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ORIGINALS

Physiological Factors Influencing Insulin Clearance by the Isolated Perfused Rat Liver

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Summary. Hepatic insulin clearance was studied in normal male and female Wistar rats using the isolated liver perfusion technique and the dextran coated charcoal radioimmunoassay for insulin. The following results were obtained: — 1. In male rats there was a progressive increase in clearance with increasing body weight, liver weight and age. — 2. In adult female rats clearance was significantly greater than in comparable males, but no relationship between liver weight and clearance was observed. — 3. With each increase in insulin concentration there was an apparent decrease in clearance.

Key words: Insulin, clearance, perfusion, radioimmunoassay.

Introduction

The mammalian liver has been shown to be one of the major organs involved in insulin degradation Mirsky [14], Williams *et al.*, [23] and Freinkel [6]. All endogenous insulin must pass through it en route to muscles and other target organs. Disturbances of hepatic insulin metabolism have been variously suggested to explain the carbohydrate intolerance of diabetes mellitus though the evidence is inconclusive and to some extent conflicting Mirsky [13], Tomizawa and Varandani [22] and Cerasi and Luft [3]. There is, however, little information on the effects of such physiological variables as age, weight, sex and substrate concentration on insulin clearance by the normal liver. The present study was undertaken to evaluate these factors, and to standardise conditions under which hepatic insulin degradation might be studied both in the normal animal and in states of carbohydrate intolerance.

Materials and Methods

The isolated intact rat liver perfusion technique of Miller *et al.* [12] and Schimassek [20] as modified by Hems *et al.* [8] was employed throughout these studies. Each insulin clearance experiment was preceded by a control perfusion period of 15 min. Pork insulin (Novo 10 times recrystallised Lot No. S23267) of potency 25 I.U./mg was then added to the perfusion medium to give a final concentration of 200 μU/ml unless otherwise stated in the protocol. A further 6 min perfusion period was allowed for equilibration Solomon *et al.* [21] after which the first of a series of approximately 0.5 ml samples was taken directly from the hepatic effluent. Further samples were taken 3, 6, 9, 14, 19, 24 and 34 min later. Samples were immediately centrifuged to remove red blood cells and stored frozen at — 20°C. Insulin in the samples was subsequently measured by the radioimmunoassay technique of Yalow and Berson [24] as modified by Herbert *et al.* [9] and Buchanan and McCarroll [1].

Calculation of results

The results were calculated as the percentage of maximal (initial) concentration determined 6 min after the insulin had been added to the perfusate and the half life determined from the linear regression equation of insulin concentration against time. No correction was made for the error introduced by the decreasing perfusate volume as this was less than 5%.

Experimental Protocol 1

The effect of age, weight and sex on hepatic insulin half life ($t\frac{1}{2}$). Eighty-four Wistar albino rats fed ad libitum and with free access to water were divided into 4 groups according to weight and age. There was no significance difference in weight between the sexes within groups. The isolated intact livers were perfused with 200 μU of pork insulin per ml of perfusate.

Experimental Protocol 2

The effect of the amount of substrate present on hepatic insulin $t\frac{1}{2}$. Thirty-one male Wistar rats 150—200 g were fed ad libitum with free access to water and divided into 4 groups. Liver perfusion studies were carried out with 50, 200, 500 and 1,000 μU of insulin per ml of perfusate. These concentrations were chosen to mimic what might be expected to be found in the portal vein

(1) under fasting conditions
(2) post prandial — low physiological response
(3) post prandial — high physiological response
(4) at the approximate upper limit of the biological range.

Results

The effect of age, weight and sex on hepatic insulin $t\frac{1}{2}$

Table 1 shows the hepatic insulin $t\frac{1}{2}$ in different weight-age-groups. It will be seen that there is a progressive decrease in $t\frac{1}{2}$ with increasing weight and age except in females of Group 4.

Analysis of variance between male groups, Table 2, shows a highly significant difference, $p < 0.001$. It is clear that both age and weight of male animals have a significant effect on insulin $t\frac{1}{2}$ except between Groups 2 and 3.

