of glucagon. In the perfusions with insulin alone (0.03 or 0.3 U.), insignificant decreases in phosphorylase activity were observed (1.0 ± 1.0 μmoles Pi/min./gm. liver). In the perfusions with 0.03 μg. of glucagon, insulin inhibited phosphorylase activation, with a good dose-effect relationship (figure 2). How-

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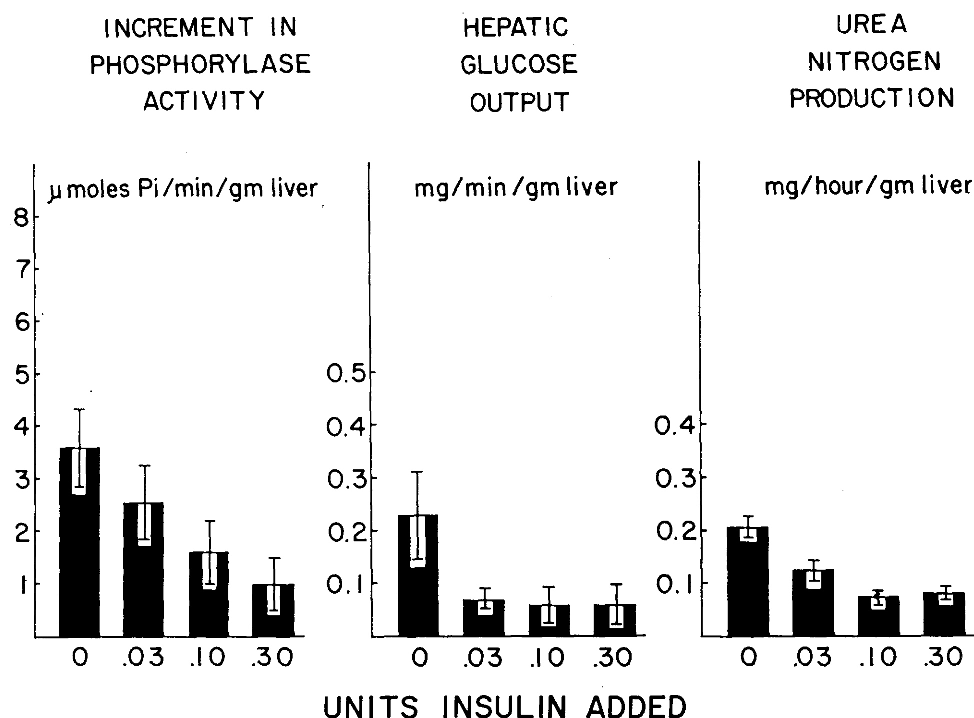

FIG. 2.

Inhibitory effects of different amounts of insulin on the actions of 0.03 μg. of glucagon. Each bar represents the mean of four to six perfusions; vertical lines indicate standard errors.

ever, in perfusions with 0.1 μg. or more of glucagon, no inhibitory effect of insulin on phosphorylase activation could be demonstrated.

Hormonal effects on glycogenolysis

In all groups of perfusions, the hepatic glucose output during the baseline periods was not significantly different from zero, indicating that net glycogenolysis was not occurring. The effects of addition of various combinations of glucagon and insulin are summarized in table 1. Insulin alone had no effect on hepatic glucose output (Section A). Glucagon alone uniformly induced unequivocal increases in hepatic glucose output, similar to those observed in previous studies of glucagon action in this laboratory. The maximal effects of glucagon were observed after addition of 0.3 μg. (peak plasma concentration 4 to 5 mμg./ml.); addition of 1.0 μg. produced identical effects, and data from both groups of perfusions are pooled in table 1.

