STUDIES ON UREA SYNTHESIS, INSULIN DEGRADATION AND PHAGOCYTOSIS BY ISOLATED RAT KUPFFER CELLS*

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SUMMARY

Isolated Kupffer cells synthesize urea from a variety of added amino acids at a rate comparable to that of hepatocytes. Urea synthesis was determined both by enzymic product analysis and by the incorporation of labeled bicarbonate into urea. Studies on uptake of radioactive insulin showed that both hepatocytes and Kupffer cells bind insulin to almost the same extent. However, much higher degradation of insulin was found with Kupffer cells than with hepatocytes. Labeled insulin-anti-insulin serum (I-AIS) complex is neither bound nor taken up by hepatocytes. Kupffer cells, on the other hand, removed the complex in a time-dependent manner from the medium and partially degraded it.

Metabolic studies with isolated Kupffer cells provide a unique opportunity for the eludication of biological characteristics such as phagocytosis and protein degradation. Although several investigators have reported the isolation of Kupffer cells (1-3) only few attempts have been made to describe protein degradation and phagocytotic activities of these cells. Isolated Kupffer cells retaining their in vivo metabolic characteristics offer an excellent experimental tool to study these properties. In this paper we present data on urea synthesis as well as some preliminary studies on their phagocytotic capabilities.

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*** Abbreviation used: I-AIS, Insulin-anti-Insulin serum.

MATERIALS AND METHODS

Female Wistar rats weighing 170-190 g (Ivanovas, Kisslegg, West Germany) were maintained on Altromin R diet (Altrogge GmbH, Lage/Lippe, West Germany). They were anaesthesized with pentobarbital, the livers removed and perfused with collagenase; hepatocytes were obtained as reported earlier (4). Kupffer cells were isolated by modifying the method of Mills and Zucker-Franklin (3) as reported previously (5). For urea synthesis, 8 mg of cells (wet weight) were incubated with various substrates in plastic tubes at 37°C in a metabolic incubator at 90 oscillations/min under an atmosphere of 95% O_2/5% CO_2. At the end of the incubation, vial contents were placed in Eppendorf cups and centrifuged at 3000 x g for 5 min. Urea was determined in the supernatant by the enzymic method of Fawcett and Scott (6); appropriate blanks for endogenous ammonia were subtracted. Radioactivity incorporated into urea was liberated by urease after removal of excess bicarbonate; the $^{14}CO_2$ evolved was trapped in 2N NaOH and determined as described previously (7,8). The data were corrected for $^{14}CO_2$ evolved without urea treatment. ^{125}I-insulin was obtained from The Radiochemical Center, Amersham. I-AIS complex was prepared by incubating 1 ml of anti-insulin serum with 5 µCi of labeled insulin for 6 hours at room temperature and an additional 6 hours at 5°C. The radioactivity in the I-AIS-complex was determined after precipitation by 90% ethanol and ethanol washing of the precipitate. In studies on the uptake of radioactive insulin and of I-AIS-complex approximately 10 mg of hepatocytes or Kupffer cells were incubated in 1 ml medium. After incubation and centrifugation cells were washed once and radioactivity retained by the cells was measured. Supernatants were assayed for insulin activity by radio immuno assay as described previously (9).

RESULTS AND DISCUSSION

Isolated Kupffer cells synthesized urea from alanine, aspartate, valine, isoleucine, glutamate, arginine, citrulline and ornithine (Table I). The rate of urea synthesis from alanine, aspartate, valine and arginine was significantly higher in Kupffer cells than in hepatocytes. This rules out a substantial contribution by contaminating hepatocytes or debris thereof to the observed urea formation. In the preparations used, only 12 $\pm$ 9 hepatocytes were present per 10,000 Kupffer cells (n = 7). Citrulline and ornithine were less effective precursors of urea synthesis in Kupffer cells than in hepatocytes. This may be due to different permeabilities of the two cell types. Kupffer cells were also shown to incorporate added labeled HCO_3^- into urea (Table II). The formation of radioactive urea in comparable amounts indicates a close similarity of the pathway of urea synthesis in Kupffer cells and hepatocytes.

Table I

SYNTHESIS OF UREA BY ISOLATED HEPATOCYTES AND KUPFFER CELLS

substrate (2 mM)	hepatocytes 30 min	hepatocytes 60 min	Kupffer cells 30 min	Kupffer cells 60 min	ratio Kupffer cells/ hepatocytes
none	11.9 ± 3.6	23.0 ± 8.7	18.2 ± 4.7	32.6 ± 8.7	1.4
alanine	15.7 ± 3.1	38.6 ± 6.1	47.9 ± 9.5	81.9 ± 16.8	2.1
aspartate	21.1 ± 5.9	27.0 ± 5.7	23.7 ± 4.1	43.5 ± 13.3	1.6
valine	22.6 ± 8.4	36.1 ± 6.5	13.1 ± 2.7	48.7 ± 8.4	1.3
isoleucine	33.6 ± 4.1	64.5 ± 13.9	28.0 ± 9.4	62.6 ± 16.4	1.0
glutamate	32.0 ± 10.2	57.3 ± 11.1	28.5 ± 8.4	56.9 ± 15.2	1.0
arginine	81.7 ± 17.9	121.7 ± 24.4	107.9 ± 14.6	157.5 ± 41.2	1.3
citrulline	49.7 ± 11.0	70.9 ± 13.1	22.5 ± 10.5	52.8 ± 8.9	0.7
ornithine	49.0 ± 2.4	99.6 ± 12.6	28.6 ± 8.8	71.5 ± 17.8	0.7

Approximately 8 mg of hepatocytes or Kupffer cells from normal rat livers were incubated in 300 µl of Krebs-Henseleit buffer. The given values represent formation of urea in µmoles/g wet wt. ± S.E.M. of 4-6 observations. The ratios are derived from the 60 min data.