In females, Table 3, analysis of variance also shows a significant difference between groups. In this case the difference lies between Group 1 and the other 3 groups.

The relationship between body weight and the weight of liver tissue in the two sexes is shown in Table 4. It will be seen that there is a direct relationship between body weight and the weight of the liver in both sexes. For any given body weight there is no difference in liver weight between the sexes, except in Group 1 in which the liver weight of females was significantly less than that of males.

The effect of sex on insulin $t\frac{1}{2}$ is also shown in Table 1. No significant difference was noted between sexes in Group 1 or in Group 4. In Groups 2 and 3, however, there was a highly significant difference between the sexes, the $t\frac{1}{2}$ being much shorter in the females.

The effect of substrate concentration on hepatic insulin $t\frac{1}{2}$

The results are given in Table 5. Insulin $t\frac{1}{2}$ increases progressively with increasing substrate concentration. The difference is highly significant between all groups except 2 and 3.

Table 1. *Hepatic insulin half-life in different weight-age groups*

Group	Sex	No. of rats	Mean t ½ min	Significance of difference between sexes within groups
1. 100—150g 8—10 wks.	M	7	22.9 ±3.3 8.7	$t = 0.9843$
	F	6	19.22+1.19 2.87	$0.2 > P > 0.15$
2. 150—200 g 10—12 wks.	M	10	18.1 ±0.81 2.57	$t = 2.2609$
	F	10	15.7 +0.7 2.25	$0.01 > P > 0.005$
3. 200—300 g 12—16 wks.	M	15	16.5 ±1.07 4.15	$t = 2.0648$
	F	15	13.71+0.8 3.1	$0.025 > P > 0.0125$
4. 300 g+ 16—20 wks.	M	15	10.97±0.79 3.07	$t = 1.6879$
	F	6	14.47±2.7 6.68	$0.1 > P > 0.05$

Table 2. *Analysis of variance of hepatic insulin half-life in males*

Groups	t	p	
1 and 2	2.1428	$0.025 > P > 0.0125$	Correlation
1 and 3	3.0769	$0.0025 > P > 0.0005$	coefficient
1 and 4	5.7692	$P < 0.0005$	$r = 0.6627$
2 and 3	0.8649	$0.2 > P > 0.15$	$P < 0.001$
2 and 4	3.8919	$P < 0.0005$	
3 and 4	3.3735	$P < 0.0005$	

Table 3. *Analysis of variance of hepatic insulin half-life in females*

Groups	t	p	
1 and 2	1.8789	$0.05 > P > 0.025$	Correlation
1 and 3	3.0955	$0.0025 > P > 0.0005$	coefficient
1 and 4	2.2405	$0.0125 > P > 0.01$	$r = 0.4748$
2 and 3	1.2933	$0.10 > P > 0.05$	$0.01 > P$
2 and 4	0.5684	$0.30 > P > 0.25$	> 0.001
3 and 4	0.4269	$0.35 > P > 0.30$	

Discussion

Body weight, liver weight and age:

These three factors are considered together because a highly significant positive correlation ($p < 0.001$) was shown to exist between them in both male and female rats (Table 4). The finding of a progressive decrease in insulin $t\frac{1}{2}$ in male rats with increasing body weight, liver weight and age is in keeping with that of Mirsky and Perisutti [16] and Diengott *et al.* [4]. However, in adult female rats no such relationship between liver weight and insulin $t\frac{1}{2}$ was observed. It would appear that for a given substrate concentration the rate of hepatic insulin clearance in male rats is directly related to the amount of available liver tissue, but that in comparable adult females other factors, perhaps hormonal, may also be operative.

The effect of sex on hepatic insulin $t\frac{1}{2}$

There are apparently no published data on the relationship between the sex of an animal and the rate of hepatic insulin clearance. The results of the experiments in this study indicate that the clearance of insulin by the livers of 150—300 g (approximately 10—16 weeks old) female rats is significantly greater than in males of similar weight and age. Rats of this age are in the most active reproductive stage of their lives, so that maximal hormonal differences between the sexes are to be expected. That female hormones do influence insulin disposal in the liver is strongly suggested by the work of Goodner and Freinkel [7] which showed that insulin turnover in pregnant rats is ac-

celerated to a degree only partly explained by the presence of the products of conception.