Insulin clearly inhibited the glycogenolytic effect of glucagon. With glucagon additions of 0.03 μg., all con-

TABLE 1

Effects of glucagon and insulin on hepatic glucose output

Hormonal additions: Glucagon (μg.)	Hormonal additions: Insulin (U.)	No. of perfusions	Baseline hepatic glucose output (mg./min./gm. liver) Mean ± S.E.M.	Increase in hepatic glucose output after hormonal additions (mg./min./gm. liver) 9-10 min.	19-20 min.	Average
			A			
0	0	6	.01±.01	−.02±.01	−.03±.01	−.02±.01
0	.03, .30	5	−.01±.01	0 ±.02	−.02±.01	−.01±.02
			B			
.03	0	4	.01±.02	.22±.07	.26±.09	.25±.07
.03	.03, .10, .30	14	0 ±.01	.10±.02	.04±.02	.07±.02
			C			
.10	0	5	−.01±.02	.39±.03	.33±.03	.36±.04
.10	.03	4	−.01±.02	.38±.05	.26±.04	.32±.04
.10	.10	4	−.01±.02	.29±.06	.28±.03	.28±.06
.10	.30	5	.01±.01	.13±.06	.10±.04	.12±.04
			D			
.30, 1.0	0	5	−.02±.01	.53±.06	.49±.06	.51±.07
.30, 1.0	.03, .30	11	0 ±.01	.48±.03	.43±.03	.46±.03

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centrations of insulin had comparable inhibitory effects, and data from three groups of perfusions are pooled in table 1 (Section B). It is evident that both the intensity and the duration of the glycogenolytic action of this amount of glucagon were decreased by insulin. In the perfusions with additions of 0.1 μg. of glucagon (Section C) only the highest insulin concentration unequivocally inhibited glycogenolysis. With glucagon additions of 0.3 μg. or more (Section D), neither modest nor high concentrations of insulin inhibited hepatic glucose release.

Direct measurement of changes in glycogen, a much less precise technic, yielded results consistent with those in table 1. No significant differences or consistent trends were seen among the inhibitory effects of different concentrations of insulin and thus, pooled data were used for statistical analysis. In both groups of perfusions summarized in Section A of the table, glycogen did not change significantly during the one-hour experimental period. All concentrations of insulin inhibited the effect of 0.03 μg. of glucagon, reducing glycogen decrements from 5.0 $\pm$ 0.2 to 1.1 $\pm$ 1.4 mg./gm. liver ($p < .05$). Insulin partially inhibited the glycogenolysis caused by 0.1 μg. of glucagon (glucagon alone, 10.8 $\pm$ 1.2 mg./gm.; glucagon plus insulin, 6.6 $\pm$ 0.7 mg./gm.; $p < .05$). No effect of insulin was demonstrable when glucagon additions of 0.3 or 1.0 μg. were used (19.8 $\pm$ 1.2 mg./gm. vs 16.0 $\pm$ 2.0 mg./gm.; $p > .1$).

Hormonal effects on urea production

Data on urea production by these isolated livers are summarized in table 2. In the six control perfusions, urea production averaged 0.21 $\pm$ .03 mg. urea N/hr./gm. liver. Both low and high concentrations of insulin depressed urea production by about 60 per cent ($p > .01$). Addition of 0.03 μg. of glucagon had no effect on urea formation. However, additions of 0.1 μg. or more induced a 74 per cent increase in urea production ($p < .02$).

All concentrations of insulin reversed glucagon-induced stimulation of amino acid catabolism, reducing urea production to rates below the control levels. The effects of the larger additions of glucagon were not completely overcome under these conditions, however. Urea production by livers exposed to insulin and 0.1 μg. or more of glucagon, though below control values, was significantly greater than that observed after addition of insulin alone ($p < .01$).