Table II

INCORPORATION OF LABEL FROM $H^{14}CO_3^-$ INTO UREA BY ISOLATED HEPATOCYTES AND KUPFFER CELLS

substrate	hepatocytes		Kupffer cells	
	30 min	60 min	30 min	60 min
alanine (2 mM) + $NaH^{14}CO_3$	23,680 ± 3500	68,500 ± 5300	31,650 ± 4320	86,500 ± 8200
arginine (2 mM) + $NaH^{14}CO_3$	41,850 ± 5360	89,420 ± 7830	38,280 ± 4600	68,280 ± 4350

Approximately 20 mg of isolated Kupffer cells or hepatocytes from normal rat livers were incubated in 1 ml of Krebs-Henseleit buffer with alanine or arginine and 2.0 µCi of $NaH^{14}CO_3$ (specific activity 58.6 mCi/mmole). The given values represent cpm/g wet weight of cells. They are the mean of 4 observations ± S.E.M.

The phagocytotic capability of Kupffer cells has been established by histological observations (10) and, more recently, by biochemical studies (11). In the light of diverging data on the stability of insulin in perfused livers (12-15) and in hepatocyte suspensions (16-18), binding, uptake and degradation of this hormone by isolated Kupffer cells and by hepatocytes was studied. As summarized in Table III, both cell types removed part of the added radioactive insulin from the medium. The uptake by hepatocytes was quite rapid but increased only slightly after 10 minutes. This suggests that the insulin was rapidly bound to the receptors but that consecutive processes, e.g. endocytosis, degradation, were proceeding slowly. After 60 min, 80% of the added insulin could still be recovered in the medium by radio-immuno assay. The uptake of insulin by Kupffer cells was only 50% of that by hepatocytes during the first minutes but it increased linearly with time of incubation. Kupffer cells appear to phagocytize the added insulin; after 60 min, less than 20% of the added insulin could be recovered in the medium indicating rapid proteolytic degradation. The large difference in insulin inactivation between Kupffer cells and hepatocytes offers a plausible explanation for the longer persistence of insulin effects in the presence of isolated hepatocytes (16-18) as compared to the intact liver (12-15).

Experiments on uptake and degradation of the complex between insulin and anti-insulin serum as outlined in Table IV serve both as a control to the results of Table III and as evidence of true phagocytosis by Kupffer cells in contrast to hepatocytes. The I-AIS-complex was not taken up at all by the hepatocytes during one hour of incubation; all radioactivity added was recovered in the medium indicating that neither binding nor uptake and degradation of the complex had occurred. In contrast, isolated Kupffer cells showed an increased uptake of the complex during the incubation. Almost 18% of the added radioactivity was incorporated into the Kupffer cells at the end of the first hour. These results suggest that isolated Kupffer cells phagocytize and degrade added proteins including protein hormones. Appreciable amounts of NH_3 liberated during amino acid degradation seem to be converted to urea.

To our knowledge this is the first report showing urea synthesis and insulin degradation in Kupffer cells. It would be also worth-

Table III

UPTAKE OF ^{125}I INSULIN BY ISOLATED HEPATOCYTES AND KUPFFER CELLS

incubation time (min)	Hepatocytes			Kupffer cells		
	insulin bound		recovered*	insulin bound		recovered*
	dpm/10 mg of cells	%	%	dpm/10 mg of cells	%	%
1	3850 ± 280	1.92	92 ± 6	2180 ± 210	1.09	88 ± 6
3	4660 ± 420	2.33	84 ± 8	4260 ± 326	2.13	82 ± 8
10	6575 ± 630	3.28	90 ± 6	7638 ± 420	3.82	71 ± 10
30	7835 ± 680	3.91	85 ± 5	11338 ± 830	5.66	36 ± 12
60	8380 ± 720	4.19	80 ± 7	12880 ± 1600	6.44	20 ± 14

Approximately 10 mg of isolated hepatocytes or Kupffer cells from normal rat livers were incubated in 1 ml of medium containing 200,000 dpm of ^{125}I insulin. The given values represent the mean of 4 observations ± S.E.M.

* Recovery denotes amount of insulin measured in the medium by radio-immuno assay at the end of the incubations (see Methods).

Table IV

UPTAKE OF ANTI-INSULIN Serum ^{125}I INSULIN COMPLEX BY ISOLATED HEPATOCYTES AND KUPFFER CELLS

	Hepatocytes		Kupffer cells		
incubation time (min)	dpm/10 mg of cells	complex recovered in medium %	dpm/10 mg of cells	incor-porated %	complex recovered in medium %
1	145 ± 80	98	4380 ± 400	2.19	88 ± 6
3	280 ± 120	98	9650 ± 850	4.82	82 ± 8
10	360 ± 180	98	18230 ± 1200	9.11	66 ± 12
30	480 ± 195	98	30500 ± 2600	15.25	40 ± 15
60	560 ± 260	98	36380 ± 3200	18.19	41 ± 12

Approximately 10 mg of isolated hepatocytes or Kupffer cells from normal rat livers were incubated in 1 ml of Krebs-Henseleit buffer containing 200,000 dpm of the complex formed by ^{125}I insulin and anti-insulin serum (see Methods). The given values represent the mean of 4 observations ± S.E.M.

while to investigate insulin degradation in isolated Kupffer cells in various disease states. The biochemical performances of Kupffer cells as observed in this and in previous (5) studies will facilitate a comparison of Kupffer cells with bone marrow, peritoneal and alveolar macrophages and to assess their ontologic relation.

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