The effect of substrate concentration on hepatic insulin $t\frac{1}{2}$

The enzyme system responsible for insulin inactivation has been shown by numerous workers to obey first order kinetics Mirsky and Broh-Kahn [15], Mirsky *et al.* [17], Bürgi *et al.* [2] and Marshall *et al.* [11]. The rate of elimination of insulin by the enzyme system should therefore be proportional to the amount of substrate presented to it. Consequently insulin $t\frac{1}{2}$ should be constant at all substrate concentrations. However, the results presented here (Table 5) show that insulin $t\frac{1}{2}$ increases progressively with each increase in substrate concentration.

There are at least two possible explanations for the discrepancy between these findings and those predicted by enzyme kinetic studies. The first is that at low substrate concentrations first order kinetics prevail, but that high concentrations produce enzyme inhibition. Dixon and Webb [5] described several examples of this phenomenon and suggested that there might be competitive inhibition by the substrate itself. A second possible explanation is that in any preparation other than the purified enzyme itself, at least two other steps are interposed between the union of substrate and enzyme. These are the uptake or 'capture' of substrate by the liver cells, and transcellular passage of the hormone to reach the reaction site. The possible influence of such factors on the measured rate of insulin disposal was first suggested by Mirsky and Perisutti [16] who demonstrated that liver slices inactivated insulin more slowly than equivalent amounts of homogenised tissue. Further, the liver has been shown to remove 20—50% of insulin in the first single passage through it, indicating its vast capacity for hormone uptake Kaplan and Madison [10] and Samols and Ryder [19]. Mortimore and Tietze [18] have con-

Table 4. *Liver weights in 4 groups*

Group	No. of rats	Sex	Mean liver wt. (g)	Significance of difference of liver weights between sexes within groups	
1. 100—150 g 8—10 weeks	4	M	5.28±0.24 0.49	$t=2.1175$	Correlation coefficient (males) $r=0.9427$ $P<0.001$
	4	F	4.47±0.28 0.57	$0.05>P>0.025$	
2. 150—200 g 10—12 weeks	5	M	6.68±0.23 0.51	$t=0.3813$	
	5	F	6.78±0.13 0.29	$0.40>P>0.35$	
3. 200—300 g 12—16 weeks	5	M	9.86±0.57 1.29	$6=1.8202$	Correlation coefficient (females) $r=0.8922$ $P<0.001$
	5	F	8.76±0.18 0.40	$0.10>P>0.05$	
4. 300 g + 16—20 weeks	4	M	12.46±0.52 1.17	$t=0.4660$	
	4	F	12.05±0.74 1.48	$0.35>P>0.30$	

Table 5. *Hepatic insulin half-life at 4 levels of substrate*

Amount of insulin	No. of rats	Mean t ½ min.	Group	Significance of difference of t ½ between groups	
1. 50 μU/ml	7	10.03±1.0 2.65	1 and 2	$t=3.4935$ $0.0025>P>0.005$	Correlation
			1 and 3	$t=3.9731$	
2. 200 μU/ml	10	18.10±1.2 2.57		$0.0005>P$	coefficient $r=0.9762$
			1 and 4	$t=11.3415$	$0.05>P>0.02$
3. 500 μU/ml	6	20.40±1.16 2.6		$0.0005>P$	
			2 and 3	$t=1.0455$	
4. 1,000 μU/ml	8	37.59±2.86 8.1		$0.2>P>0.15$	
			2 and 4	$t=8.7792$ $0.0005>P$	
			3 and 4	$t=6.7944$ $0.0005>P$	

cluded from their investigations on the capture mechanisms of the liver that the ultimate rate limiting factor in hepatic clearance of insulin may be its transfer from the site of uptake to the degrading enzyme system. Finally, it is clear that insulin concentration should be taken into account in any experiment using the isolated perfused rat liver before valid comparisons can be made.

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