Although, in perfusions with glucagon alone, stimulation of urea production persists without change for at least an hour after addition of the hormone,[15] it seemed quite possible that under the conditions of these experiments, with persistent insulin action but rapidly falling glucagon levels, the situation might be different. The apparent dominance of insulin effect, shown in table 2, might have been due simply to the longer half-life of insulin and, thus, the longer duration of hepatic exposure to this hormone. Therefore, in order to make

TABLE 2

Effects of glucagon and insulin on hepatic urea production

Hormonal additions Glucagon (μg.)	Insulin (U.)	No. of perfusions	Urea nitrogen produced (mg./hr./gm. liver) Mean $\pm$ S.E.M.
0	0	6	.21 $\pm$ .03
0	.03, .30	5	.08 $\pm$ .01
.03	0	4	.21 $\pm$ .02
.03	.03	6	.12 $\pm$ .02
.03	.10, .30	8	.08 $\pm$ .02
.10	0	5	.37 $\pm$ .04
.10	.03, .10	8	.18 $\pm$ .01
.10	.30	5	.14 $\pm$ .01
.30, 1.0	0	5	.36 $\pm$ .02
.30, 1.0	.03, .30	12	.16 $\pm$ .02

certain that valid data were obtained for hormonal antagonism on urea production, a supplementary series of experiments was performed, in which a high concentration of glucagon was maintained throughout the experimental period. Small and large insulin additions were made as before (0.03 U., supplemented by 0.015 U.; and 0.3 U.), but 1.0 μg. of glucagon was added to the blood reservoir every ten minutes. The effects on urea production in these experiments (table 3) were only slightly different from those observed after single glucagon additions of 0.3 or 1.0 μg., described above. Urea production rates with glucagon alone were a little higher, but not significantly so (0.43 vs 0.36 mg./hr./gm. liver). Modest, as well as high concentrations of insulin completely blocked the glucagon effect on urea production. However, rates below control levels were not achieved under these conditions.

Summary of hormonal effects

Although antagonism between insulin and glucagon

TABLE 3

Effects of insulin on hepatic urea production in the presence of high, sustained glucagon levels. Insulin additions as in previous experiments; 1.0 μg. of glucagon added every ten minutes.

Insulin added U.	No. of perfusions	Urea nitrogen produced (mg./hr./gm. liver) Mean $\pm$ S.E.M.
0	4	.43 $\pm$ .04
.03	4	.21 $\pm$ .02
.30	4	.21 $\pm$ .03

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was demonstrated for each parameter of hormonal action studied, there were marked differences between the effects of different combinations of the two hormones on hepatic phosphorylase activity, glucose output and urea production. These are best appreciated by comparing the inhibitory effects of different concentrations of insulin on the actions of individual glucagon additions.

Figure 2 summarizes the effects of different insulin additions on the actions of 0.03 μg. of glucagon. It is evident that phosphorylase activation was least sensitive to insulin inhibition; only a slight effect was seen with 0.03 U. of insulin, and 0.3 U. were required for 70 per cent inhibition of this action of glucagon. Glycogenolysis was more sensitive; 70 per cent inhibition of glucagon effects on hepatic glucose release was obtained at the relatively modest insulin concentrations achieved with addition of 0.03 U. Urea production proved to be very sensitive to insulin action. It was reduced to values well below control levels in all experimental groups and, with insulin additions of 0.1 U. or more, urea production rates were clearly indistinguishable from those observed after addition of insulin alone (see table 2).

Figure 3 summarizes the effects of insulin on the actions of 0.1 μg. of glucagon. Inhibitory effects follow the same order seen in figure 2. At this concentration of glucagon, none of the insulin additions inhibited phosphorylase activation. However, addition of 0.3 U. of insulin significantly inhibited glycogenolysis. Again, all concentrations of insulin markedly inhibited hepatic urea production, though levels as low as those seen with insulin alone were not achieved.

Figure 4 summarizes the effects of insulin on the action of 0.3-1.0 μg. of glucagon. At these concentrations of glucagon, even the largest additions of insulin completely failed to inhibit hepatic phosphorylase activation or glucose release. However, a modest concentration of insulin was still effective in reducing urea production to rates somewhat below those observed in control perfusions. Inhibition by insulin of the effects on urea production of still higher, and sustained, glucagon concentrations has already been described (table 3).

It is clear that the antagonism between the actions of these two hormones is not a uniform one. Insulin exerted weak inhibitory effects on glucagon-induced activation of phosphorylase and had only moderate effects on glycogenolysis. However, even modest levels of insulin completely suppressed the effects of high concentrations of glucagon on urea production.

DISCUSSION

The effects of glucagon observed in this study were similar to those previously reported from this and other laboratories. It is universally agreed that glucagon

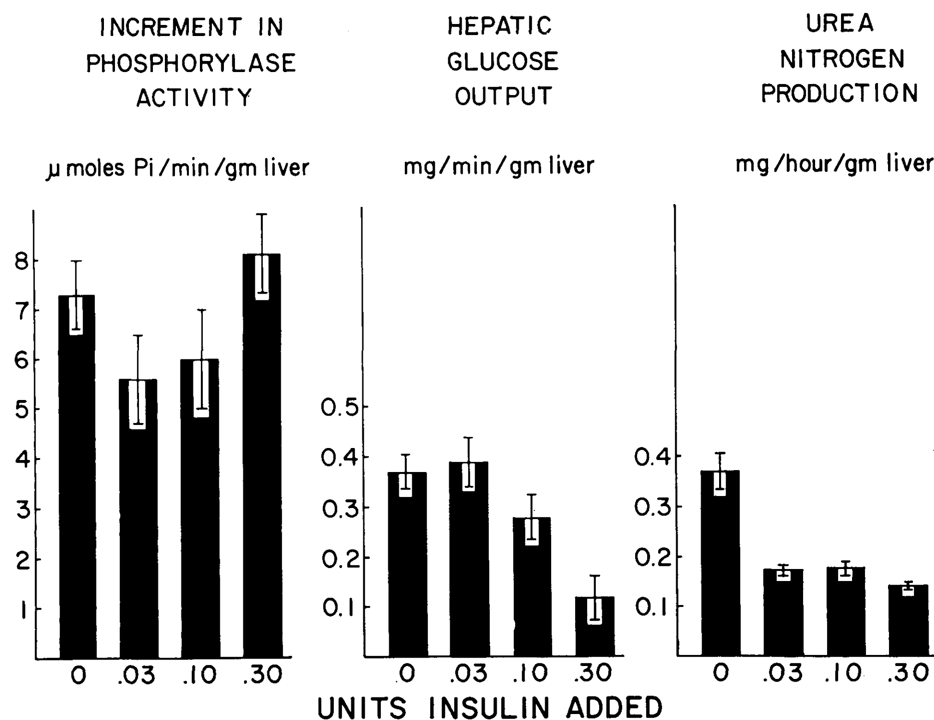

FIG. 3.

Inhibitory effects of different amounts of insulin on the actions of 0.10 μg. of glucagon. Each bar represents the mean of four to five perfusions; vertical lines indicate standard errors.

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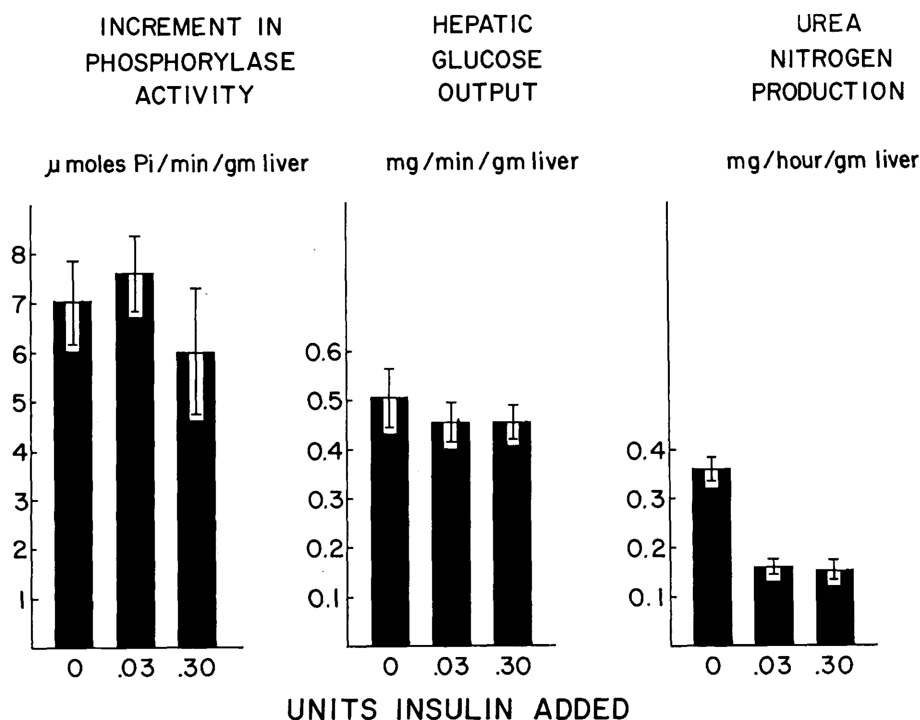

FIG. 4.

Inhibitory effects of different amounts of insulin on the actions of 0.3 or 1.0 μg. of glucagon. Each bar represents the mean of five to six perfusions; vertical lines indicate standard errors.

stimulates hepatic phosphorylase activity, glycogenolysis and amino acid catabolism. However, there has not been complete agreement regarding the effects of insulin on the liver, particularly with respect to glycogen metabolism and glucose output. Madison et al.[17] found that insulin reduced hepatic glucose output of dogs with portocaval shunts. However, Shoemaker et al.,[18] studying dogs with intact portal circulation, found no effect of insulin on the hepatic glucose output. In 1963, Mortimore[19] reported that insulin produced a prompt and unequivocal decrease in glucose release by perfused livers, under a variety of experimental conditions. Subsequently, Haft[4] reported only marginal and late effects. In Mortimore's control experiments, the perfusate glucose concentration rose progressively, indicating that active glycogenolysis was occurring, whereas in Haft's experiments, like ours, blood glucose was stable or declining after the first thirty minutes. More recently, Glinsmann and Mortimore[20] restudied this point, using an experimental design somewhat similar to ours, and concluded that insulin inhibits glycogenolysis but does *not* stimulate hepatic glucose uptake or glycogen deposition. The same conclusion was reached by Mondon and Burton.[21] Our data are in agreement with this view. In perfusions without glucagon, net glycogenolysis was not occurring and no insulin effect on hepatic glucose release was seen (table 1, Section A). However, insulin inhibited the glycogenolysis induced by small additions of glucagon (table 1, Section B). Thus, there now appears to be a consensus that insofar as major changes in glucose balance across the liver are concerned, the principal effect of insulin is to restrict glycogenolysis and glucose outflow from the liver, rather than to stimulate hepatic glucose uptake and glycogen deposition. When net glycogenolysis is occurring, an unequivocal effect of insulin on the hepatic glucose output is seen; when glycogen levels are stable, no effect can be demonstrated.

The rate of urea production in control perfusions in this study was quite similar to that reported by Haft,[4] but somewhat lower than that observed by Mortimore during in situ liver perfusion. Mortimore used considerably younger animals than did Haft or we, and the higher urea production rates he reported may be due, at least in part, to the presumably higher metabolic rates in his animals. The insulin-induced decrease in urea production observed in the present study is comparable to that reported by Mortimore, but greater than that observed by Haft. Of interest is the fact that over thirty years ago, Bach and Holmes[22] reported inhibition of urea production by liver slices, in the presence of insulin, of the same order of magnitude as that recorded in the present study.

The simultaneous addition of insulin to perfusing

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blood resulted in inhibition of all of the effects of glucagon measured in the present study. Although there is no longer serious doubt that insulin exerts direct action on the liver, these findings provide additional evidence on this point and show that effects are exerted on several different reactions in the liver. This study appears to be the first in which insulin inhibition of glucagon-induced phosphorylase activation has been demonstrated. Others have also described inhibition of glucagon effects on glycogenolysis[20,21] and urea production, [20,21,23] as well as on potassium release from the liver.[20]

It is evident that the effects of insulin, like those of glucagon, can be observed promptly after exposure of the liver to the hormone. The degree of antagonism between these two hormones appears to be dependent on their concentrations, rather than on "preconditioning" of the liver, since (a) we obtained virtually identical results on hepatic glucose output and phosphorylase activity, in experiments in which livers were exposed to insulin thirty to sixty minutes before addition of glucagon (unpublished data) and (b) Miller[23] demonstrated prompt reduction in the rate of urea production after addition of insulin, in experiments in which continuous glucagon infusion had been started ninety to 120 minutes previously.

An important finding of this study is that there is a clearcut hierarchy in the effects of each hormone on the liver, as demonstrated by the striking differences in their antagonism on the three reactions studied. Insulin concentrations approaching 4,000 μU./ml., probably somewhat above the upper limit of the physiologic range for portal vein blood, had *no* inhibitory effect on the phosphorylase activation induced by 0.1 μg. of glucagon (figure 3). However, concentrations of insulin below 400 μU./ml. completely suppressed the effect on urea production of much higher and sustained glucagon concentrations, well above the biologic range (table 3). Glycogenolysis occupied an intermediate position with respect to this antagonism.

It is difficult to reconcile these observations with a single site of hormonal action on the liver cell. If both hormones acted on a single receptor, then any particular combination of glucagon and insulin should have had effects in the same direction on all the reactions studied. This was clearly not the case. After addition of 0.03 μg. of glucagon and 0.03 U. of insulin, for example, phosphorylase activity *increased* while urea production *decreased* (table 2, figure 2). These findings are inconsistent with the hypothesis that these hormones act via a single common pathway, with their effects determined by net changes in the intracellular concentration of cyclic 3′,5′-AMP.[24] Obviously, the combined action of insulin and glucagon at one receptor site could not result in both an increase (for phosphorylase activation) and a decrease (for inhibition of amino acid catabolism) of cyclic 3′,5′-AMP generation at that site.

Our findings could be reconciled with primary action of both hormones via cyclic 3′,5′-AMP as a "second messenger,"[25] only by postulating that the adenyl cyclase system exists in different compartments in the liver cell and that insulin and/or glucagon reach such different compartments inhomogeneously—or that adenyl cyclase in different compartments has different sensitivities to these hormones. Under such circumstances, it is conceivable that there might be different insulin:glucagon concentration ratios—or "biological effectiveness" ratios—at different loci, resulting in increase of cyclic 3′,5′-AMP concentration at one site and decrease at another. However, this would require revision of the current concept that adenyl cyclase in one type of cell is of a single species, located principally at the cell membrane.[25]

We conclude that the various effects of insulin and glucagon on the liver cell are not mediated via a single common pathway. Either two or more primary mechanisms, or different effects on the same mechanism (e.g., cyclic 3′,5′-AMP generation) at different sites, must be involved.

ACKNOWLEDGMENT

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REFERENCES

[1] Levine, R., and Fritz, I. B.: The relation of insulin to liver metabolism. Diabetes *5*:209-17, 1956.

[2] Levine, R.: Cell membrane as a primary site of insulin action. Fed. Proc. *24*:1071-74, 1965.

[3] Krahl, M.: The Action of Insulin on Cells. New York and London, Academic Press 1961.

[4] Haft, D. E.: Effects of insulin on glucose metabolism by the perfused normal rat liver. Amer. J. Physiol. *213*:219-28, 1967.

[5] Sokal, J. E.: Glucagon—An essential hormone. Amer. J. Med. *41*:331-41, 1966.

[6] Foâ, P. P.: Glucagon. Ergebn. Physiol. *60*:142-219, 1968.

[7] Samols, E., Marris, G., and Marks, V.: Promotion of insulin secretion by glucagon. Lancet *2*:415-16, 1965.

[8] Porte, D., Jr., Graber, A. L., Kuzuya, T., and Williams, R. H.: The effect of epinephrine on immunoreactive insulin

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levels in man. J. Clin. Invest. *45*:228-36, 1966.

[9] Sokal, J. E., and Weintraub, B.: Failure of the isolated liver to react to hypoglycemia. Amer. J. Physiol. *210*:63-68, 1966.

[10] Sokal, J. E., and Ezdinli, E. Z.: Basal plasma glucagon levels of man. J. Clin. Invest. *46*:778-85, 1967.

[11] Weintraub, B., Sarcione, E. J., and Sokal, J. E.: Effect of glucagon on phosphorylase activity of the isolated perfused liver. Amer. J. Physiol. *216*:521-26, 1969.

[12] Good, C. A., Kramer, H., and Somogyi, M.: The determination of glycogen. J. Biol. Chem. *100*:485-89, 1933.

[13] Chaney, A. L., and Marbach, E. P.: A phenol-hypochlorite method for determination of urea in plasma. Clin. Chem. *8*: 131-37, 1962.

[14] Sokal, J. E., Sarcione, E. J., and Henderson, A. M.: Relative potency of glucagon and epinephrine as hepatic glycogenolytic agents. Studies with the isolated perfused rat liver. Endocrinology *74*:930-38, 1964.

[15] Sokal, J. E.: Effect of glucagon on gluconeogenesis by the isolated perfused rat liver. Endocrinology *78*:537-48, 1966.

[16] Unger, R. H., Eisentraut, A. M., McCall, M. S., and Madison, L. L.: Measurements of endogenous glucagon in plasma and the influence of blood glucose concentration upon its secretion. J. Clin. Invest. *41*:682-89, 1962.

[17] Madison, L. L., Combes, B., Adams, R., and Strickland, W.: The physiological significance of the secretion of endogenout insulin into the portal circulation. III. Evidence for a direct immediate effect of insulin on the balance of glucose across the liver. J. Clin. Invest. *39*:507-22, 1960.

[18] Shoemaker, W. C., Mahler, R., and Ashmore, J.: The effect of insulin on hepatic glucose metabolism in the unanesthetized dog. Metabolism *8*:494-511, 1959.

[19] Mortimore, G. E.: Effect of insulin on release of glucose and urea by isolated rat liver. Amer. J. Physiol. *204*:699-704, 1963.

[20] Glinsmann, W. H., and Mortimore, G. E.: Influence of glucagon and 3',5'-AMP on insulin responsiveness of the perfused rat liver. Amer. J. Physiol. *215*:553-59, 1968.

[21] Mondon, C. E., and Burton, S.: Insulin—An anticatabolic agent in regulation of hepatic carbohydrate metabolism. Diabetes *17*, *Suppl. 1*:336, 1968.

[22] Bach, S. J., and Holmes, E. G.: The effect of insulin on carbohydrate formation in the liver. Biochem. J. *31*:89-100, 1937.

[23] Miller, L. L.: Direct actions of insulin, glucagon and epinephrine on the isolated perfused rat liver. Fed. Proc. *24*: 737-45, 1965.

[24] Exton, J. H., Jefferson, L. S., Jr., Butcher, R. W., and Park, C. R.: Gluconeogenesis in the perfused liver. The effects of fasting, alloxan diabetes, glucagon, epinephrine, adenosine 3',5'-monophosphate and insulin. Amer. J. Med. *40*:709-15, 1966.

[25] Sutherland, E. W., Oye, I., and Butcher, R. W.: The action of epinephrine and the role of the adenyl cyclase system in hormone action. Recent Progr. Hormone Res. *21*:623-42, 1965.

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